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The Role of Angiotensin II and of Its Receptor Subtypes in the Acetic Acid-Induced Abdominal Constriction Test

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GEORGIEVA, D. AND V. GEORGIEV. *The role of angiotensin II and of its receptor subtypes in the acetic acid-induced abdominal constriction test.* PHARMACOL BIOCHEM BEHAV **62**(2) 229–232, 1999.—The effects of angiotensin II (AngII), the AngII analogues saralasin—[Sar[1], Ala[8]]AngII, sarmesin—[Sar[1]Tyr(Me)[4]]AngII, the nonpeptide AngII receptor antagonists DuP753 (losartan) (for AT_1 receptor subtype) and PD123319 (for AT_2 receptor subtype), as well as combinations of AngII and each of its analogues and receptor antagonists, administered intracerebroventricularly (ICV), were studied on mice using the acetic acid-induced abdominal constrictions test (acetic acid 1% intraperitoneally, IP). The abdominal constrictions were counted at 5-min intervals for 30 min. AngII at doses of 0.05, 0.1, and 1 μg exerted a dose-dependent antinociceptive effect. Saralasin, sarmesin, losartan, and PD123319 exhibited a dose-dependent effect on nociception: they either increased or decreased it. PD123319 antagonized the antinociceptive effect of AngII while losartan was ineffective. The importance of AT_2 receptor subtype for the nociception reducing effect of AngII is considered. © 1999 Elsevier Science Inc.

Abdominal constrictions Acetic acid Mice Saralasin Sarmesin Losartan PD123319 AT_2 receptor subtype

THE octapeptide angiotensin II (AngII) participates in membrane functions, maintenance of salt and volume homeostasis, blood pressure control, release of pituitary hormones, and control of behavioral responses like thirst and drinking (14). In addition to these well-known central actions AngII injected intracerebroventricularly (ICV) enhances exploratory behavior and locomotor activity, improves learning and memory (4,5), increases the threshold of seizures, and decreases the intensity of PTZ-kindled seizures (6). Renin and AngII administered ICV exert analgesic effects in rats (a short-lasting increase of latency in the tail-flick test). The stress-induced analgesia is usually accompanied by a release of renin–angiotensin, and is decreased by saralasin and naloxone (9,10). The central effects of AngII are mainly realized via two receptor subtypes, i.e., AT_1 and AT_2, which are differently distributed in the brain regions (14). The AT_1 receptor subtype is blocked by losartan (DuP 753), and the AT_2 receptor subtype, by PD123319. The present work was undertaken to further evaluate the role of AngII, and particularly of its receptor subtypes (AT_1 and AT_2) in nociception. The effects of the AngII analogues as well as saralasin ([Sar[1], Ala[8]]AngII) and sarmesin (Sar[1], Tyr (Me)[4]]), AT_1 receptor antagonist losartan and AT_2 receptor antagonist PD123319, were also studied.

METHOD

Animals

The experiments were conducted on male albino mice ICR strain (18–20 g), obtained from the breeding house of the Bulgarian Academy of Sciences, in an air-conditioned room at a temperature of 24 ± 1°C. Food and water were available ad lib except during hte experiments. All tests were performed between 0900 and 1200.

Drugs

Acetic acid (diluted with distilled water) at a concentration of 1% was administered in the volume 2of 0.1 ml/10 g/b.wt.,

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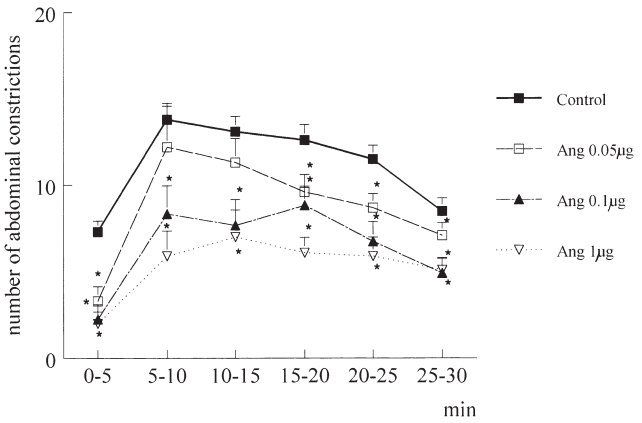

FIG. 1. Time course for the antinociceptive effect coused by ICV treatment of animals (male mice) with AngII on the writhing responses (abdominal constrictions) induced by IP injection of acetic acid. The number of abdominal constrictions (mean ± SEM) was measured for 5 min (at each 5-min interval) for the whole period of measurement (30 min). The control values are from animals injected ICV with the vehicle ($n = 12$). The asterisks (*) denote the significant difference from the control group.

IP, Angiotensin II (Ciba-Geigy Pharmaceuticals), saralasin ([Sar^1, Ala^8]AngII), and sarmesin (Sar^1, Tyr $(Me)^4$]AngII; generously supplied by J. Matsoukas, Department of Chemistry, Patras University, Patras, Greece), losartan (DuP 753, 2-n-butyl-4-chloro-5 hydroxymethyl-1-[2-(1H-tetrazol-5-yl) biphenyl-4-yl) methyl] imidazole potassium salt; generously supplied by Dr. R. D. Smith, Du Pont Merck, Wilmington, DE), and PD123319 (1-[-4-(dimethylamino)-3-methylphenyl] methyl]-5-diphenylacethyl)-4,5,6,7-tetrahydro 1H-4imidazo [4,5 c]pyridine-6 carboxylic acid, ditrifluoroacetate, dihydrate; Parke-Davis, Ann Arbor, MI) were dissolved in 0.9% using an injection volume of 10 μl. The injections were given free hand directly into the right cerebral ventricle of conscious mice (8). 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. The equivalent volume of vehicle was administered to the control groups. Each group consisted of 10 to 12 mice.

Acetic Acid-Induced Abdominal Constriction Test

The mice were placed in individual cages and the number of abdominal constrictions (writhings) of each mouse were counted at 5-min intervals for 30 min. AngII, saralasin, sarmesin,

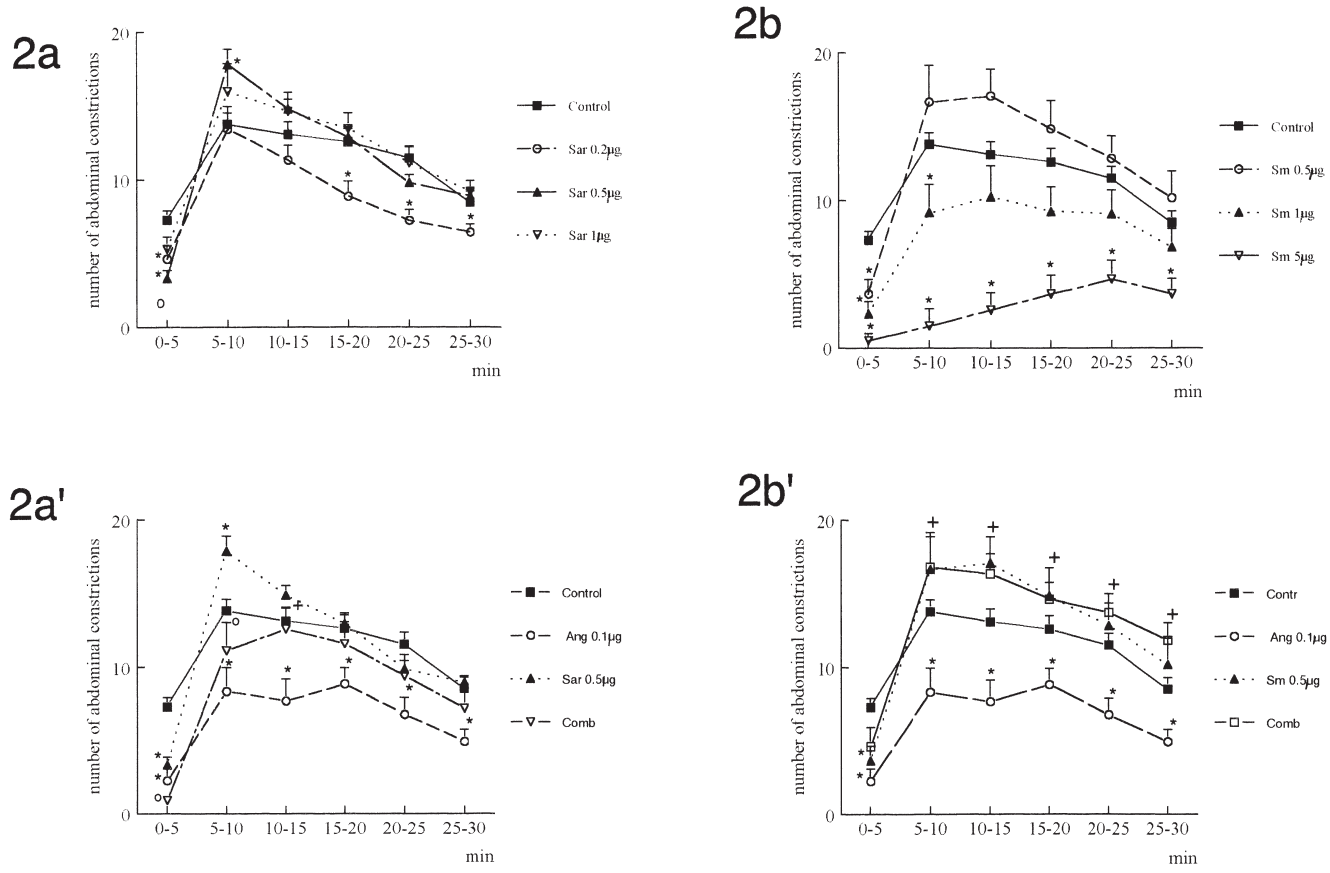

FIG. 2. Time course for the effect of of ICV treatment of animals (male mice) with saralasin, Sar (a), combination of AngII with Sar (a′), sarmesin, Sarm (b), and combination of AngII with Sarm (b′), on the writhing responses (abdominal constrictions) induced by IP injection of acetic acid. The number of abdominal constrictions (mean ± SEM) was measured for 5 min (at each 5-min interval) for the whole period of measurement (30 min). The control values are from animals injected ICV with the vehicle ($n = 12$). The asterisks (*) denote the significant difference from the control group ($^0p < 0.05$ vs. saralasin; $^+p < 0.05$ vs. AngII).

and losartan (DuP753) were injected 10 min and PD123319 30 min before acetic acid. Counting of abdominal constrictions started immediately after injection of acetic acid. The mice with decreased number of writhings were considered protected by the test agent (3). The experimental procedurers were carried out in accordance with institutional guidelines and general recomendations on the use of animals for scientific purposes.

Statistical Analysis

The data were analyzed by a multifactor analysis of variance (one-way ANOVA), followed by Duncan test for comparison of differences at $p < 0.05$.

RESULTS

The data revealed that AngII at doses of 0.05, 0.1, and 1 μg exerted a significant dose-dependent antinociceptive effect, which was particularly pronounced with the dose of 1 μg. (Fig. 1). The ANOVA showed that saralasin at a dose of 0.2 μg significantly decreased the number of writhings at 0–5-, 15–20-, 20–25-, and 25–30-min intervals. Saralasin, at a dose of 0.5 μg, also significantly decreased the number of writhings at the 0–5-min interval, while at the 5–10 min interval saralasin significantly increased the number of writhings. Saralasin, at a dose of 1 μg, was without effect (Fig. 2a). Saralasin (0.5 μg) administered 5 min before AngII (0.1 μg) antagonized the antinociceptive effect of AngII at the 5–10-min interval only (Fig. 2a′). The time course of the response to sarmesin is shown in Fig. 2b. The ANOVA revealed that sarmesin at a dose of 0.5 μg significantly decreased the number of writhings at the 0–5-min interval and then slightly increased it at the 10- to 30-min interval. Sarmesin, at a dose of 1 μg, significantly decreased the number of writhings at the 0–5- and 5–10-min intervals. Sarmesin, at a dose of 5 μg, exerted a particularly pronounced antinociceptive effect during the whole 30-min period. Sarmesin (0.5 μg), administered 5 min before AngII (0.1 μg), significantly reduced the antinociceptive effect of AngII at the 10–30-min interval (Fig. 2b′). The ANOVA revealed that losartan, at a dose of 10 μg, significantly increased the number of abdominal constrictions at the 5–10- and 25–30-min intervals, while at doses of 25 and 50 μg it significantly decreased the number of writhings for all the intervals (Fig. 3a). Losartan (25 μg), injected 5 min before AngII (0.1 μg), failed to antagonize the effect of AngII (Fig. 3a′). Figure 3b shows the effect of PD123319 on the writhing responses. The ANOVA

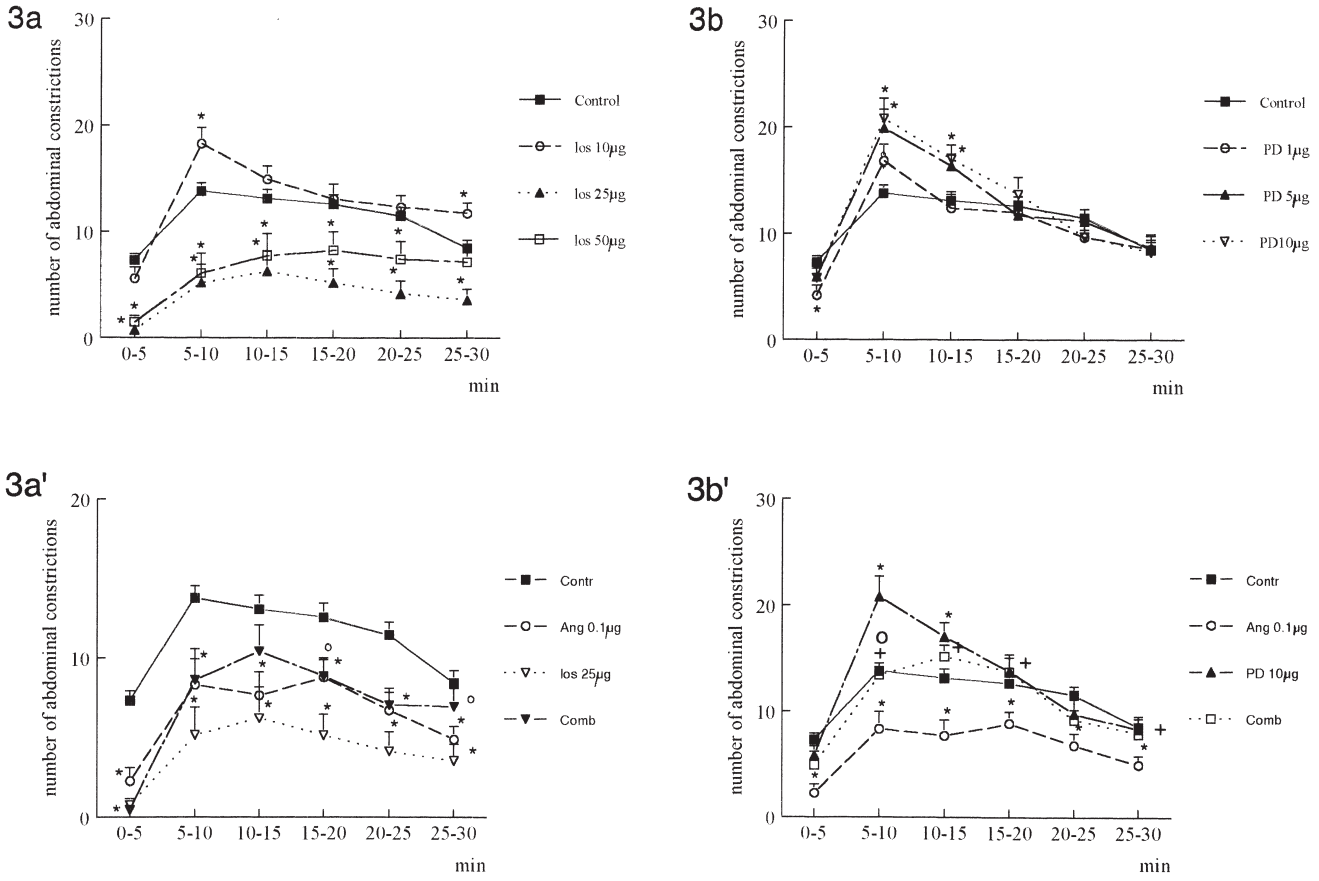

FIG. 3. Time course for the effect of of ICV treatment of animals (male mice) with losartan, los (a), combination of AngII with los (a′), PD123319, PD (b), and combination of AngII with PD (b′), on the writhing responses (abdominal constrictions) induced by IP injection of acetic acid. The number of abdominal constrictions (mean ± SEM) was measured for 5 min (at each 5-min interval) for the whole period of measurement (30 min). The control values are from animals injected ICV with the vehicle ($n = 12$). The asterisks (*) denote the significant difference from control group. $^{0}p < 0.05$ vs. losartan or PD; $^{+}p < 0.05$ vs. AngII.

showed that PD123319 at a dose of 1 μg significantly decreased the number of writhings within 0–5 min, but at doses of 5 and 10 μg increased it at the 5–10-min and 10–15-min intervals. PD123319 (10 μg), injected 5 min before AngII (0.1 μg), abolished the effect of AngII during the whole period of observation exept the time of 20–25 min (Fig. 3b′).

DISCUSSION

The main finding of the present study is the well-pronounced dose-depending antinociceptive effect of AngII. The effects of saralasin, sarmesin, losartan, and PD123319 on nociception in the acetic acid-induced abdominal constriction test, as well as the influence of these agents on the antinociceptive effect of AngII, suggest the participation of AngII receptor subtypes. Sarmesin proved to be a stronger antagonist than saralasin, because its influence on the antinociceptive effect of AngII was more pronounced. In an earlier work of ours (7) sarmesin has also been found to antagonize the inhibitory effect of AngII (0.1 μg) on exploratory behavior (ambulation and rearing) and on apomorphine stereotypy. Sarmesin and saralasin bind to different regulation sites on the AngII receptor. Saralasin, as a peptide analogue of AngII, performs as a partial agonist (12); a partial agonistic action of saralasin on nociception was observed in the present study also. We have data to show that saralasin behaves as an AngII agonist in retention of active avoidance paradigm (5), and in defensive burying behavior (13). AT_1 and AT_2 receptor subtypes are distributed in different brain areas some of them being related with nociception (14). In the present experiments, PD123319 alone exerted a writhings-increasing effect, but when administered before AngII PD123319 abolished its antinociceptive effect. This suggests interaction with AT_2 receptors. It is not quite clear whether the effects of AngII and its interaction with Ang receptor antagonists are due to global sedation. There are data in the pertinent literature that the phenilquinone-induced writhing, which is rather similar to the acetic acid-induced abdominal constriction, is sensitive to sedative drugs (2). Losartan is known to be an anxiolytic drug without sedative effects in mice (1). PD123177 (a drug similar to PD123319) does not show any anxiolytic activity (1,12). Noteworthy is the finding that AngII (0.1–10 pmol or 0.1–10 ng), injected ICV in mice has no effect on nociception but dose dependently attenuates the morphine-induced analgesia in tail-pinch and hot-plate tests (11). Saralasin (1 pmol or 0.9 ng), which alone has no analgesic effect in these tests, potentiates the morphine-induced analgesia (11). The differences between the present results and those of the above-cited authors could be due to the different doses and nociception tests used.

In conclusion, AngII influences nociception in the acetic acid-induced abdominal constriction test, i.e., it dose dependently decreases the number of writhings. The finding that PD123319 antagonizes the antinociceptive effect of AngII and that losartan does not influence this effect of AngII suggests the importance of the AT2 receptor subtype for nociception.

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REFERENCES

1. Barnes, N. M.; Costall, B.; Kelly, M. E.; Murphy, D. A.; Naylor, R. J.: Anxiolitic-like action of DuP753, a nonpeptide angiotensin II receptor antagonist. Neuroreport 1:20–21; 1990.
2. Chernov, H. I.; Wilson, D. E.; Fowler, F.; Plummer, A. J.: Nonspecificity of the mouse writhing test. Arch. Int. Pharmacodyn. Ther. 167:171–178; 1967.
3. Collier, H. O. J.; Dinneen, L. C.; Johanson, C. A.; Schneider, C.: The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br. J. Pharmacol. Chemother. 32:295–310; 1968.
4. Georgiev, V.; Getova, D.; Opitz, M.: Mechanisms of the angiotensin II effects on the exploratory behavior of rats in open field. I. Interaction of angiotensin II with saralasin and catecholaminergic drugs. Methods Find. Exp. Clin. Pharmacol. 9:297–301; 1987.
5. Georgiev, V.: Involvement of transmitter mechanisms in the behavioural effects of angiotensin II. Pol. J. Pharmacol. Pharm. 42:553–562; 1990.
6. Georgiev, V. P.; Lazarova, M. B.; Kambourova, T. S.: Further evidence for the interactions between angiotensin II and GABAergic transmission in pentylenetetrazol kindling seizures in mice. Neuropeptides 28:29–34; 1995.
7. Georgiev, V.; Matsoukas, J. M.; Georgieva, D.; Tchekalarova, J.; Todorov, I.: Influence of sarmesin on some dopamine-related types of behaviour. Lett. Peptide Sci. (LIPS) 3:185–190; 1996.
8. Haley, T. J.; McCormick, W. G.: Pharmacological effects produced by intracerebral injections of dugs in the conscious mouse. Br. J. Pharmacol. 12:12–15; 1957.
9. Haulica, I.; Neamtu, C.; Stratone, A.; Petrescu, G.; Branisteanu, D.; Rosca, V.; Slatineanu, S.: Evidence for the involvement of cerebral renin–angiotensin system (RAS) in stress analgesia. Pain 27:237–245; 1986.
10. Hayes, A. G.; Sheehan, M. J.; Tyers, M. B.: Differential sensitivity of models of antinociception in the rat, mouse and guinea pig to μ- and κ-opioid receptor agonists. Br. J. Pharmacol. 91:823–832; 1987.
11. Kaneko, S.; Mori, A.; Tamura, S.; Satoh, M.; Takagi, H.: Intracerebroventricular administration of angiotensin II attenuates morphine-induced analgesia in mice. Neuropharmacology 24:1131–1134; 1985.
12. Timmermans, P. B. W. M.; Wong, P. C.; Chiu, A. T.; Herblin, W. F.; Benfield, P.; Carini, D. J.; Lee, R. J.; Wexler, R. R.; Saye, A. M.; Smith, R. D.: Angiotensin II receptors and angiotensin II receptor antagonists. Pharmacol. Rev. 45:205–251; 1993.
13. Tsuda, A.; Tanaka, M.; Georgiev, V.; Emoto, H.: Effects of angiotensin II on behavioral responses of defensive burying paradigm in rats. Pharmacol. Biochem. Behav. 43:729–732; 1992.
14. Wright, J. W.; Harding, J. W.: Brain angiotensin receptor subtypes AT_1, AT_2 and AT_4 and their functions. Regul. Pept. 59:269–295; 1995.