Bulgarian Chemical Communications, Special Issue E, (pp. 118 – 123) 2017

Involvement of hippocampal angiotensin II type 1 receptors in locomotor activity in rats with a model of depression

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Received October 08, 2016; Revised February 13, 2017

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. The olfactory bulbectomy (OBX) model is an animal model of depression that produces behavioural, physiological, and neurochemical alterations resembling clinical depression. To examine the involvement of Ang II and AT1 receptors in locomotion we studied the effects of Ang II, losartan (AT1 receptor antagonist) infused uni- and bilaterally into hippocampal CA1 area of OBX-rats. The changes in locomotor activity were registered in an Opto Varimex apparatus. The increased locomotor activity is a typical behavioural phenomenon in OBX rats. Microinjected bilaterally and left-side into the hippocampal CA1 area Ang II (0.5 μg) increased the number of horizontal and vertical movements in bulbectomized rats, while losartan (100 μg) infused bilaterally and the left-side, but not into the right-side, decreased the number of both horizontal and vertical movements in OBX rats, as compared to saline-treated OBX controls. It was found that the effects of Ang II and losartan were opposite and asymmetric in left and right CA1 area. These data reveal a pronounced lateralized Ang II effect on the locomotor activity of OBX rats and suggest a possible involvement of AT1 receptors in the mechanisms of the olfactory bulbectomy syndrome in rats.

Key words: Angiotensin II, Losartan, Locomotor activity, Asymmetry, Hippocampus, Depression

INTRODUCTION

The octapeptide angiotensin II (Ang II) is the major effector of the renin-angiotensin system (RAS). The brain RAS is independent of the circulating RAS. The brain RAS includes the biologically active angiotensin peptides: Ang II, Ang III, Ang IV and Ang-(1-7) [1]. There are four types of angiotensin receptors:Ang II type 1 and type 2 receptors (AT1, AT2), Ang IV-specific receptor (AT4), and Ang-(1–7)-selective receptor [2, 3, 4]. AT1 and AT2 are structurally similar, G-protein coupled receptors, AT4 is a protein, which is not G-protein-linked, while Ang-(1–7) exerts its actions via the G protein-coupled Mas receptor.

It is known that the concentration of Ang II and the expression of its different receptor types are particularly high in the hippocampus [4, 5]. In the CA1 region of the hippocampus, Ang II directly excites pyramidal neurons [6]. Ang II is a full agonist at the AT1 and AT2 receptors in accordance with the nomenclature (Guide to Receptors and Channels).

It has been reported that ATII injected intracerebroventricularly (i.c.v.) or into hippocampal CA1 area affects exploratory behaviour, locomotor activity, learning and memory in rats [7, 8]. Previous studies showed behavioural asymmetries in locomotor-exploratory activity, anxiety, learning and memory following unilateral infusions of Ang II into CA1 area [9]. We have found that losartan, a specific antagonist of AT1 receptors, microinjected bilaterally or into the left hippocampal CA1 area suppressed the exploratory activity, while the right-side losartan administration showed no effect as compared to the controls.

Recently, it has been reported that orally administered losartan can suppress the enhancing effect of voluntary running on cell proliferation in the rat hippocampus [10].

The bilateral olfactory bulbectomy (OBX) in rats is widely accepted as an animal model of depression. The OBX in rats leads to numerous behavioural, physiological, neurochemical and neuroendocrine changes that are used to model of major depression, but may also be a valuable tool in the study of neurodegenerative disorders such as Alzheimer's disease. Behavioural abnormalities of

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OBX rats include exploratory hyperactivity in response to a novel environmental stress, memory deficits, anxiety symptom-resembling behaviour, etc. [11, 12]. The bulbectomy-induced behavioural deficits are attributable to a retrograde degeneration of neurons from the olfactory bulbs which project to cortical, amygdala, and hippocampal regions [11, 13]. Saavedra and co-workers [14] have showed that blockade of brain AT1 receptors ameliorates stress, anxiety, brain inflammation and ischemia. In addition, losartan has been shown to ameliorate depression in mice, as determined by the forced swim test [15].

Baring in mind the suggested role for hippocampal dysfunction in depression, as well as our previous findings on the behavioral effects of Ang II and losartan administration into the hippocampal CA1 area, the aim of the present study was to investigate the effects of Ang II and losartan after uni-and bilateral infusion into CA1 hippocampal area on locomotor activity of rats with a model of depression.

EXPERIMENTAL

Animals

Male Wistar rats (200 - 220g at the time of surgery) were housed individually in polypropylene boxes with free access to food and water. The animals were maintained in a constant temperature environment (22 ± 2°C) on a 12 h light/dark cycle (lights on at 6:00am). The behaviour experiments were carried out between 10:00am and 1:00pm.

The experiments were carried out according to the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985), and the rules of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences.

Experimental model of depression

Bilateral olfactory bulbectomy (OBX) was carried out according to the method depicted by Kelly et al. [13].

Stereotaxic implantation and drug injection into hippocampal CA1 area of OBX rats

Seven days after bilateral olfactory bulbectomy guide cannulae (right and left) were implanted into CA1 hippocampal area according coordinates to the stereotaxic atlas of Pellegrino and Cushman [16] (P = 3.8 mm; L = ± 3.0 mm; h = - 3.0 mm) as described previously [17].

Rats were microinjected into both hippocampal CA1 areas with Angiotensin II (0.5 μg), Losartan (100 μg) or saline (doses active in earlier experiments) [17]. Angiotensin II (Sigma) or Losartan (Sigma) were dissolved ex tempore in saline and microinjected into CA1 hippocampal area. The substances were injected through an injection cannula connected by polyethylene tubing with a constant rate microsyringe (Hamilton, Reno, NV, USA). 0.5 μl of the Ang II (pH 7.4) solution or 0.5 μl of the losartan (pH 7.4) solution or 0.5 μl saline were infused over a period of 1 min and the injection cannula was left in place for another 30s.

Just before scarification, the animals were injected with 0.5 μl 2% Fast green dye through the injection cannula. Brains were removed, and successful bulbectomy was verified macroscopically by comparison with the bulbs of an intact rat brain. Animals in which < 80% of the bulbs had been removed were omitted from the analysis. Injection sites were then verified histologically post-mortem in 25 μm coronal brain sections cut through the hippocampus. Animals excluded by the cannula placement and the diffusion of dye were beyond the CA1 area of the hippocampus as depicted in the stereotaxic atlas [16] or were not symmetrical. Animals with cannulae placement outside the CA1 area or not symmetrical within both CA1 areas were excluded from the statistical analysis.

Locomotor activity

Locomotor activity was recorded in an Opto Varimex apparatus (Columbus Instruments, USA). The experimental chamber was 50 cm X 50 cm X 25 cm. This apparatus records the number of photobeam interruptions during the movements of the animal. It provides selective counting of the number of horizontal and vertical movements in arbitrary units (AU). The information obtained was recorded automatically every 5 minutes in the observation period (5-30 min). The experiments were carried out at one and the same time (between 10:00 a.m. and 1:00 p.m.). The rats were placed in the central quadrant of the activity monitor 15 min after the microinjection of Ang II or losartan.

Statistical analysis

One way ANOVA was used to analyze the data obtained for effect of bilateral Ang II and losartan microinjections. Two-factor ANOVA analysis with factors:drug - 3 levels (Ang II, losartan, saline,) and side of injection - 2 levels (right, left) was used for evaluation of data about unilateral injections. ANOVA data were further analyzed by post hoc t-test, where appropriate.

RESULTS AND DISCUSSION

Effects of bilateral microinjection of Ang II and losartan into CA1 area of OBX rats

Separate one way ANOVA analysis of the total number of horizontal or vertical movements for a 30-minute period of observation showed a significant effect for factor "drug" ($F_{3,27}$ = 99.775; P ≤ 0.001) for the number of horizontal movements, and for the number of vertical movements ($F_{3,27}$ = 37.149; P ≤ 0.001).

Post hoc t-test showed that infused bilaterally into CA1 area of OBX rats, Ang II increased, while losartan decreased, the total number of both horizontal (P ≤ 0.003; P ≤ 0.001, respectively) and vertical movements (P ≤ 0.005; P ≤ 0.01, respectively), as compared to the respective saline-treated OBX rats (Fig. 1, Fig. 2).

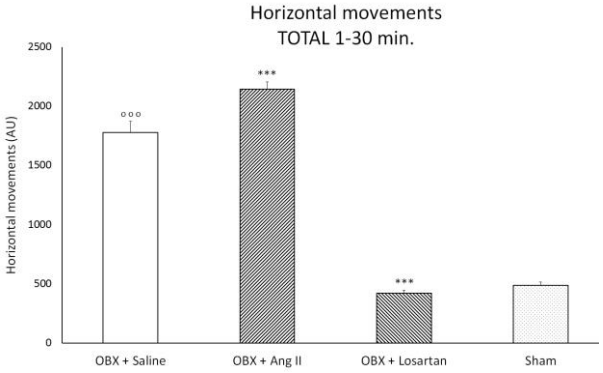

Fig. 1. Effect of Ang II and losartan microinjected bilaterally into the hippocampal CA1 area on the total number of horizontal movements for the whole period of observation (30 min). n=7. Means (± S.E.M.) are presented. Asterisks depict - drug treated OBX rats vs. respective OBX saline-treated. ***P ≤ 0.001. Circles depict - sham vs. OBX saline-treated. ^{ooo}P ≤ 0.001.

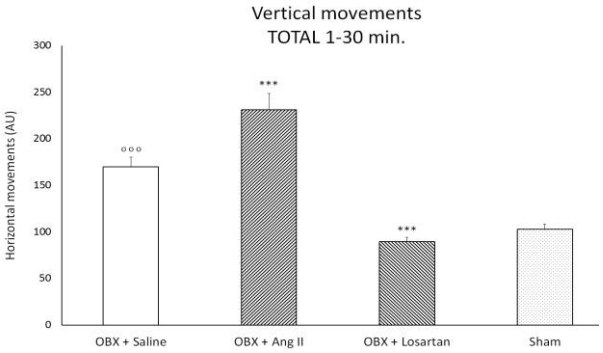

Fig. 2. Effect of Ang II and losartan microinjected bilaterally into the hippocampal CA1 area on on the total number of vertical movements for the whole period of observation (30 min). n=7. Means (± S.E.M.) are presented. Asterisks depict - drug treated OBX rats vs. respective OBX saline-treated. ***P ≤ 0.001. Circles depict - sham vs. OBX saline-treated ^{ooo}P≤ 0.001.

Effects of unilateral microinjection of Ang II and losartan into CA1 area of OBX rats

Two-way ANOVA analysis on the effect of unilateral Ang II (0.5 μg) and losartan (100 μg) microinjection on the total number of horizontal movements in OBX rats for a 30-min period of observation showed a significant effects for the factor "drug" ($F_{2,47}$ = 92.508; P ≤ 0.001), the factor "side" ($F_{1,47}$ = 71.183; P ≤ 0.001) and a significant interaction between the factors "drug" X "side" ($F_{2,47}$ = 36.967; P ≤ 0.001).

Ang II administered into the left CA1 area of OBX rats increased the total number of horizontal movements (P ≤ 0.05), while the microinjection of Ang II into the right CA1 area had no significant effect on the total number of horizontal movements (P =NS) as compared to the saline-treated OBX controls (Fig.3). Losartan (100 μg) microinjected into the left CA1 area significantly decreased the total number of horizontal movements as compared to the left saline-treated OBX controls (P ≤ 0.001) and compared to the right-side infused losartan (P ≤ 0.001) (Fig.3).

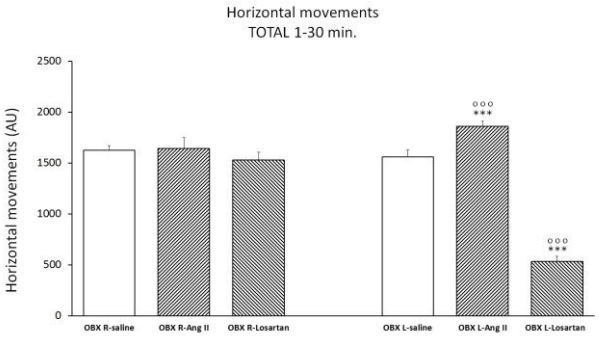

Fig. 3. Effect of Ang II and losartan microinjected unilaterally (right or left) into the hippocampal CA1 area on the total number of horizontal movements for the whole period of observation (30 min). n=8. Means (± S.E.M.) are presented. Asterisks depict - drug treated OBX rats vs. respective OBX saline-treated. ***P ≤ 0.001. Circles depict - left-side vs. right-side ^{ooo}P ≤ 0.001.

Two-way ANOVA on the effect of Ang II and losartan on the total number of vertical movements of OBX rats demonstrated a significant effects of factors "drug" ($F_{2,47}$ = 18.250; P ≤ 0.001), "side" ($F_{1,47}$ = 172.601; P ≤ 0.001) and an interaction between "side" X "drug " ($F_{2,47}$ = 40.032; P ≤ 0.001).

The number of vertical movements was significantly increased upon microinjection of Ang II into the left CA1 area (P ≤ 0.001). The injection of losartan into left-side significantly decreased the number of vertical movements compared to the left

saline-treated OBX rats ($P \leq 0.001$) and compared to the right-side injected losartan ($P \leq 0.001$) (Fig.4).

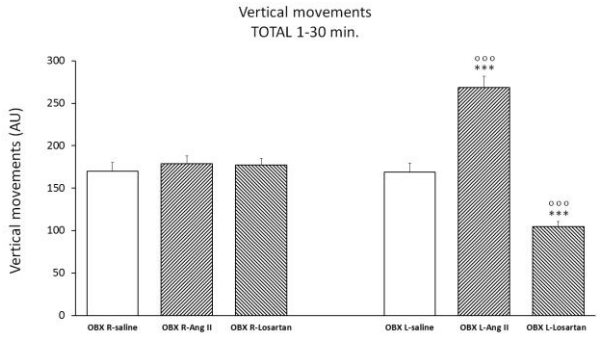

Fig. 4. Effect of Ang II and losartan microinjected unilaterally (right or left) into the hippocampal CA1 area on the total number of vertical movements for the whole period of observation (30 min). n=8. Means (± S.E.M.) are presented. Asterisks depict - drug treated OBX rats vs. respective OBX saline-treated. $^{***}P \leq 0.001$. Circles depict - left-side vs. right-side $^{ooo}P \leq 0.001$.

In our previous studies we have found that bilateral administration of losartan at a dose of 100 μg into CA1 areas decreased exploratory activity of rats [18]. It has been also demonstrated that bilateral bulbectomy leads to hyperactivity of rats in an Opto Varimex apparatus (increased total number of both horizontal and vertical movements) [18]. This locomotor hyperactivity is a typical behavioural phenomenon in OBX rat and it is accepted as an index of depressive-like behaviour [13]. Considering these findings, we were interested to investigate the role of Ang II and Ang II receptors in the locomotor hyperactivity induced by OBX.

The bilateral microinjection of Ang II into hippocampal CA1 stimulated locomotor activity of OBX rats, expressed by an increase of both horizontal and vertical movements. The bilateral infusion of losartan significantly decreased the total number of both horizontal and vertical movements, i.e. suppressed the locomotion of OBX rats as compared to the saline-treated OBX controls. Thus, Ang II administration augmented the hyperactivity of OBX rats and aggravated the depression-like state, while the inhibition of AT1 receptors by losartan demonstrated antidepressant effect.

Bilateral olfactory bulbectomy is accompanied by changes in many neurotransmitter systems [13]. After bulbectomy, degeneration of neurons in cortex, hippocampus [19, 20], impaired neurogenesis in hippocampal dentate gyrus [21] and changes in the expression of neuropeptides in some brain areas [11] have been reported. It is possible that the increased expression of Ang II or Ang II receptors in the hippocampal neurons might contribute to the increased hyperactivity observed in our study.

The brain RAS has been implicated in the pathophysiological mechanisms of dementia and neurodegenerative diseases [22, 23]. It has also been related to the mechanisms of depression as shown in studies demonstrating antidepressant effect of captopril in hypertensive patients that also suffered from depression [24, 25, 26].From the viewpoint of asymmetry we investigated the effects of equal doses of Ang II or losartan microinjected unilaterally (left or right) into CA1 hippocampal area on the locomotor activity. The unilateral topical administration of the drugs showed different effects on locomotion. Thus,when injected into the left CA1 area, Ang II increased locomotor activity, while losartan decreased it. The microinjection of the drugs into the right CA1 area induced no changes in locomotion.

The present findings are in agreement with studies showing asymmetric effects on exploratory behaviour after administration of Ang II into CA1 area. Belcheva and co-workers [9] have shown that only left-side injections affected exploratory behaviour, while Ang II administered into the right CA1 area had no effect.

Several studies support the hypothesis that the RAS is a part of the neurochemical dysregulation underlying negative affective states, anxiety disorders, and ethanol dependence [27]. Something more, it has been suggested that the increased RAS activity may increase the relative risk of depression [22, 28], while the blockade of AT1 receptors could be potentially useful for the treatment of stress-induced disorders [29].

The different behavioural effects of Ang II microinjected into the left or right CA1 hippocampal area suggest a different distribution of Ang II receptors, high concentrations of which are found in the hippocampus [4]. The presence of AT1 and AT2 receptors, involved in the modulation of exploratory behaviour, learning and memory processes, etc., has been demonstrated in rat hippocampus [22, 23].

CONCLUSION

This study the first time provides information on the locomotion-stimulatory effect of Ang II and locomotion-inhibitory effect of losartan when injected into the left but not into the right CA1 hippocampal area of OBX rats. This implies that the injection of the drugs in the left side only can modulate the depressive-like behavior. The asymmetry of the Ang II- related behavioral

responses upon administration in the left or right hippocampus could be related to a different distribution of AT1 receptors in the two hemispheres.

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УЧАСТИЕ НА ХИПОКАМПАЛНИТЕ АНГИОТЕНЗИН II ТИП 1 РЕЦЕПТОРИ В ДВИГАТЕЛНАТА АКТИВНОСТ НА ПЛЪХОВЕ С МОДЕЛ НА ДЕПРЕСИЯ

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Постъпила на 08 октомври, 2016 г.; Коригирана на 13 февруарии, 2017 г.

(Резюме)

Октапептидът ангиотензин II (Ang II) е основният ефектор на ренин-ангиотензиновата система. Ang II упражнява ефектите си чрез свързване с Ang II тип 1 (AT1) и Ang II тип 2 (AT2) рецепторите. Олфакторната булбектомия (OBX) е животински модел на депресия, която води до поведенчески, физиологични и неврохимични промени, наподобяващи клиничната депресия. За да проучим участието на Ang II и AT1 рецепторите в двигателната активност ние сме изследвали ефектите на Ang II и лосартан (антагонист на AT1 рецепторите), въведени едностранно и двустранно в хипокампалното CA1 поле на OBX плъхове. Промените в локомоторната активност са регистрирани в апарат Opto Varimex. Повишената двигателна активност е типичен поведенчески феномен при OBX плъхове. Ang II (0.5 μg), микроинжектиран двустранно и само в лявото CA1 поле на хипокампа, повишава броя на хоризонталните и вертикалните движения на булбектомизираните плъхове, докато лосартан (100 μg) въведен двустранно и в лявото CA1 поле, но не и в дясно, намалява броя на хоризонтални и вертикални движения на OBX плъхове, в сравнение с третираните с физиологичен разтвор OBX контроли. Установено е, че ефектите на Ang II и лозартан са противоположни и асиметрични в лявото и дясното CA1 хипокампално поле. Тези данни показват ясно изразен латерализиран ефект на Ang II върху двигателната активност на OBX плъховете и предполагат възможното участие на AT1 рецепторите в механизмите на синдрома на олфакторната булбектомия при плъхове.