

LATERALIZED EFFECTS OF INTRA-AMYGDALAR LOSARTAN INFUSION ON ANXIETY IN RATS WITH A MODEL OF DEPRESSION

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Abstract

This study investigated the effects of Angiotensin II (Ang II) and losartan, a selective Ang II type 1 receptor (AT1) antagonist when unilaterally infused (left or right) into the central nucleus of the amygdala (CeA) on anxiety-like behaviour in male Wistar rats with a model of depression (bilateral olfactory bulbectomy, OBX). Microinjections of Ang II (0.5 µg/side) into the CeA of OBX rats did not alter anxiety-like behaviour, as assessed in the elevated plus-maze test. In contrast, inhibition of AT1 receptors in the left or right CeA by losartan (100 µg/side) induced an anxiolytic-like effect, evidenced by an increased number and time of open-arm entries, a higher open/total entries ratio, a higher open/total time ratio, and a decreased number and time of closed- arm entries. For the first time, our study demonstrates the anxiolytic-like effect of losartan when infused unilaterally into the CeA of OBX rats. Additionally, our findings suggest a differential distribution of AT1 receptors in the left and right CeA. This asymmetric distribution may contribute to the lateralized effects of AT1 receptor antagonist and indicates that AT1 receptor expression levels could play an important role in depression.

Key words: depression, losartan, amygdala, AT1 receptors, elevated plus-maze, rat

Introduction. In recent years, increasing evidence has suggested that the renin-angiotensin system (RAS) is involved not only in hypertension but also

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in anxiety and depression. The presence of a renin-angiotensin system in the brain is now well established. The primary effector hormone of this system is angiotensin II (Ang II), which is widely distributed in limbic system structures. This distribution suggests its involvement in processing autonomic, endocrine, and sensory-motor information, as well as in regulating emotions and motivation [1,2]. Ang II is the most significant angiotensin peptide and exerts its effects by binding to selective AT1 and AT2 receptors, which belong to the class of G protein-coupled receptors [3,4]. These receptors have been identified in brain structures such as the amygdala, hippocampus, lateral septum, and frontal cortex – regions implicated in the pathophysiology of depression and anxiety [4,5]. Emerging evidence indicates that RAS plays a role in the mechanisms underlying depression and anxiety [6]. AT1 and AT2 blockers have been widely used as antihypertensive drugs. A meta-analysis on the effects of antihypertensive medications on mental health revealed that AT1 receptor blockers and ACE inhibitors have beneficial effects on anxiety and overall mental health, in contrast to other antihypertensive drugs [7,8]. In a complex and multifunctional mechanism, Ang II influences and regulates the behaviour of experimental animals. Losartan, a selective, non-peptide competitive AT1 receptor antagonist, is commonly used to treat hypertension.

The amygdala plays a key role in behavioural responses related to memory, learning, anxiety, nociception, and stress. It is also one of the brain structures whose dysfunction contributes to various disorders associated with depression. Notably, the concentration of the amygdalar Ang II and angiotensin receptors is particularly high. The central amygdaloid nucleus (CeA) is a crucial regulatory centre for emotional processing, playing a significant role in integrating sympathetic and behavioural responses to stress [9]. Stress is a major etiopathogenetic factor in the development of depression. Studies suggest Ang II is involved in stress response and the hypothalamic-pituitary-adrenal (HPA) axis regulation. Despite evidence indicating neuropeptide imbalances in certain affective disorders, the role of Ang II and its receptors in depression and depression models remains largely unexplored. There is a lack of data in the literature regarding the involvement of amygdalar Ang II in depressive states or its specific role in depression. Furthermore, no studies have investigated hemispheric differences in behavioural responses to amygdalar Ang II and its AT1 receptors in an experimental model of depression, specifically bilateral olfactory bulbectomy (OBX). OBX is considered one of the most suitable experimental models for studying the neurochemical mechanisms underlying the pathophysiology of depression.

Our study aimed to examine the involvement of AT1 receptors in the central nucleus of the amygdala in modulating anxiety-like behaviour in OBX rats through the unilateral microinjection of Ang II and losartan, an AT1 receptor antagonist.

Materials and methods. *Animals.* Male Wistar rats (240 g at the time of surgery) were housed individually in polypropylene boxes with free access to food and water.

The experiments were carried out according to the rules of the Research Ethics Commission of the Medical University of Sofia (KENIMUS). All efforts were made to minimize animal suffering and reduce the number of animals used in the study.

Experimental model of depression – bilateral olfactory bulbectomy (OBX). Bilateral olfactory bulbectomy was carried out according to the method first described by KELLY et al. [10,11]. The surgical procedure, performed using a stereotaxic apparatus (Stoelting, USA), involved drilling two burr holes according to the coordinates from the stereotaxic atlas of Pellegrino and Cushman. The olfactory bulbs were aspirated using a stainless steel needle attached to a water pump, as previously described [11].

Stereotaxic implantation and drug injection into (CeA). Seven days after bilateral olfactory bulbectomy, following anesthesia with Calypsol (50 mg/kg, i.p.), the rats were placed in a stereotaxic apparatus, and guide cannulae were implanted into the right and left CeA according to the coordinates from the stereotaxic atlas of Pellegrino and Cushman. ($P = 0.6$ mm, $L = \pm 4.5$ mm, $h = -7.5$ mm) [11]. After 15 days, as a depression-like state developed, the rats were microinjected with Angiotensin II (0.5 μ g/side), losartan (100 μ g/side), or saline into the left or right CeA, using doses previously shown to be active in earlier experiments [11]. The substances were administered in a volume of 0.3 μ l over a period of 1 min, and the injection cannula was left in place for an additional 1 min. The sham operation was performed similarly to the OBX procedure but without the removal of the olfactory bulbs.

Elevated plus maze (EPM) test. The plus-maze consisted of two open arms (50×10 cm) positioned opposite each other and two closed arms ($50 \times 50 \times 40$ cm) and was elevated to a height of 50 cm. The number of entries into the open and closed arms and the time spent in the open arms were recorded during the five-minute test.

Verification. Injection sites were verified post-mortem. Animals with misplaced or asymmetrical cannulae and dye-diffusion beyond the CeA were excluded.

Statistical analysis. A two-way ANOVA was used to analyze the data. Findings from the ANOVA were post-hoc analyzed using the Student–Newman–Keuls (SNK) test.

Results. Effects of Ang II or losartan microinjected into the CeA. ANOVA analysis of the behavioural responses of OBX rats microinjected unilaterally (left or right) with Ang II or losartan in the CeA in the plus maze test showed a significant effect for: the number of entries into the open arms - for the two factors “substance” ($F_{1,47} = 17.027$; $P \leq 0.001$) and “side” ($F_{1,47} = 5.25$; $P < 0.001$), as well as for the interaction between them “substance” X “side” ($F_{2,47} = 4.189$; $P \leq 0.03$); for the time spent in the open arms - for the two factors “substance” ($F_{1,47} = 8.075$; $P \leq 0.01$), “side” ($F_{2,47} = 10.975$; $P \leq 0.001$); for the number of entries into the closed arms for the two factors “substance” ($F_{1,47} =$

15.638; $P \leq 0.001$), “side” ($F_{1,47} = 6.796$; $P \leq 0.01$); for the ratio of the number of entries into the open arms to the total number of entries into the open and closed arms for the two factors “substance” ($F_{2,47} = 22.458$; $P \leq 0.001$), “side” ($F_{1,47} = 4.164$; $P \leq 0.03$); for the ratio of the time spent in the open arms to the time spent in the open and closed arms for the two factors “substance” ($F_{2,47} = 10.564$; $P \leq 0.001$), “side” ($F_{2,47} = 5.186$; $P \leq 0.04$); for the total number entries (entries into the open arms + entries into the closed arms) for the factor “substance” ($F_{1,47} = 9.003$; $P \leq 0.05$) and no significant for factor “side” ($F_{2,47} = 2.265$; $P = \text{NS}$).

Post-hoc SNK test demonstrated that Ang II infused into the right or left CeA of OBX rats had no significant effect on the behavioural parameters reported in the plus maze test, both compared to the corresponding OBX controls and sham-operated OBX rats (Fig. 1).

Losartan infused unilaterally into the right or left CeA of OBX rats increases the number of exits into the open arms ($P \leq 0.04$; $P \leq 0.001$, respectively) and the time spent in them ($P \leq 0.03$; $P \leq 0.001$, respectively), the ratio of the number of entries into the open arms to the total number of entries into the arms ($P \leq 0.003$; $P \leq 0.001$, respectively), as well as the ratio of the time spent in the open arms to the time spent in the open and closed arms ($P \leq 0.03$; $P \leq 0.001$, respectively) and decreases the time spent in the closed arms ($P \leq 0.005$; $P \leq 0.001$, respectively), and total number entries ($P \leq 0.03$; $P \leq 0.002$, respectively) compared to the corresponding OBX rats, infused with saline. The post-hoc test showed also a significant difference between the effects of losartan infused into the right-side and left-side, i.e. the effects of losartan were more pronounced after its microinjection into the left vs. the right CeA of OBX rats (Fig. 1).

Discussion. The present study is the first to provide data on the effects of Ang II and losartan infused into the right or left central nucleus of amygdala (CeA) on anxiety-like behaviour in OBX rats. Microinjections of Ang II (0.5 pg/side) into the right or left CeA of OBX rats increased the number of closed-arm entries without affecting other tested parameters compared to both the sham-operated and saline-treated OBX controls.

We have demonstrated anxiety-like behaviour in OBX rats tested in an elevated plus maze [12]. Previously, we found that Ang II infusion into the hippocampal CA1 area of OBX rats did not affect anxiety-like behaviour, whereas losartan (100 µg/side) exhibited an anxiolytic-like effect, suggesting the involvement of AT1 receptors in the pathogenesis of anxiety [11]. It is currently believed that the RAS system plays a role in the pathogenesis of depression and anxiety [2]. Ang II is thought to be involved in the information transmission from the hippocampus, amygdala, and other brain regions to the limbic and prefrontal cortex – areas associated with regulating complex behaviours. The limbic system, including the amygdala, has been found to contain a high density of AT1 receptors [5]. Stimulation of AT1 receptors has been associated with depression and

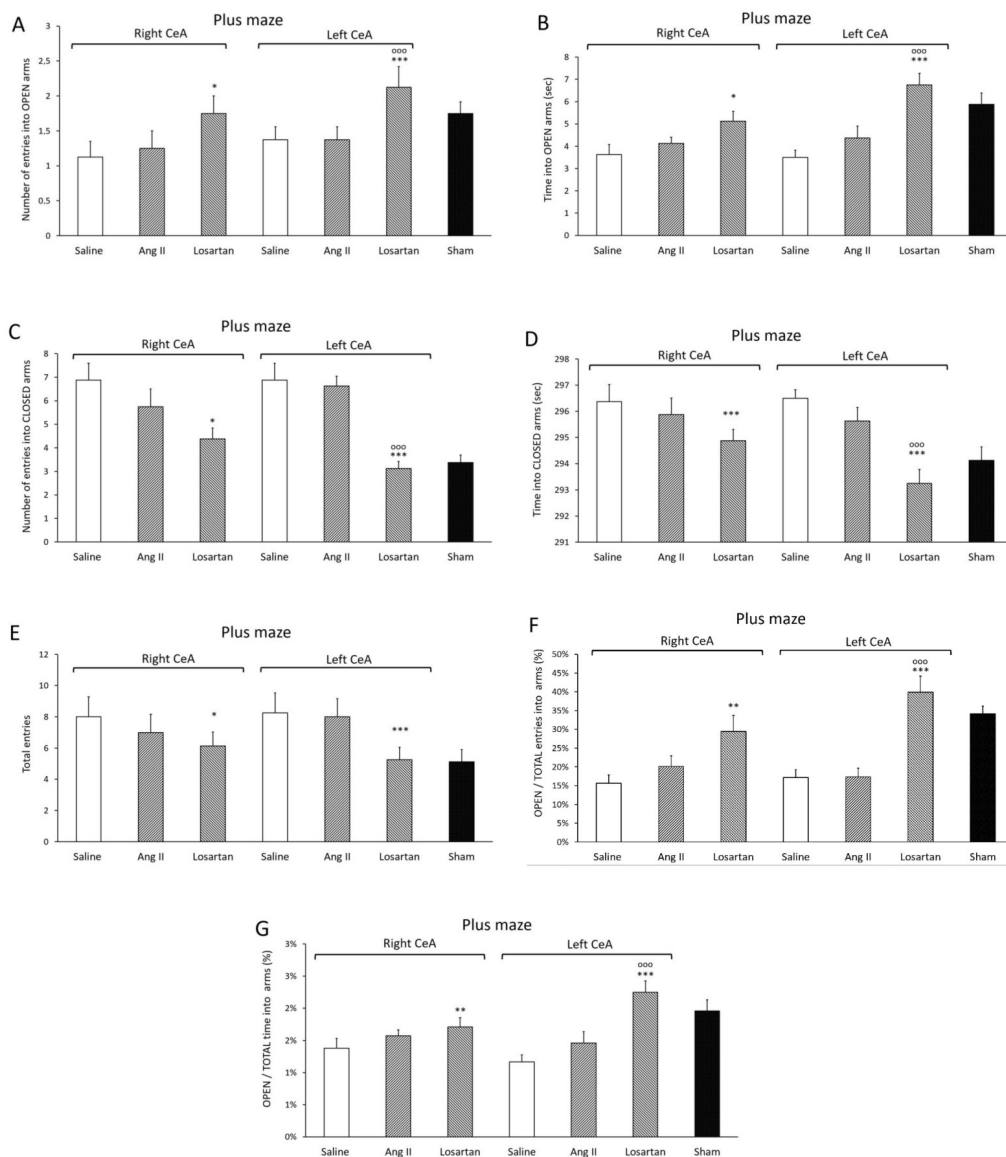


Fig. 1. Effects of Ang II (0.5 $\mu\text{g}/\text{side}$) and Losartan (100 $\mu\text{g}/\text{side}$) microinjected unilaterally into the CeA of OBX rats: **A**) on the number of open arms entries; **B**) on the time spent into open arms; **C**) on the number of closed arms entries; **D**) on the time spent into closed arms; **E**) on the total entries; **F**) on the ratio open/total entries; **G**) on the ratio open/total time into arms. $n = 8$. Means ($\pm$ S.E.M.) are presented. Asterisks depict drug treated vs. respective OBX saline-treated. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. Circles depict left-side vs. right-side; ° $P \leq 0.05$; °° $P \leq 0.01$; °°° $P \leq 0.001$

anxiety, while their blockade reduces stress, anxiety, and depression [7,13]. Additionally, the behavioural effects of AT1 receptor activation or inhibition may vary depending on the brain structure into which they are infused.

The amygdala, particularly its central nucleus (CeA), is a key structure implicated in disorders associated with depression, anxiety, and stress responses. Notably, Ang II concentration and angiotensin receptor expression are high in this region. The relationship between hemispheric brain asymmetry and depression has been extensively studied, revealing functional deficits in both hemispheres that are affected by antidepressant therapy. Of particular interest to us was examining the effects of losartan infused unilaterally into the CeA in a rat model of depression. This study extended our understanding of the asymmetric anxiety-like effects of losartan microinjected into the left or right CeA. Research suggests functional differences between the right and left amygdala. Electrical stimulation of the right amygdala induces negative emotions, particularly fear and sadness, whereas stimulation of the left amygdala can evoke either pleasant (happiness) or unpleasant (fear, anxiety, sadness) emotions [14]. Other evidence suggests that the left amygdala plays a role in the brain's reward system [15].

According to recent findings, there is a coordinated interaction among the different peptides of the RAS system, with their functional status depending on the action of various aminopeptidases (APs), also called angiotensinases. BANEGAS et al. [16] investigated the hemispheric distribution of angiotensinases, including alanyl- (AlaAP), cystinyl- (CysAP), and glutamyl- (GluAP) aminopeptidases involved in the metabolism of angiotensin and cholecystokinin. Their findings indicate an asymmetric distribution of these enzymes in the amygdala, hippocampus, and prefrontal cortex. Notably, aminopeptidase activity was greater in the right amygdala. This interhemispheric difference in enzymatic activity suggests an uneven distribution of endogenous substrates, potentially contributing to asymmetry in brain functions. It could be suggested, that the asymmetries in depression-like or anxiety-like behaviours may result from the asymmetric distribution of aminopeptidases.

Our study demonstrated that losartan, a specific AT1 receptor antagonist, microinjected into the right or left CeA of OBX rats produced an anxiolytic-like effect. Notably, unilateral infusion of losartan resulted in asymmetric behavioural effects, with the anxiolytic-like behaviour being more pronounced following microinjection into the left CeA compared to the right. These results indicate that losartan alleviates the heightened anxiety state induced by bulbectomy, exerting an anxiolytic effect in OBX animals. Our findings align with previous studies showing asymmetric effects on exploratory behaviour and nociception following losartan microinjection into the CeA, depending on the side of drug administration. This suggests distinct roles for the right and left CeA [17,18]. The more pronounced anxiolytic-like effect of losartan in the left CeA is consistent with a recent report on asymmetry in the altered activity of specific amygdalar nuclei in

patients with depression. That study established a correlation between increased anxiety in a depressive state and the amplitude of low-frequency fluctuations in the left central amygdala [19].

In conclusion, for the first time, our study demonstrated an anxiolytic-like effect of losartan when microinjected unilaterally into the CeA in a rat model of depression. Our findings suggest a differential distribution of AT1 receptors in the left and right CeA. This asymmetric distribution may contribute to the observed asymmetry in the effects of ATI receptor antagonism and suggests that AT1 receptor expression levels play an important role in depression.

REFERENCES

- [1] MCKINLEY M. J., A. L. ALBISTON, A. M. ALLEN et al. (2003) The brain renin-angiotensin system: location and physiological roles, *Int. J. Biochem. Cell. Biol.*, **35**(6), 901–918.
- [2] WRIGHT J. W., J. W. HARDING (2013) The brain renin-angiotensin system: a diversity of functions and implications for CNS diseases, *Pflugers Arch. Europ. J. Physiol.*, **465**(1), 133–151.
- [3] SONG K., A. M. ALLEN, G. PAXINOS, F. A. MENDELSON (1991) Angiotensin II receptor subtypes in rat brain, *Clin. Exper. Pharmacol. Physiol.*, **18**(2), 93–96.
- [4] VON BOHLEN UND HALBACH O., D. ALBRECHT (2006) The CNS renin-angiotensin system, *Cell Tissue Res.*, **326**(2), 599–616.
- [5] VON BOHLEN UND HALBACH O., D. ALBRECHT (1998) Mapping of angiotensin AT1 receptors in the rat limbic system, *Regul. Pept.*, **78**(1–3), 51–56.
- [6] GONG S., F. DENG (2023) Renin-angiotensin system: The underlying mechanisms and promising therapeutical target for depression and anxiety, *Front. Immunol.*, **13**, 1053136.
- [7] SAAVEDRA J. M., E. SÁNCHEZ-LEMUS, J. BENICKY (2011) Blockade of brain angiotensin II AT1 receptors ameliorates stress, anxiety, brain inflammation and ischemia: therapeutic implications, *Psychoneuroendocrinology*, **36**(1), 1–18.
- [8] BROWNSTEIN D. J., E. SALAGRE, C. KÖHLER et al. (2018) Blockade of the angiotensin system improves mental health domain of quality of life: A meta-analysis of randomized clinical trials, *Aust. N. Z. J. Psychiatry*, **52**(1), 24–38.
- [9] MARINZALDA M. A., P. A. PÉREZ, P. A. GARGIULO et al. (2014) Fear-potentiated behaviour is modulated by central amygdala angiotensin II AT1 receptors stimulation, *BioMed Res. Int.*, **2014**, Article ID 183248, <https://doi.org/10.1155/2014/183248>.
- [10] KELLY J. P., A. WRYNN, B. E. LEONARD (1997) The olfactory bulbectomized rat as a model of depression: an update, *Pharmacol. Ther.*, **74**(3), 299–316.
- [11] TASHEV R., M. IVANOVA (2018) Involvement of hippocampal angiotensin 1 receptors in anxiety-like behaviour of olfactory bulbectomized rats, *Pharmacol. Rep.*, **70**(5), 847–852, <https://doi.org/10.1016/j.pharep.2018.03.001>.
- [12] IVANOVA M., S. BELCHEVA, I. BELCHEVA et al. (2014) Modulatory effect of VIP injected into hippocampal CA1 area on anxiety in olfactory bulbectomized rats, *Acta Neurobiol. Exp.*, **74**(3), 317–327.

- [13] SAAVEDRA J. M. (2021) Angiotensin receptor blockers are not just for hypertension any more, *Physiology* (Bethesda), **36**(3), 160–173.
- [14] XIE T., S. J. H. VAN ROOIJ, C. S. INMAN et al. (2025) The case for hemispheric lateralization of the human amygdala in fear processing, *Mol. Psychiatry*, **30**, 2252–2259, <https://doi.org/10.1038/s41380-025-02940-2>.
- [15] ZALD D. H. (2003) The human amygdala and the emotional evaluation of sensory stimuli, *Brain Res. Rev.*, **41**(1), 88–123.
- [16] BANEGAS I., I. PRIETO, F. ALBA et al. (2005) Angiotensinase activity is asymmetrically distributed in the amygdala, hippocampus and prefrontal cortex of the rat, *Behav. Brain Res.*, **156**(2), 321–326.
- [17] TASHEV R., H. NOCHEVA, M. TZVETKOVA, S. BELCHEVA (2017) Differential effects of angiotensin II and losartan on exploratory behaviour after microinjections into rat amygdala, *C. R. Acad. Bulg. Sci.*, **70**(6), 863–870.
- [18] SABIT Z., H. NOCHEVA, R. TASHEV (2019) Modulation of nociception by angiotensin II type 1 receptors antagonist losartan infused into amygdala of rats with a model of depression, *C. R. Acad. Bulg. Sci.*, **72**(8), 1121–1129.
- [19] QIAO J., S. TAO, X. WANG et al. (2020) The difference of amplitude of low frequency fluctuation in amygdala subregion and its correlation with clinical characteristics in patients with anxious depression, *CJBMBS – Chinese Journal of Behavioral Medicine and Brain Science*, **12**, 400–405.

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