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Differential involvement of hippocampal angiotensin 1 receptors in learning and memory processes in bulbectomized rats

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Abstract

There is conflicting evidence regarding the effect of AT1 receptor antagonists on learning and memory processes. The effects of angiotensin II and losartan administration into CA1 hippocampal area on the avoidance performance in olfactory bulbectomized (OBX) rats using active avoidance (shuttle box) test and passive avoidance (step through) test were investigated. Rats were microinjected unilaterally through implanted guide cannulas into the CA1 area of the dorsal hippocampus and the drugs were administered separately, 5 minutes before each training session. The microinjections of losartan into the left, but not the right CA1 hippocampal area improved the acquisition and retention of active and passive avoidance learning, thus suggesting dependence on the side of injection. The unilateral (left or right) administration of angiotensin II did not significantly affect the performance of OBX rats in the avoidance tasks. A differential distribution of the AT1 receptors in the left and right hemisphere could contribute for the asymmetry in the behavioral effects of the AT receptor antagonist.

Keywords: angiotensin II, losartan, learning, memory, asymmetry, hippocampus, olfactory bulbectomy

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Introduction

The octapeptide angiotensin II (Ang II) is a potent effector hormone of the renin-angiotensin system (RAS). The brain has its own intrinsic RAS and most of its components are expressed in the central nervous system. The brain RAS includes the biologically active angiotensin peptides: Ang II, Ang III, Ang IV and Ang-(1-7) (1). Four receptor types have been proposed within the RAS: the Ang II type 1 and 2 receptors (AT1, AT2), Ang IV-specific receptor(AT4), and Ang-(1–7) selective receptor (2- 4). The AT1 and AT2 types are structurally similar, G-protein coupled receptors. The AT4 receptor is a protein, which is not G-protein-linked, while Ang-(1–7) exerts its actions via the G protein-coupled Mas receptor.

The hippocampus is a part of the limbic system, which is important for cognitive processes. Both the concentration of Ang II and the expression of angiotensin receptors are particularly high in the hippocampus and the CA1 hippocampal neurons are reported to be sensitive to Ang II (5, 6). Ang II being a part of the brain RAS, acts as a neurotransmitter or a neuromodulator. Increasing evidence supports the involvement of Ang II and its receptors in learning and memory processes, although the behavioral consequences apparently depend upon the methods, doses and location of the treatment (7-9). Previous studies have found behavioral asymmetries (in locomotor activity, anxiety, learning and memory) following microinjections of Ang II into the CA1 hippocampal area (9- 11).

The role of Ang II in brain pathology is poorly understood and not widely studied. Ang II has been suggested to participate in the pathophysiology of stress, Alzheimer's disease and depression. The first evidence that brain RAS may be involved in depressive disorder was observed in hypertensive patients undergoing captopril treatment (12). Investigations demonstrated that high angiotensin-converting enzyme activity led to high Ang II formation, which was associated with depression, hypothalamic-pituitary axis hyperactivity and anxiety (13-15). It was found that the blockage of angiotensin II synthesis with angiotensin-converting enzyme inhibitors (ACEI) in transgenic rats with high production of brain Ang II reduces the increased anxiety (16, 17). There is increasing evidence indicating beneficial effects of angiotensin II receptor blockers (ARBs) in brain disorders such as stroke, Alzheimer's disease, depression and stress (18). In an experimental study, the AT1 antagonist losartan improved depression-like behavior in mice, as determined by the forced swim test (19). Clinical research demonstrated that the treatment with ARBs decreases anxiety and depression in patients (20, 21). The ACEI led to similar therapeutic effects - improved the efficacy of antidepressants and decreased anxiety and depression in normotensive rats (22, 23).

Olfactory bulbectomy (OBX) provides a well-validated animal model of depression. It is considered most suitable for studying the neurochemical mechanisms underlying the pathophysiology of depression. Bilateral removal of the olfactory bulbs in rats induces a syndrome of behavioral deficits that reflect a disruption of cortical and limbic regions, accompanied by neurochemical, endocrine and immune alterations, reminiscent of symptoms of human depression (24, 25). OBX leads to extensive cognitive impairments, with deficits in learning and memory (24, 26, 27). Borre et al. and Douma et al. have suggested that OBX in rats can serve also as an animal model of neurodegeneration (28, 29). According to Yehuda and Rabinovitz, OBX mimics a complex of Alzheimer's symptoms, including cognitive decline, increased locomotor activity, decreased food intake, etc. (30). The neurodegeneration of the septo-hippocampal cholinergic pathways (31), which accompany memory impairments and the elevation of *amyloid* beta level in the brain (32) also point to the usefulness of OBX as an animal model of Alzheimer's disease.

Bearing in mind the above considerations, the aim of our study was to examine the effects of Ang II and the AT1 receptor antagonist losartan on learning and memory processes after unilateral application into CA1 hippocampal area in OBX rats.

Materials and methods

Animals

Male Wistar rats (200 – 220 g, 2 month old 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 12h light/dark cycle (lights on at 6.00 am). The behavior experiments were carried out between 10:00 am and 1:00 pm. After the testing procedure, the rats were returned to their respective home cages.

The experiments were performed according to the "Rules for care and experiments on laboratory animals" of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences. All efforts were made to minimize animal suffering and reduce the number of animals used in the study.

Surgical procedures

Bilateral olfactory bulbectomy was carried out according to the method described by Kelly et al. (24). Seven days after bilateral olfactory bulbectomy a cannula was surgically implanted into the CA1 hippocampal area as described previously (9).

Behavioral methods

The animals were tested in a two-way active avoidance test (shuttle box) and a passive

avoidance test (step-through) as described previously (7).

Statistical analysis

Separate two-factor analysis of variance (ANOVA) was used to analyze the data obtained for the number of avoidances (shuttle-box) in the learning test (1st and 2nd training day) and the memory test (24 h after the 2nd training day) between subject factors: drug (three levels: Ang II, losartan or saline) and side of injection (two levels: left and right). ANOVA data were analyzed further by post-hoc Student-Newman–Keuls (SNK). Analysis of the passive avoidance data was performed using $\chi 2$ tests.

Results

Shuttle box test

The bilateral removal of the olfactory bulbs resulted in an impairment of the learning and memory in the active avoidance task (shuttle box).The number of avoidances of rats with developed depression was significantly lower as compared to the sham-operated controls on the 1st training day ($P \leq 0.001$), 2nd training day ($P \leq 0.001$) and on the retention test ($P \leq 0.001$) (Fig. 1A, B, C).Two way ANOVA demonstrated significant effects for the factors "drug" (Ang II and losartan) on the number of avoidances ($F_{2,35}$= 13,819, P<0.001)and "side" of injection ($F_{1,35}$= 8.533, P≤ 0.04) on the 1st training day. There was also significant interaction between "drug"×"side" of injection ($F_{2,\ 35}$=8,402, P≤0.001). On the 2nd training day ANOVA demonstrated significant effects for the factors "drug" ($F_{2,35} = 32,439$, $P \leq 0.001$), "side" ($F_{1,35} = 19.756$, $P \leq 0.001$) and a significant interaction between the factors "drug"× "side " ($F_{1,35} = 18.756$, $P \leq 0.001$). At the retention test ANOVA revealed a significance for "drug" ($F_{2,35} = 25.833$, $P \leq 0.001$), side of injection ($F_{1,35}$ = 15,873, $P \leq 0.001$) and a significant interaction between "drug" × "side" ($F_{2,35}$ = 18,373; $P \leq 0.001$).

Post-hoc SNK comparisons demonstrated that losartan infused into CA1 area produced significantly greater number of avoidances when injected into the left CA1 area on 1st day ($P \leq 0.001$), 2nd day ($P \leq 0.001$) and at the retention test ($P \leq 0.001$), as compared to the respective saline-treated OBX-controls (Fig. 1A, B, C). Microinjected into the right side, losartan did not affect significantly the number of avoidances on the training days and at the retention test (Fig. 1A, B, C).Upon left-side or right-side administration, Ang II did not significantly affect the number of avoidances, as compared to the respective OBX saline-treated controls during the whole experimental period(Fig. 1A, B, C).

The unilateral injection of losartan showed a clear lateralized improvement of learning and memory. Microinjected into the left CA1 area losartan increased significantly the number of avoidances as compared to the right-side

injections: on the 1st training day ($P \leq 0.001$), on the 2nd day ($P \leq 0.001$) and at the retention test($P \leq 0.001$) (Fig. 1A, B, C).

Step through test

One way ANOVA on the latent time of OBX rats showed a significant effect on the retention tests: on the 3rd h after training ($F_{1,11}$ = 155,702; $P \leq 0.001$) and on the 24th after training ($F_{1,11}$ = 218,256; $P \leq 0.001$). At the passive avoidance task, bulbectomized rats demonstrated a significant decrease of the latent time on the retention tests: on the 3rd h ($P \leq 0.001$) and on the 24th ($P \leq 0.001$) as compared to the sham-OBX group. The number of OBX rats fulfilling the learning criteria diminished to 0%,as compared to the sham operated controls which reached50%learning criteria on the 3rdh and 63% on the 24th h (Fig. 1A, B). Separate two way ANOVA demonstrated significant effects of the factors "drug"($F_{2,47}$ = 45,014; $P \leq 0.001$) and "side"($F_{1,47}$= 42,103, $P \leq 0.04$)for the 3rdhThere was also significant interaction between "drug"×"side" of injection ($F_{2,47}$=34,715, $P \leq 0.001$). At the retention test ANOVA showed significance for "drug" ($F_{2,47}$ =150,410, $P \leq 0.001$), "side " ($F_{1,47}$ = 115,394, $P \leq 0.001$), as well as a significant interaction between "drug" × "side" ($F_{2,47}$ = 118,530; $P \leq 0.001$).

Post hoc test showed that the microinjection of losartan into the left CA1 area prolonged the latency time on the 3rdh ($P \leq 0.001$) and on the 24th h ($P \leq 0.001$) and increased the percentage of the rats that reached the learning criteria on the 3rdh (50%)and on the 24th h(63%).Losartan infused into the right side did not affect the performance of the OBX rats in the step-through task (Fig. 2A, B).The injection of losartan into the left CA1 area increased significantly the latent time on the 3rdh ($P \leq 0.001$) and 24th h ($P \leq 0.001$) as compared to the right-side infusions (Fig. 2A, B). The effect of Ang II administered unilaterally (left- or right side) did not differ significantly from the respective saline treated OBX controls (Fig. 2A, B).

Discussion

The bilateral olfactory bulbectomy markedly impaired the performance of rats in both avoidance tasks compared to the sham-operated controls, as has been reported previously (24, 27). Our results showed a lateralized ameliorating effect of the AT1 receptor antagonist losartan on learning and memory deficits in OBX rats. The microinjections of losartan into the left but not the right CA1 hippocampal area improved the acquisition and retention of active and *passive avoidance* learning, thus suggesting dependence on the side of injection. In addition, the unilateral (left or right) administration of angiotensin II did not affect significantly the performance of OBX rats in the avoidance tasks.

Studies have shown controversial results about the effects centrally administered AngII on learning and memory processes. In the avoidance paradigms, used to evaluate learning and memory

of rodents, it has been observed that intracerebroventricular (i.c.v.) injection of Ang II impairs (34), improves (35-37), or has no effect (38) on the cognitive performance. The administration of Ang II into CA1 hippocampal area in a step-down inhibitory avoidance task produced a dose-dependent amnesic effect, which was blocked by the AT2 receptor antagonist, PD 123319, but not by the AT1 receptor antagonist losartan (39, 40). The conflicting results about the cognitive effects of Ang II can be explained by the fact that these effects are very sensitive to the different methodological approaches (time interval between the injection of Ang II and the performing of the test, type of administration, differences in dosage, duration of treatment, number of training sessions, and type of memory task evaluated).

Concerning the type of receptors involved in the effects of Ang II on cognitive processes, data are contradictory too. Braszko reported that both AT1 and AT2 receptors may contribute to the effects of Ang II in passive avoidance, object recognition and active avoidance tests, with an exception of lack of influence of AT1 receptor inhibition on active avoidance acquisition (41), while other authors (37, 42) observed no effect of centrally applied losartan on active avoidance tests.

By comparing the effect of captopril (ACEI) and losartan (a selective AT_1 receptor antagonist) in an active avoidance task, Raghavendra et al. found that both drugs were equally effective in enhancing retention of memory when administered prior to training. Hence, it was concluded that the decrease in endogenous Ang II activity in the brain might result in improved cognitive performance (43).

There are many reports about the beneficial effects of AT1 blockers in different *experimental models* of learning and *memory impairments.* DeNoble et al. showed that the impaired performance on a passive avoidance task in rats treated i.c.v. with renin could be offset with ACEI treatment, or by the application of the AT1 receptor antagonists (44). Orally administered telmisartan (AT1 receptor blocker) improved spatial learning and memory in a type 1 diabetic mouse model, streptozotocin-induced diabetic mice (45), while pretreatment with a non-hypotensive dose of telmisartan significantly inhibited cognitive decline in an experimental model of Alzheimer disease (46). Inaba et al. demonstrated that olmesartan (another AT1 blocker) diminished the cognitive alterations observed ina shuttle-box avoidance task for the human renin and human angiotensinogen gene chimeric transgenic mice and suggested that continuous activation of the brain RAS impairs cognitive function via stimulation of AT1 receptor (47). Clinical data with Ang II antagonists tend to support experimental findings. It has been shown that losartan improves cognitive functions in hypertensive patients as well as in normotensive adults (48, 49).

Our results showed no effects of Ang II on the avoidance behavior of OBX rats. Previously, using the same experimental protocol, Belcheva et al. reported that Ang II infused into CA1 area enhanced acquisition and retention in a two-way active avoidance test (9). The bilateral removal of the olfactory bulbs results in neurodegeneration in the projection areas of the bulbs such as cortex, amygdala and hippocampus (25). Although data are lacking about the expression of RAS components in the brain of OBX rats, the neurodegenerative changes might be accompanied with abnormalities in RAS in different brain regions, similarly to the observed alterations on AT receptors in patients with neurodegenerative disorders (50, 51). It could be suggested that the neurodegenerative changes in the hippocampus and the following compensatory neuronal reorganization could explain the lack of effects of angiotensin II on the performance of OBX rats.

The improvement of memory deficits after injection of losartan into CA1 area in OBX rats may be related to its neuroprotective, anti-inflammatory and anti-oxidant effects. Neurodegenerative changes, inflammation and oxidative stress were found to play role in the etiology of OBX-induced depression. Increased production of oxygen reactive species, saturation of antioxidant enzymes, lipid peroxidation and generation of pro-inflammatory cytokines in the brain of OBX rats have been reported (52- 55).

Angiotensin II type 1 (AT1) receptor blockers have shown powerful neuroprotective effects in vivo (56, 57). The latest research provided evidence that ARBs may protect the brain from different types of injury resulting in parenchyma inflammation and neuronal damage. Beneficial effects on memory functions for the AT1 receptor blockers have been demonstrated in traumatic animal models. For example, candesartan treatment reduced the lesion volume after controlled cortical impact injury on mice and improved performance in the Morris water maze 4 weeks after injury (58). The AT1 blockers candesartan and telmisartan exhibited neuroprotective and anti-inflammatory effects in different models of brain injury in rodents (58- 61). The systemic administration of candesartan decreased the acute brain inflammatory response to a bacterial endotoxin lipopolysaccharide and reduced the inflammatory reaction by decreasing the production of cytokines and other inflammatory factors in the brain cortex (62, 63). The anti-inflammatory effects were widespread, occurring in many limbic areas such as hypothalamus, prefrontal cortex, hippocampus, and amygdala.

Left–right asymmetries of brain and behavior are widespread among both vertebrates and invertebrates. Knowledge of the bilateral distribution of a system in brain is important to appropriately understand how it functions in health

and pathology. Data have accumulated concerning anatomical, functional and neurochemical asymmetries in the rat brain. The research on the impact of hippocampal left–right asymmetry on higher-order brain functions using "split-brain" mice have shown that the usage of the right hippocampus improves the accuracy of spatial memory, while performance of other hippocampus-dependent tasks (fear conditioning) was not influenced by the laterality of the hippocampus (64). Recently, a dissociation of hippocampal long-term memory function between hemispheres has been reported, suggesting that memory is routed via distinct left–right pathways within the mouse hippocampus (65).

Other evidence regarding the lateralization in the expression of different components of RAS has been provided by the research of Wu et al., who found asymmetrical distribution of angiotensinase activity in the amygdala, hippocampus and prefrontal cortex of the rat (66). The aminopeptidases (APs) termed angiotensinases are involved in the metabolism of angiotensin peptides. In the hippocampus, there was a left predominance for AlaAP, CysAP and AspAP activities, while GluAP predominated in the right hippocampus (66, 67). It was also suggested that the asymmetric activity of angiotensinases in the rat hippocampus might be associated with both the direction and the intensity of behavioral lateralization as expressed by paw preference (66).

Our results are in line with the reports about asymmetry in the effects of drugs acting on angiotensin receptors. Belchevaet al. observed lateralization in the effects of angiotensin II, injected into the CA1 area of rats, where left-side administration enhanced acquisition and retention in an active avoidance task (9). Previous research of our laboratory also demonstrated lateralized memory enhancing effects of Ang IV and Ang II after pretreatment with losartan in the CA1 area (7, 68).

Based on the above-mentioned findings, we suggest that the lateralized ameliorative effect of losartan on learning and memory in OBX rats could be attributed to a hemispheric asymmetry in the components of the hippocampal RAS, including the expression and/or activity of different peptides, enzymes and receptors.

In conclusion, our study demonstrated lateralized *beneficial effects* of losartan, injected into the CA1 hippocampal area, on learning and *memory deficits* induced by the olfactory bulbectomy in rats. Ang II did not affect the behavior of OBX rats tested in the avoidance paradigms. A differential distribution of the AT1 receptors in the left and right hemisphere could contribute for the asymmetry in the behavioral effects of the AT receptor antagonist.

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Figure captions

Fig. 1 (A, B, C). Effects ofAng II (0,5 μg) and losartan (100 μg) microinjected unilaterally into the left or right CA1 hippocampal area of OBX rats on the number of avoidances (shuttle box). **A)** 1st training day; **B)** 2nd training day; **C)** retention test. Asteriscsdepict comparisons of the number of avoidances, following injections of the drug vs respective saline injections into CA1 area, ***P≤0.001; circles depict comparisons of the number of avoidances, following injections into the left vs right-side. n=6. Means (± S.E.M.) are presented.

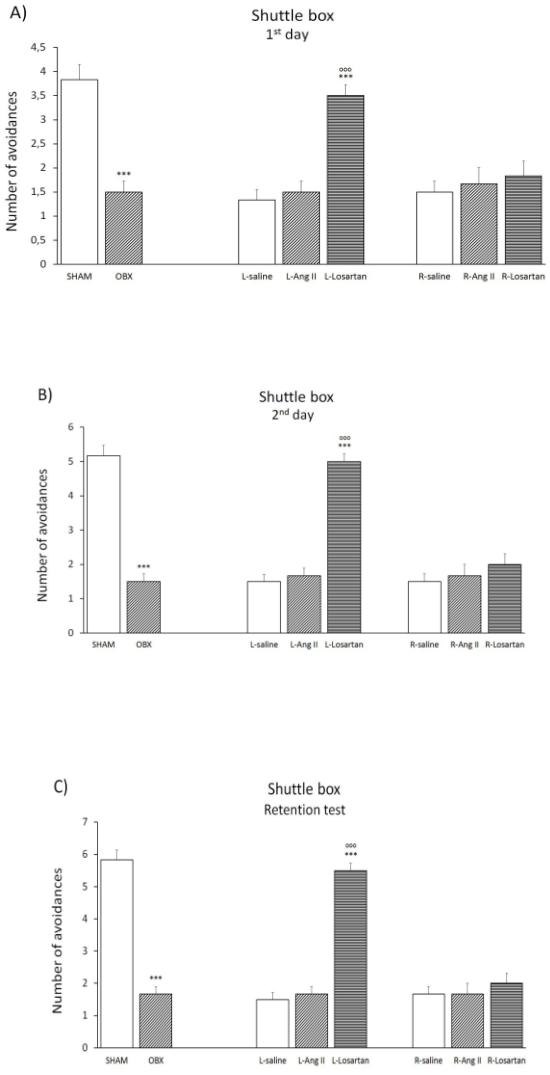

Fig. 2 (A, B). Effects of Ang II (0,5μg) and losartan (100 μg)microinjectedunilaterally into the left or right CA1 hippocampal area ofOBX rats on the latent time (step through). **A)** 3rd hour. **B)** 24th hour.***P≤0.001; Asteriscsdepict comparisons of the latent time (sec) following injections of the drugs vs respective saline injections intoCA1areas; circles depict comparisons of the latent time (sec), following injections into the left vs right-side. n=8. Means (± S.E.M.) are presented.