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LATERALIZED ACTIVE AVOIDANCE LEARNING AND MEMORY TO ANG II AND LOSARTAN MICROINJECTED INTO AMYGDALA IN RATS DEPRESSION MODEL

Roman Tashev[1], Hristina Nocheva[1], Margarita Velikova[2]✉

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Abstract

Depression is a widespread socially significant disease. Studies aiming to reveal the pathogenesis of depression have lasted for decades, but the specific mechanisms remain unclear. Olfactory bulbectomy (OBX) in rats provides a well-validated animal model of depression and Alzheimer's disease. The aim is to evaluate the involvement of Ang II and AT1 receptors in learning and memory after unilateral infusion of Ang II and losartan (specific AT1 antagonist) into CeA (central nucleus of the amygdala) in rats with a model of depression (bilateral olfactory bulbectomy, OBX). The effects of Ang II and losartan infused into CeA on the avoidance performance in OBX rats using the active avoidance (shuttle box) test were investigated. A stereotaxic technique was used for bilateral implantation of guide cannulae into CeA. Fourteen days after OBX, Ang II, or losartan were microinjected unilaterally into CeA of rats with depressive-like behaviour. For the first time it was found that Ang II infused into the left CeA impaired learning and memory, while losartan infused into the left CeA significantly improved these processes and prevented the memory deficits induced by the bulbectomy. The data suggest an involvement of amygdala Ang II and AT1 receptors in learning and memory of rats and a differential distribution of the AT1 receptors in the left and right central nucleus of the amygdala in rats with a model of depression.

Key words: angiotensin II, losartan, learning, asymmetry, amygdala, depression

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Introduction. Depression is a widespread socially significant disease. According to the World Health Organization, depression is a leading cause of disability worldwide. Studies aimed at detecting the etiopathogenesis of depression have lasted for decades, but specific pathogenetic mechanisms remain unclear.

The olfactory bulbectomized rat (OBX) is a widely used animal model of depression [1, 2]. OBX in rats induces various behavioural, physiological, neurochemical, etc. disturbances. The behavioural changes are similar to those seen in depressed patients [1, 3]. The bilateral removal of the olfactory bulbs in rats affects a substantial number of brain structures such as the cortex, amygdala, hippocampus, thalamus, etc; similar findings are observed in patients with major depression [4]. In the last years, many researchers suggested that OBX in rodents can be used also as an animal model of neurodegeneration. OBX mimics some of the Alzheimer's disease symptoms, including cognitive decline, increased locomotor activity, decreased food intake, etc. The neurodegeneration after OBX is accompanied by memory impairments and an elevation of amyloid beta level in the brain [5], which also point to the usefulness of OBX as a model of Alzheimer's disease.

A growing body of evidence indicates that the renin-angiotensin system (RAS) and especially the main effector hormone of RAS – the octapeptide angiotensin II (Ang II) participate in the pathophysiology of stress, Alzheimer's disease, and depression [6].

Ang II exerts its actions in the CNS via AT1, AT2, and AT4 receptor subtypes, which are differently distributed in the brain regions [7,8]. The major effects of Ang II are mediated by the AT1 receptor. AT1 receptors are expressed in brain structures including the amygdala, hippocampus, lateral septum, and frontal cortex, functionally associated with anxiety, learning, and memory [9]. Numerous data have shown that Ang II involvement in learning and memory processes is associated with AT1 and/or AT4 receptors [8, 10]. In addition, AT1 receptors are implicated in the pathophysiology of depression and play a role in cognitive impairment in Alzheimer's disease [8, 9].

In previous studies, we have found that the inhibition of angiotensin AT1 receptors in the left CA1 hippocampal area provoked changes in the behavioural responses of rats: exploratory behaviour, locomotor activity, nociception, anxiety, learning, and memory. Losartan microinjected into the left, but not the right CA1 area of OBX rats exerted lateralized learning and memory effects [11]. Clinical studies have shown that the antihypertensive drug captopril (angiotensin-converting enzyme, ACE, inhibitor) improved mood and attenuated depressive symptoms in major depression, and this effect was not associated with its antihypertensive action [12]. Reports have indicated possible beneficial effects of RAS blockade on cognitive processes [8, 9, 13].

The amygdala is a structure of the limbic system primarily associated with emotional processes and emotional learning and memory; it is also involved in

the pathophysiology of diseases such as epilepsy, anxiety, and depression. The central nucleus of the amygdala (CeA) is an important regulatory centre for the behavioural responses to stress [14]. Among the different neurotransmitter systems, the CeA contains Ang II, ACE, and AT1 receptors, but their role in the development of behavioural disturbances observed in depression is not fully understood.

The lack of data supporting an asymmetry in the behavioural effects of Ang II and the AT1 receptor antagonist losartan motivated us to evaluate the effects of unilateral, intra-amygdala (CeA) microinjections of the drugs on learning and memory in rats with an experimental model of depression – bilateral olfactory bulbectomy.

Materials and methods. ***Animals.*** The experiments were carried out on male Wistar rats (200—220 g at the time of surgery). Experiments were approved by the local ethical committee of MU-Sofia following EC Directive 2010/63/EU for animal experiments.

Bilateral olfactory bulbectomy (OBX) was performed according to the method of Kelly et al. [1] as described previously [2].

Stereotaxic implantation and drug injection into CeA. Seven days after the olfactory bulbectomy the rats were anesthetized and placed in a stereotaxic apparatus. Guide cannulae were implanted (right, R and left, L) into the CeA. On the 15th day after OBX, rats were microinjected into the left or right CeA with Ang II (0.5 µg), losartan (100 µg), or saline. Ang II (Sigma) or Losartan (Sigma) were dissolved ex tempore in saline before the injection. The sham operation was performed in the same way as in the case of OBX, without the removal of the olfactory bulbs. The drugs were injected 5 min before each training session. Injection sites were verified post-mortem.

Behavioural method – two-way active avoidance (shuttle-box). The behavioural tests were carried out 14 days after OBX. The animals were trained for two-way active avoidance in a shuttle-box apparatus as described previously [2, 11]. A criterion for improved learning and memory was the significant increase in the avoidance responses in the training sessions and at the retention test.

The experimental animals were divided into 8 groups ($n = 6$ for each group):
4 control groups: Sham-operated rats without treatment; OBX rats without treatment; OBX saline-treated rats, microinjected into the right CeA or left CeA.
4 experimental groups: OBX-Ang II treated rats, microinjected into the right CeA or left CeA; OBX-Losartan treated rats, microinjected into the right or left CeA.

Statistical analysis. One-way ANOVA was used to analyze the data obtained for bilateral olfactory bulbectomy. Two-way ANOVAwas used to analyze the data obtained for the number of avoidances (N Av) between subject factors: "drug" (Ang II, losartan, saline) and "side"of injection (L and R). Findings from the ANOVA were post-hoc analyzed by the Student–Newman–Keuls test.

Results. ANOVA on the N Av of OBX rats demonstrated a significant

effect on the 1st training day (T day) ($F_{1,14} = 15.566$; $P \leq 0.001$), 2nd T day ($F_{1,14} = 19.877$; $P \leq 0.001$) and on the retention test (RT) ($F_{1,18} = 49.543$; $P \leq 0.001$). SNK test demonstrated that the N Av of OBX rats was lower as compared to the sham-operated controls on the 1st T day $P \leq 0.001$), 2nd T day ($P \leq 0.001$), and at the RT ($P \leq 0.001$) (Fig. 1A, B, C). Two-way ANOVA after Ang II or losartan infused unilaterally into CeA on the N Av showed a significant effect on the 1st T day for the factors "drug" ($F_{2,35} = 15.306$; $P \leq 0.001$) and "side" of injections ($F_{1,35} = 5.184$; $P \leq 0.03$). On the 2nd T day ANOVA demonstrated significant effects for "drug" ($F_{2,35} = 44.684$; $P \leq 0.001$), "side" ($F_{1,35} = 11.842$; $P \leq 0.001$) and significant interaction between "drug" X "side" ($F_{2,35} = 14.684$; $P \leq 0.001$). At the RT, (24 h after the 2nd training day) ANOVA revealed a significance for "drug" ($F_{2,35} = 28.957$; $P \leq 0.001$), "side" ($F_{1,35} = 4.235$; $P \leq 0.05$) and "drug" X "side" ($F_{2,35} = 12.540$; $P \leq 0.001$) interactions. Ang II decreased the N Av of OBX-rats when it was infused into L-CeA on the 1st and 2nd T days ($P \leq 0.05$; $P \leq 0.05$, respectively) and at the RT ($P \leq 0.05$) as compared to the OBX-saline controls and the sham-controls ($P \leq 0.001$) in all testing days. Ang II did not produce any significant effect on the R-CeA, compared to the OBX–saline controls. Comparisons between L-side and R-side Ang II demonstrated that the L-CeA microinjections significantly decreased the N Av ($P \leq 0.05$) only upon the RT (Fig. 1A, B, C). The losartan microinjection into L-CeA increased the N Av in OBX rats on the 1st ($P \leq 0.001$), 2nd ($P \leq 0.001$) T days, and at the RT ($P \leq 0.001$) in comparison with the OBX-saline rats; as compared to sham-rats it increased the N Av at the RT only ($P \leq 0.05$). The effect of losartan microinjected into the R-CeA did not differ from that of the OBX-saline rats. Infusions of losartan into the L-CeA produced a significant increase in the N Av compared to the R-side injections on the 1st ($P \leq 0.001$), 2nd ($P \leq 0.001$) T days, and at the RT ($P \leq 0.001$) (Fig. 1A, B, C).

Discussion. The present study is the first to provide data about the effects of Ang II and losartan (specific antagonist of AT1 receptors) infused unilaterally into the CeA on the learning and memory deficits in OBX rats. In previous studies we showed impaired learning and memory in OBX rats [2, 11], thus supporting the reports about learning impairments following olfactory bulbectomy [1, 3]. We have also found that Ang II microinjected into the left, but not into the right CeA suppressed exploratory activity. The microinjections of losartan into the L-CeA stimulated the exploratory activity and it was significantly higher as compared to the effects on the R-CeA [15].

Our present results demonstrated that the unilateral microinjections of Ang II and losartan into CeA exerted an opposite effect on rats' cognitive performance in the active avoidance task. The infusion of Ang II into the L-CeA further impaired learning and memory of OBX, i.e. while there was no significant effect upon Ang II right-side administration. We observed again a lateralized effect of the AT1 receptor antagonist losartan microinjected into the CeA on the performance of

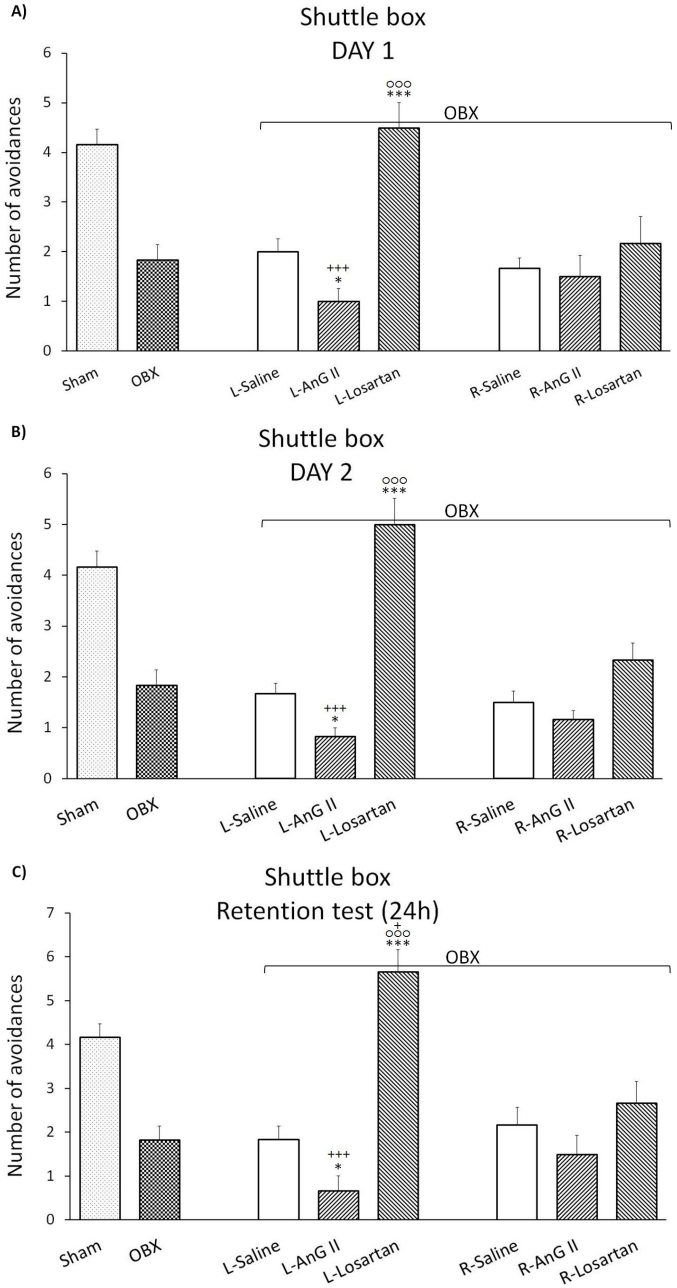


Fig. 1. Effects of Ang II and losartan microinjected into the left or right CeA of OBX rats on the number of avoidances (N av). **A)** 1st training day; **B)** 2nd training day; **C)** retention test. $^{*}P \leq 0.05$; $^{***}P \leq 0.001$ – comparisons of the N av, following drug injections vs. saline injections into CeA of OBX rats; $^{+}P \leq 0.05$, $^{+++}P \leq 0.001$ – comparisons of the N av, following drug injections to OBX rats vs. sham-operated rats; $^{\circ\circ\circ}P \leq 0.001$ – comparisons of the N av, following injections into the left CeA vs. right CeA; $n = 6$; Means (± S.E.M.) are presented

OBX rats. Losartan infused into the L-CeA not only ameliorated the learning and memory deficits but also reversed them. The inhibition of the AT1 receptors in the left CeA improved memory compared to the right CeA. The results indicate that Ang II and losartan influence rat cognition in a manner depending on the side of drug administration and suggest a different role of right and left CeA. Our results suggest that Ang II and AT1 receptors in CeA play a crucial role in rats' cognitive processes. Furthermore, CeA might likely be involved in the cognitive deficits accompanying depressive-like behaviour.

Microinjection of Ang II in the rat's amygdala increases the discharge rate of amygdala neurons and the increase can be blocked by AT1 receptor antagonists [16]. The amygdala modulates memory in various tasks such as inhibitory avoidance and motor or spatial learning and exerts a modulatory influence on the memory systems of the hippocampus and caudate-putamen [14, 17]. Based on the abovementioned findings, we suppose 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 amygdalar RAS, including the expression and/or activity of different peptides, enzymes, and receptors.

Conclusion. We demonstrated that Ang II infused into left CeA in OBX rats impaired learning and memory, while losartan, infused into left CeA improved these processes and reversed the memory deficits induced by the olfactory bulbectomy. Differential distribution of the AT1 receptors in the left and right hemispheres could contribute to the asymmetry in the memory-enhancing effects of the AT receptor antagonist.

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[1]*Department of Physiology and Pathophysiology, Medical University of Sofia*
2 Zdrave St, 1431 Sofia, Bulgaria
e-mails: romantashev@gmail.com, dr_inna@yahoo.com

[2]*Department of Physiology and Pathophysiology, Medical University of Varna*
55 M. Drinov St, 9002 Varna, Bulgaria
e-mail: msvelikova@yahoo.com