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Intrahippocampal administration of losartan improves learning and memory in rats with model of depression

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The brain renin-angiotensin system is involved in learning and memory, but the role of angiotensin II and its receptors in these processes is not well established. The effects of losartan (angiotensin type 1 receptor antagonist) and angiotensin II, microinjected bilaterally into CA1 hippocampal area on learning and memory in rats with a model of depression (bilateral olfactory bulbectomy), using two avoidance paradigms: active avoidance (shuttle box) and passive avoidance (step through) were investigated. After stereotaxic implantation of guide cannulas into the CA1 area of dorsal hippocampus angiotensin II (0.5 μg) and losartan (100 μg) were microinjected separately, 5 minutes before each training session. It was found that intra-CA1 losartan reverses memory deficits induced by bulbectomy unlike angiotensin II which did not show any effect. The data suggest an involvement of angiotensin type 1 receptors in modulating memory processes in rats with model of depression.

Key words: Losartan, Angiotensin II, Hippocampus, Depression, Learning, Memory

INTRODUCTION

The brain renin–angiotensin system (RAS) includes a number of bioactive angiotensin (Ang) peptides (Ang II, Ang III, Ang IV and Ang-(1-7) which show variable neurological activities [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 a putative Ang-(1–7)-selective receptor. Angiotensin II (Ang II) is the most important angiotensin peptide, which binds selectively AT1 and AT2 receptors.

Recent studies have revealed that Ang II regulates synaptic transmission in several brain regions including the hippocampus [2]. The hippocampus is known to be involved in a variety of learning tasks and there the concentration of Ang II and the expression of the various angiotensin receptor subtypes are particularly high [3, 4].

There are few reports about the involvement of hippocampal angiotensin receptors in cognitive processes using the avoidance paradigms. It was demonstrated that when administered to the hippocampus, Ang II impaired retention of the single trial step through shock avoidance response by activation of AT1 receptors [5]. Other studies provided evidence that Ang II applied to the CA1 area blocked memory formation through a mechanism involving the activation of AT2 receptors [6]. Recently, a possible role of hippocampal Ang II receptors in voluntary exercise-induced enhancement of learning and memory in rats was suggested [7]. It has been reported that orally administered losartan (an antagonist of the AT1 receptors) suppresses the enhancing effect of voluntary running on cell proliferation in the rat hippocampus [8].

The first suggestion that brain RAS may be important in depression was observed in hypertensive patients undergoing captopril treatment [9-11]. Captopril treatment has also been shown to protect animals against the forced swim induction method of learned helplessness. Evidence accumulates that the brain RAS is involved in the mediation of stress responses and depression [12, 13].

The olfactory bulbectomized rat (OBX) is a well-validated animal model of depression.OBX is associated with a variety of behavioral abnormalities such as hyperactivity in the "open-field" test, appetite-motivated behaviors, decreased fear-related behavior, extensive cognitive impairments, and

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others [14-16]. Hippocampal degeneration has been suggested to be the basis for the cognitive deficits in Alzheimer's disease (AD) [17]. As far as bulbectomy is associated with increased levels of beta-amyloid protein in neocortex and hippocampus [18] and induces some behavioral and biochemical phenotypes of Alzheimer's disease, such as an increase of locomotor activity and cognitive defects [19, 20] it has been used also as an AD model.

Taking into account the high density of AT1 receptors in the hippocampus and the role of this limbic structure in the cognitive processes, the aim of the present study was to examine the effects of Ang II and losartan (a selective AT1 receptor antagonist) after bilateral infusion into CA1 hippocampal area on learning and memory processes in rats with an OBX model.

MATERIAL AND METHODS

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 behavioral 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 (registration FWA 00003059 by the US Department of Health and Human Services).

Surgical procedures

Bilateral olfactory bulbectomy (OBX), stereotaxic implantation and drug microinjections into the hippocampal CA1 area were published previously [14, 21, 22]. OBX was performed according to the method described by Kelly et al. [14]. Seven days after OBX, guide cannulas were implanted bilaterally (right and left) into CA1 hippocampal area of OBX rats (P = 3.8 mm; L = ± 3.0 mm; h = - 3.0 mm). After surgery, the animals were allowed 7 days to recover before the beginning of the behavioral tests, e.g. 15 days after OBX. During the recovery period, the rats were handled daily.

Rats were microinjected into both CA1 areas with 0.5 μl of Ang II (pH 7.4) or 0.5 μl of losartan (pH 7.4) or 0.5 μl saline. Following the termination of the experiments and immediately prior to the sacrifice, the rats were injected through the injection cannula with 0.5 μl 2 % Fast Green dye for verification of cannula placement into hippocampal CA1 area. Animals with cannula placement outside the CA1 area or not symmetrical within both CA1 areas were excluded from the statistical analysis.

Behavioral methods

The behavioral tests were carried out 15 days after the bilateral olfactory bulbectomy. The animals were tested in two learning and memory tests: two-way active avoidance test (shuttle box) and passive avoidance test (step-through) as described previously [21]. The experimental rats were divided into 2 main groups for each of avoidance test (shuttle box and step through): A) - rats without cannulas and without treatments - OBX operated rats and sham-operated rats B) - OBX rats with bilaterally implanted cannulas into CA1 areas microinjected with Ang II; losartan and saline. The drugs were injected 5 minutes prior to each training session.

Statistical analysis

One-way ANOVA was used to analyze the data obtained for bilateral olfactory bulbectomy. Separate one-way ANOVA was used to analyze the data obtained for number of avoidances for learning (1st and 2nd training day) and memory test (24 hours after the 2nd training day). ANOVA data were analyzed further by post-hoc Student-Newman–Keuls (SNK). T-test was used for post-hoc comparisons between left- and right-side injections. Analysis of the passive avoidance data was performed using $\chi 2$ tests.

RESULTS AND DISCUSSION

Shuttle box test

One-way ANOVA on the number of avoidances of OBX rats (without implanted cannulas) demonstrated a significant effect on the 1st training day ($F_{1,11} = 34{,}090$; $P \leq 0.001$), 2nd training day ($F_{1,11} = 60{,}500$; $P \leq 0.001$) and on the retention test ($F_{1,11} = 74{,}387$; $P \leq 0.001$) at the active avoidance paradigm. Post-hoc SNK showed that the avoidances of OBX rats were significantly lower as compared to the sham-OBX controls on 1st day ($P \leq 0.001$), 2nd day ($P \leq 0.001$) and on the retention test, 24 h after the 2nd day ($P \leq 0.001$) (Fig. 1).

One way ANOVA after bilateral infusions of Ang II and losartan on the number of avoidances of OBX rats showed a significant effect for "drug" on the 1st day ($F_{2,17} = 4{,}078$; $P \leq 0.03$), on the 2nd day ($F_{2,17} = 6{,}0465$; $P \leq 0.01$), and at the retention test ($F_{2,17} = 5{,}248$; $P \leq 0.01$). Post-hoc test revealed that losartan significantly increased the number of avoidances during the 1st day ($P \leq 0.005$), the 2nd day ($P \leq 0.005$), and at the retention test ($P \leq 0.0001$), as compared to the saline-treated OBX-controls, while Ang II did not produce any significant effect (Fig. 2).

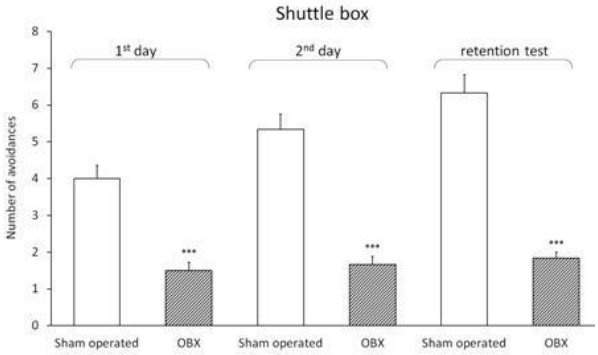

Fig. 1. Effect of olfactory bulbectomy (OBX) on the number of avoidances (shuttle box). ***P < 0.001. Asterisks depict comparisons of the number of avoidances in OBX rats vs. sham operated controls. n=6. Means (± S.E.M.) are presented.

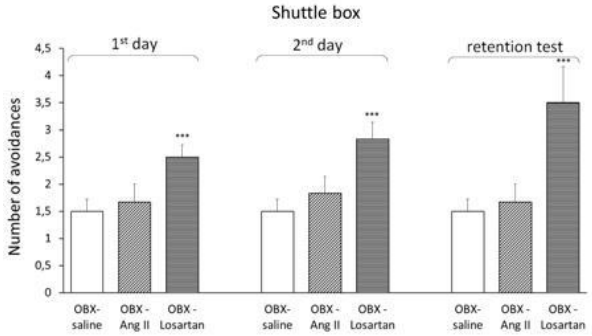

Fig. 2. Effects of Ang II and losartan microinjected bilaterally into the CA1 hippocampal area of OBX rats on the number of avoidances (shuttle box). ***P < 0.001. Asteriscs depict comparisons of the number of avoidances, following infusions of the drugs vs. respective OBX saline treated controls. n=6. Means (± S.E.M.) are presented.

Step-through test

ANOVA on the latent time of OBX rats (passive avoidance task) demonstrated a significant effect on the retention tests: 3rd h after training ($F_{1,11} = 182{,}931$; $P \leq 0.001$) and 24th after training ($F_{1,11} = 250{,}372$; $P \leq 0.001$). The OBX rats showed a significant decrease of the latent time on 3rd h ($P \leq 0.001$) and 24th h ($t = 3.98$, $P \leq 0.001$) as compared to the sham-OBX controls. The number of OBX rats to fulfill the learning criteria diminished to 0 % at both retention tests ($P \leq 0.001$) as compared to the controls (Fig.3).

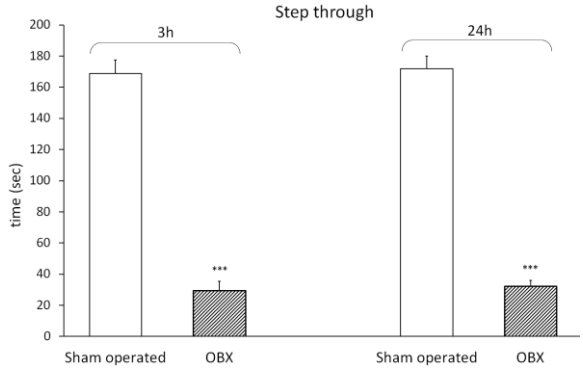

Fig. 3. Effect of olfactory bulbectomy (OBX) on the latent time (step through). ***P < 0.001. Asterisks depict comparisons of the latent time in OBX rats vs. respective sham operated controls. n=6. Means (±SEM) are presented.

ANOVA after infusions of Ang II and losartan on the latent time of OBX rats showed a significant effect for "drug" on the 3rd h ($F_{2,23} = 19{,}917$; $P \leq 0.001$) and 24th h ($F_{2,23} = 71{,}941$; $P \leq 0.001$). Losartan significantly enhanced the cognitive performance of OBX rats. It prolonged the latent time on 3rd h ($P \leq 0.04$) and 24th h ($P \leq 0.001$) and increased the percentage of the rats reaching the learning criteria on 3rd h (38% - $\chi^2 = 5.333$; $P \leq 0.02$) and 24th h (63% - $\chi^2 = 7.237$; $P \leq 0.01$) as compared to the saline-treated OBX rats. Ang II administered into the CA1 areas did not produce significant effects on memory-related behavior of OBX rats (Fig.4).

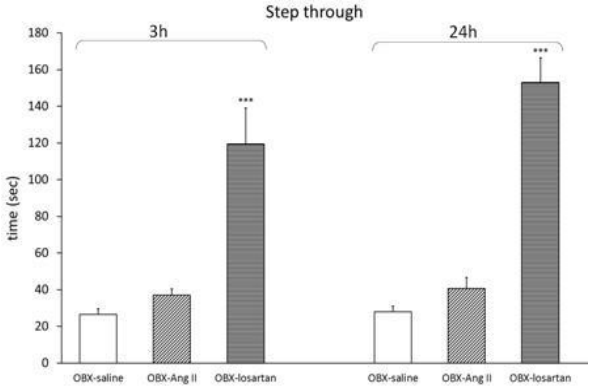

Fig. 4. Effects of Ang II and losartan microinjected bilaterally into the CA1 hippocampal area of OBX rats on the latent time (step through). ***P < 0.005. Asteriscs depict

comparisons of of the latent time, following infusions of the drugs vs. respective OBX saline treated controls. n=8. Means (± S.E.M.) are presented.

The present study extended our understanding about the learning and memory effects of Ang II and losartan infused separately into the hippocampal CA1 area of OBX rats. The bilateral bulbectomy impaired the performance of rats in both avoidance paradigms as it has been demonstrated previously [23, 24]. The microinjections of Ang II failed to produce any effect on the performance of OBX rats as compared to the saline-treated OBX controls, while losartan significantly ameliorated the learning and memory-related behavior impairment. Based on our results we can make only some speculative assumptions to explain the memory ameliorating effect of losartan on OBX-induced learning deficits in the avoidance tests.

The brain RAS has been implicated in the pathophysiological mechanisms of dementia and neurodegenerative diseases. However, its role on the impairment of learning and memory-related behavior induced by OBX has not been examined yet. The bilateral removal of bulbi olfactorii is associated with a variety of behavioral abnormalities in rodents including cognitive impairments, with deficits in learning and memory [14, 16, 23, 24]. After bulbectomy degeneration of neurons in cortex, hippocampus [25, 26] and impaired neurogenesis in hippocampal dentate gyrus have been reported [27]. The present findings provided new insights concerning the modulatory role of RAS on cognitive processes in rats with olfactory bulbectomy.

Data are lacking about the expression of angiotensin receptors in the brain of OBX rats, but the neurodegenerative changes might be accompanied with abnormalities in RAS in different brain regions, similarly to the observed alterations on AT receptor subtypes in patiens with neurodegenerative disorders [28, 29]. It could be suggested that the above-mentioned neurodegenerative changes in the hippocampus after bulbectomy and the following compensatory neuronal reorganization could explain the effects of the drugs on the performance of OBX rats. The memory enhancing effects of losartan in OBX rats may indicate that it is able to ameliorate the impaired cognitive functions only in the conditions of neurodegeneration and impaired activity of many neurotransmitter systems, increased oxidative stress and inflammation, which have been reported following bulbectomy.

AT1 receptor blockers have shown powerful neuroprotective effect in vivo and their use may be beneficial for the treatment of many brain disorders [30, 31]. Recent studies showed that telmisartan protects mouse dopaminergic neurons, inhibits the microglial response in a mouse MPTP of Parkinson's disease [32] and attenuates hypertension-induced learning and memory deficits [33, 34].

Brain inflammation has been implicated in the pathophysiology of brain diseases such as major depression, Parkinson's disease, Alzheimer's disease, and traumatic brain injury. Evidence accumulates suggesting that ARBs may protect the brain from different types of injury resulting in parenchyma inflammation and neuronal damage. Inflammation has been linked to the etiology of OBX-induced depression. Ablation of olfactory bulbs is associated with production of oxygen reactive species, saturation of antioxidant enzymes, increased lipid peroxidation, etc.[35]. In addition to oxidative stress, OBX syndrome involves generation of pro-inflammatory cytokines in brain [36, 37] and promotes pathological damage by accompanying inflammatory reactions [38]. Oxidative stress contributes to the cognitive impairments in experimental animals [39]. Reports indicate that oxidative stress is increased in the brain of Alzheimer's disease and other neurodegenerative disorders [40]. The neuroprotective effects of ARBs may be partially related to their ability to decrease oxidative stress. Recently, complex interactions between Ang II, behavioral processes and neuronal oxidative stress have been reported. Bild et al. [2] found significant correlations between some memory-related behavioral parameters and the oxidative stress markers from the hippocampus. The central administration of Ang II induced memory deficits in two different cognitive tasks and increased oxidative stress status in the hippocampus, while the administration of losartan significantly improved the performance of rats [2]. Following this line of reasoning, it is likely the anti-inflammatory and anti-oxidant effects of losartan to contribute for its ability to ameliorate the OBX–induced deficits in the avoidance paradigms.

The ability of Ang II receptor antagonists to interfere with the activity of some neurotransmitter systems, all being involved in the cognitive processes might also contribute to the memory enhancing effect of losartan in the OBX model. As far as in rodents AT1 receptors are expressed in brain regions

involved with fear memory such as hippocampus and amygdala, the implication AT1 receptor inhibition in the mechanisms of fear memory and extinction [3, 41, 42] could also be taken into account.

CONCLUSION

This study demonstrated that the bilateral administration of the AT1 receptor antagonist losartan into the CA1 hippocampal area of OBX rats significantly ameliorated the memory deficits in both active and passive avoidance tasks. These findings could contribute to understanding the potential of the central RAS manipulation for the treatment of cognitive disorders.

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ЛОСАРТАН ВЪВЕДЕН В ХИПОКАМП НА ПЛЪХОВЕ С МОДЕЛ НА ДЕПРЕСИЯ ПОДОБРЯВА ОБУЧЕНИЕТО И ПАМЕТТА

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(Резюме)

Мозъчната ренин-ангиотензиновата система е въвлечена в обучителните и паметовите процеси, но ролята на ангиотензин II и неговите рецептори в тези процеси все още не е добре установена. Изследвани са ефектите на лосартан (антагонист на ангиотензин тип 1 рецепторите) и ангиотензин II, микроинжектирани двустранно в CA1 полето на хипокампа върху обучението и паметта на плъхове с модел на депресия (двустранна олфакторна булбектомия), при два метода за памет и обучение: активно избягване (shuttle box) и пасивно избягване (step through). След стереотаксично имплантиране на водещи канюли в CA1 полето на задния хипокамп, ангиотензин II (0.5 μg) и лосартан (100 μg) се микроинжектират поотделно, 5 минути преди всяка тренировъчна сесия. Установено е, че лосартан въведен в CA1 полето премахва паметовия дефицит, предизвикан от булбектомията, за разлика от ангиотензин II, който не показва ефект. Получените данни говорят, че ангиотензин тип 1 рецепторите са въвлечени в процесите на обучение и памет на плъховете с модел на депресия.