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Differential Involvement of Hippocampal Angiotensin II Type 1 Receptors in Nociception

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ABSTRACT

Background: The octapeptide angiotensin II (Ang II) is the major effector of the renin-angiotensin system. Ang II exerts its effects by binding to Ang II type 1 (AT1) and Ang II type 2 (AT2) receptors. To examine the involvement of Ang II and AT1 receptors in nociception the effects of Ang II, losartan (AT1 receptor antagonist) and combination (losartan+Ang II) infused uni-and bilaterally into hippocampal CA1 area of male Wistar rats were studied. **Materials and Methods:** After stereotaxic implantation of guide cannulae into hippocampal CA1 area Ang II (50 μg), losartan (100 μg) and combination (losartan 100 μg+Ang II 50 μg) were separately microinjected uni-and bilaterally into CA1 area. Nociception was examined applying mechanical pressure on the hind paw of the rat (paw pressure test). **Results:** It was found that bilateral and left-side microinjections of Ang II (50 μg) exerted a nociceptive effect. In contrast, the inhibition of AT1 receptors by losartan (100 μg), microinjected bilaterally and into the left CA1 area, produced an antinociceptive effect. The pretreatment with AT1 antagonist losartan infused bilaterally and into the left CA1 area reversed the nociceptive effect of Ang II as compared to the respective controls, i.e., elicited antinociception. The effect of all treatments was more pronounced after injection into the left CA1 area as compared to the right-side. **Conclusion:** The results suggest an involvement of hippocampal AT1 receptors in nociception of rats and a differential distribution of AT1 receptors in the left and right CA1 area.

Key words: Angiotensin II, losartan, hippocampus, nociception, asymmetry, rat

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INTRODUCTION

The octapeptide angiotensin II (Ang II) is the major effector of the Renin-Angiotensin System (RAS). It is formed in many tissues, including most peripheral organs and the brain and its actions are due to binding at Ang II type 1 (AT1) and Ang II type 2 (AT2) receptors[1]. Both receptor types belong to the superfamily of seven membrane-spanning G-protein coupled receptors[2,3]. The various components of the renin-angiotensin system (angiotensinogen, renin, angiotensin-converting enzyme, Ang II and Ang II receptors) are found in the brain, where they actively modulate functions such as stress[4,5] exploratory behavior, anxiety, learning and memory[6-14].

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The AT1 and AT2 receptors have been detected in brain areas responsible for the above mentioned functions, including the amygdala, hippocampus, lateral septum and frontal cortex[15-20]. Ang II has been shown to participate also in the antinociceptive processes. Toma *et al*.[21] provided evidence that Ang II may be involved in the modulation of nociceptive formation at the level of the rat spinal cord through the activation of AT1 receptors. In the brain, endogenous Ang II and/or an angiotensin-peptide acting on AT1 and/or AT2 receptors may be implicated in the tonic nociceptive control mediated by the periaqueductal gray matter[22].

Taken together, the above considerations suggest that the components of the RAS system might be involved in the pain control mechanisms in some brain structures. However, there are no data about asymmetry in the effects Ang II receptor ligands injected in brain

structures. Previous studies of our laboratory have found behavioral asymmetries (in locomotor-exploratory activity, anxiety, learning and memory) following microinjections of Ang II into the CA1 hippocampal area[7,8].

The aim of the present study was to examine the involvement of Ang II and AT1 receptors in nociception after unilateral and bilateral microinjection into hippocampal CA1 area.

The hippocampal CA1 area was chosen as a region with high density of AT1 receptors and as a region being implicated in processing of nociceptive information[6,23-26]. For the experiments was used losartan (DuP-753), a selective antagonist of AT1 receptors[27], applied topically uni-or bilaterally into the hippocampal CA1 area. Therefore, the present study has been planned to examine the involvement of Ang II and AT1 receptors in nociception the effects of Ang II, losartan (AT1 receptor antagonist) and combination (losartan+Ang II) infused uni-and bilaterally into hippocampal CA1 area of male Wistar rats.

MATERIALS AND METHODS

Animals: Male Wistar rats (200-240 g at the time of surgery) were housed individually in polypropylene boxes with free access to food and water. The animals were maintained at a constant temperature environment (22±2°C) on a 12 h light/dark cycle (lights on at 6.00 am). The behavior experiments were carried out between 10:00 am and 1:00 pm.

The experiments were carried out according to the rules of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences.

Stereotaxic implantation and drug injection into the CA1 hippocampal area: After anaesthesia (Calypsol 50 mg kg^{-1} i.p.,) the rats were placed in a stereotaxic apparatus (Stoelting, USA) and guide cannulae were implanted into the CA1 area (right and left) according to the coordinates of the stereotaxic atlas of [28] (P = 4.3, L = ± 2.0, h = -3.0 mm). After surgery the animals were allowed 7 days to recover before the behavioral test. During the recovery period the rats were handled daily.

The experimental rats were divided into 3 groups. Ist group-Ang II (50 μg) was microinjected uni-or bilaterally into hippocampal CA1 area. Angiotensin II (Sigma) was dissolved *ex tempore* in saline. One milliliter of Ang II solution (pH 7.4) was injected over a period of 1 min and the injection cannula was left in place for additional 15 min and 30 sec after the injection the rats were tested with the paw-pressure test. The rats were microinjected into the CA1 area every third day with randomly selected drug (Ang II or saline) and side (left, right or bilateral). Thus, each rat received six microinjections in total. The IInd group 1 mL of losartan solution (pH 7.4) (Losartan , Sigma, 100 μg) was microinjected uni-or bilaterally into hippocampal CA1 area in the same pattern as Ang II group. Fifteen minutes after the injection the rats were tested with the paw-pressure test. IIIrd group-Combination (losartan 100+Ang II 50 μg) was microinjected uni-or bilaterally into hippocampal CA1 area. Losartan and Ang II were dissolved individually in 0.5 μL saline. The combination (losartan+Ang II) was applied by separate injections with a 10 min lag (i.e., 10 min after the losartan injection, Ang II was microinjected into the same side). In the combination group the rats were tested in the paw-pressure test 15 min after the losartan injection and 5 min after the Ang II microinjection. The rats were injected into the CA1 area every third day with randomly selected drug (combination or saline) and side (left, right or bilateral). Thus, each rat received total of six microinjections.

Prior to sacrificing the animals were injected with 1 μL 2% Fastgreen dye through the injection cannula. Injection sites were verified histologically postmortem in 25 μm coronal brain sections. Animals with misplaced or asymmetrical cannulae and diffusion of dye beyond the CA1 area were excluded.

Paw-pressure test: The changes in the nociceptive response were determined by the foot-pressure method[29]. A constantly increasing pressure was applied to the dorsal surface of the hind paw. The actual load applied was recorded in Arbitrary Units (AU) when the animal made its first escape attempt. The experiments were performed between 9.00 and 13.00 h.

Statistical analysis: Results were expressed as Mean±SEM and analyzed by two way repeated analysis of variance (ANOVA). Separated two-factor ANOVA with factors: Drug (saline and Ang II, Losartan or combination) and side of injection (left, right and bilateral) was used for evaluation of data about unilateral injections. Findings from the ANOVA were *post-hoc* analyzed by Student-Newman-Keuls test. A level of $p \leq 0.05$ was considered significant.

RESULTS

Effects of Ang II on nociception: ANOVA showed statistical significance for the two factors-Ang II

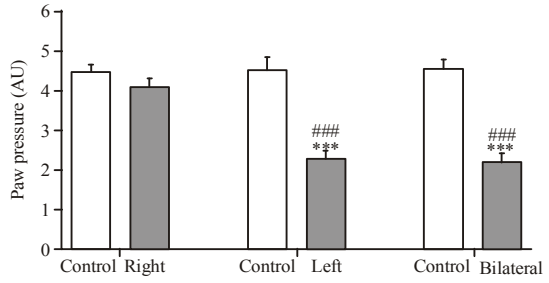

Fig. 1: Effects of Ang II (50 μg) microinjected bilaterally, left or right into the hippocampal CA1 area on nociception. Hatched bars-Ang II treated rats; open bars-respective controls. Means±SEM are presented, n=8. Asterisks depict comparisons after bilateral, left or right Ang II microinjections versus respective saline microinjections into CA1 area, ***p≤0.001, circles depict comparisons after the left-side versus right side microinjected Ang II, ###p≤0.001

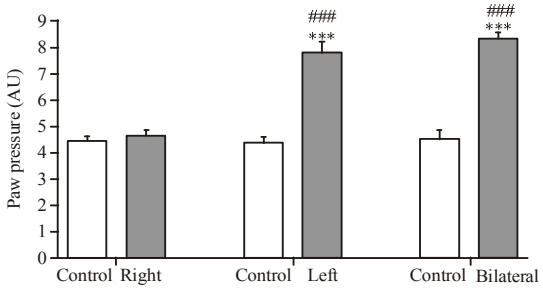

Fig. 2: Effects of losartan (100 μg) microinjected bilaterally, left or right into the hippocampal CA1 area on nociception. Hatched bars-losartan treated rats; open bars-respective controls. Means±SEM are presented, n = 8. Asterisks depict comparisons after bilateral, left or right losartan microinjections versus respective saline microinjections into CA1 area, ***p≤0.001, circles depict comparisons after the left-side versus right side microinjected losartan, ###p≤0.001

($F_{1,30}$ = 25.0; p≤0.001) and "side" ($F_{2,30}$ = 22.102; p≤0.001) and the interaction between them ($F_{2,30}$ = 16.556; p≤0.001).

Post-hoc tests showed that bilateral (p≤0.001) and left-side (p≤0.001) microinjections of Ang II (1 μg) into the CA1 hippocampal area, decreased pain threshold as compared to the respective controls while right-side applied Ang II (P = NS) showed no significant difference compared to the right-side saline injections (Fig. 1). Comparisons between left and right-side microinjections demonstrated that Ang II applied in the left-side significantly decreased the pain threshold as compared to the right-side (p≤0.001) (Fig. 1).

Effects of losartan on nociception: Two-way repeated measures ANOVA revealed significant effects for losartan ($F_{1,30}$ = 51.116; p≤0.001), "side" ($F_{2,30}$ = 21.576; p≤0.001) and losartan X "side" interactions ($F_{2,30}$ = 43.338; p≤0.001). A *post-hoc* test demonstrated a significant increase in the pain threshold induced by microinjection of losartan (100 μg) bilaterally and into left-side (p≤0.001) but not into the right-side (p = NS) as compared to the respective controls. The comparison between left-side and right-side effects of treatment with losartan showed that microinjection of losartan into the left CA1 area produced a significantly greater increase in the pain threshold as compared to the right CA1 area (p≤0.001) (Fig. 2).

Effects of the combination (losartan 100 μg+Ang II 50 μg) on nociception: ANOVA demonstrated significant main effect for the factor "drug" ($F_{1,30}$ = 51.116; p≤0.001) and for the factor "side" ($F_{2,30}$ = 21.576; p≤0.001). There was a significant interaction between the two factors "side" X "drug" ($F_{2,30}$ = 43.338; p≤0.001). *Post-hoc* SNK test demonstrated that bilateral (p≤0.001) and left-side (p≤0.001) but not right-side (P = NS) microinjections of the combination into the CA1 hippocampal area increased pain threshold as compared to the respective controls (Fig. 3). Comparisons between left-side and right-side applications demonstrated that the microinjection of the combination into the left CA1 area produced a significantly greater increase in the pain threshold as compared to the right CA1 area (p≤0.001) (Fig. 3).

DISCUSSION

The present study extended the understanding about the asymmetric behavioral effects of Ang II, microinjected into hippocampal CA1 area. It was found, for the first time, that Ang II microinjected bilaterally into the CA1 area decreased the pain threshold compared to the controls. The more important finding was that Ang II produced a different and asymmetric effect on the pain. Thus, Ang II infused into the left CA1 area decreased pain threshold, i.e., exerted a nociceptive effect but did not produce an effect into the right CA1 area.

Most of the studies reporting analgesic effects of Ang II were performed after intra cerebro ventricular (i.c.v.) administration[30,31]. Anti nociceptive effect of

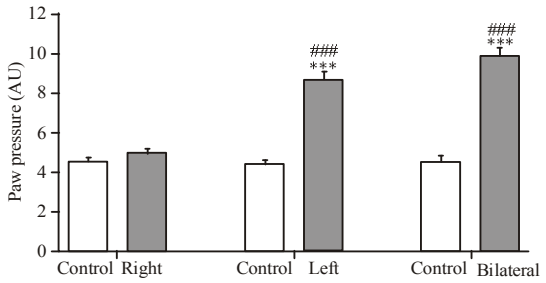

Fig. 3: Effects of combination (losartan 100+Ang II 50 μg) microinjected bilaterally, left or right into the hippocampal CA1 area on nociception. Hatched bars-combination treated rats; open bars-respective controls. Means±SEM are presented, n = 8. Asterisks depict comparisons after bilateral, left or right combination microinjections versus, respective saline microinjections into CA1 area ***$p \leq 0.001$, circles depict comparisons after the left-side versus right-side microinjected combination, ###$p \leq 0.001$

Ang II, Ang I and Ang III was established after topical injections into periacqueductal gray matter[32]. It is shown that the analgesic effect of Ang could be blocked by prior treatment with the receptor antagonist saralasin[31,33] and by the opioid antagonist naloxone[34,35].

Gebhart[36] has demonstrated that the local administration of Ang II into the periaqueductal gray or into the Rostral Ventromedial Medulla (RVM), induces antinociceptive effects which are reversed by losartan[22,30,32,37]. The authors supposed that the analgesic effect of Ang II after i.c.v. administration could be due to the release of β-endorphins from adenohypophysis[34,35] and stimulation of γ-aminobutyric acid (GABA) receptor system[38]. On the other hand Marques-Lopes *et al.*[39] have reported that the microinjection of Ang II into the caudal ventrolateral medulla (CVLM) induces hyperalgesia through AT1 receptors. The above data suggest that Ang II may participate in both inhibition and facilitation of the nociceptive transmission and its effect is region-dependent.

It was suggested that the stimulation of Ang II receptors (AT1, AT2 and AT4 receptor subtypes) in the hippocampal CA1 area might be implicated in the nociceptive response.

To determine whether the Ang II induced nociception is related to AT1 receptors, we examined the effect of antagonist of AT1 receptors-losartan and effect of combination (Ang II pretreated with losartan).

It was found that losartan (100 μg) applied bilaterally and into the left hippocampal CA1 area increased the pain threshold as compared with controls, i.e., produced an antinociceptive effect. This antinociceptive effect was lateralized, being more pronounced in the left CA1 area.

The data also suggest that the unilateral infusion of losartan at a dose of 100 μg blocked the Ang II (50 μg) elicited antinociception (i.e., decreased pain sensitivity).

The effect was present upon microinjections into the left side while the infusions into the right side had no significant effect. Thus, it was found an asymmetric and opposite effects of Ang II and losartan microinjected separately and upon pretreatment with AT1 receptor antagonist in the hippocampal left and right CA1 areas. These findings lead to suggestion that the antinociceptive effect elicited by the combination (losartan 100 μg+Ang II 50 μg) is mediated by AT1 receptors. Thus, in the nociceptive response of Ang II, the hippocampal AT1 receptors are involved.

The present study is the first to investigate the effects of AT1 receptors in hippocampal CA1 area on nociception of rats. Takai *et al.*[40] have revealed that repeated oral administration of AT1 receptor antagonist and ACE inhibitors showed antinociceptive effect in hot-plate test. Recently, Nemoto *et al.*[41] have reported that intrathecally (it) administered losartan produces antinociceptive effect in a mouse formalin test and that the Ang II induced nociceptive behavior was inhibited by losartan but not by AT2 receptor antagonist PD123319 thus suggesting an involvement of AT1 receptors. Another study has demonstrated that systemic continuous delivery of Ang II (150 ng kg^{-1} min) induced tactile, heat and cold hyperalgesia. Blockade of the AT1 receptor with losartan (2.5 mg kg^{-1} day^{-1}) prevented the tactile hyperalgesia and attenuated the cold hyperalgesia but did not affect the response to noxious heat stimulus. The authors suggested that Ang II through AT1 receptor activation is an important regulatory factor in neuropathic pain perception[42].

The importance of the AT2 receptor subtype in antinociception has also been demonstrated by Georgieva and Georgiev[30] have found that losartan alone at a dose 10 μg (i.c.v.), enhances nociception in acetic acid-induced abdominal constriction test but at higher doses (25, 50 μg) decreases the nociception when administered prior to Ang II (0.1 μg). According to the authors the AT1 receptor is not involved in the antinociceptive effect of Ang II. Selective non-peptide AT2 receptor antagonist PD123319 at a dose 1 μg (i.c.v.) decreased the number of writings but at higher doses (5, 10 μg) increased them administered before Ang II. The ability of the antagonist to reverse the antinociceptive effect Ang II suggested an involvement of AT2 receptors in the antinociceptive effect of Ang II.

It was demonstrated that part of the tonic nociceptive control mediated by the periaqueductal gray matter is carried out locally by endogenous Ang II and/or angiotensin-peptide acting on AT1 and/or AT2 receptors[42].

The results suggest that the hippocampal AT1 receptors are responsible for the nociceptive response to Ang II and that the right and left CA1 hippocampal areas have different roles in nociception. The asymmetry in the observed effects is probably due to the uneven distribution of AT1 receptors and their interactions with other asymmetrically distributed neurotransmitter systems in the hippocampus. However, the role of Ang II in the modulation of nociceptive transmission in the hippocampus has not been reported until this study.

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