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Effects of angiotensin II receptor antagonists on anxiety and some oxidative stress markers in rat

Research Article

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Abstract: In addition to its known classical roles, the renin angiotensin system (RAS) has more subtle functions which include the regulation of emotional responses. Previous studies regarding the anxiety related behavior of RAS have showed controversial results. There is also evidence that oxidative stress accompanies angiotensin II infusion, but the role of AT1/AT2 specific receptors is not clear. The aim of this study was to evaluate the effects of central angiotensin II receptor blockers on anxiety state and oxidative stress. Behavioral testing included elevated plus maze, while oxidative stress status was measured though the extent of a lipid peroxidation product (malondialdehyde-MDA) and the specific activity of some defense antioxidant enzymes (superoxide dismutase-SOD and glutathione peroxidase-GPx). The rats treated with angiotensin II spent significantly less time in the open-arms of elevated-plus-maze, while the administration of losartan resulted in a significant increase of this time. We observed a significant increase of MDA concentration in the angiotensin II group and a decrease of MDA levels in both losartan and PD-123177 groups. In addition, a significant correlation was seen between the time spent in the open arms and oxidative stress markers. These findings could lead to important therapeutic aspects regarding the use of angiotensin II receptor blockers in anxiety-related disorders.

Keywords: *Angiotensin II • Losartan • PD-123177 • Anxiety • Oxidative stress*

1. Introduction

The discovery that all components of the renin angiotensin system (RAS) are present in the brain led investigators to postulate the existence of a local brain RAS [1]. The brain RAS mediates several classic physiological effects including body water balance and maintenance of blood pressure [2]. However, besides the classical functions, this system has more subtle functions involving complex mechanisms such as learning, memory and regulation of emotional functions [3-5].

RAS generates a family of bioactive peptides which mainly include angiotensin II (Ang II), angiotensin IV and angiotensin 1-7 [1,2]. The biological actions of Ang II are mediated by specific angiotensin receptors. Numerous studies have led to the identification of two pharmacologically specific angiotensin receptors type 1 (AT 1) and type 2 (AT 2), which are well represented in various brain areas [6,7].

The Ang II receptor antagonists, losartan and PD-123177, which are selective for the AT 1 and AT 2 receptor subtypes respectively, constitute important pharmacological tools for the assessment of behavioral consequences through the modulation of Ang II function [1,8].

Previous studies regarding the anxiety related behavior of RAS have showed controversial results. Some of the performed studies have suggested that blocking AT 1 receptors with losartan results in anxiolytic-like effects, as shown by specific tests, such as light-

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dark aversion test and elevated plus maze, in both mice and rats [5,9-12]. This was supported by other studies showing reversal of Ang II-anxiogenic action by the intracerebroventricular (icv) administration of losartan [13]. Still, other authors failed to find similar data [14] or even demonstrated opposed results [15].

There is also evidences that oxidative stress accompanies Ang II infusion, but the role of AT 1 vs. AT 2 receptors is not very clear [16,17]. It was previously reported that administration of Ang II increases the formation of free radicals like superoxide (O^{2-}) [18], while losartan inhibits oxidative stress and exerts antioxidant effects [19], most likely through the inhibition of Ang II [20,21]. There are also controversies regarding the effects of angiotensin II on oxidative stress, considering that in some experiments losartan significantly decreased angiotensin II-induced oxidative stress, while PD-123319 did not [21].

Recent reports demonstrated a linear relationship between peripheral blood oxidative stress markers and anxiety behaviour [22]. In this context, the aim of the present work was to evaluate the effects of Ang II receptor blockers icv administration on anxiety state and oxidative stress status in the temporal cortical area of rat brain, the most vulnerable cortical area to oxidative stress effects [23]. Also, we were interested in determining a possible correlation between the anxiety state response in elevated plus maze and central oxidative stress markers.

2. Material and Methods

2.1. Subjects

The subjects (n=48) were experimentally naive, male Wistar rats, weighing approximately 200-250 g at the beginning of the experiment. The animals were housed in a temperature and light-controlled room (22°C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water ad libitum. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). This study was approved by the local Ethic Committee and also, efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Neurosurgery

All surgical procedures were conducted under aseptic conditions, under sodium pentobarbital (45 mg/kg b.w., i.p., SIGMA) anesthesia. Rats were mounted in the stereotaxic apparatus with the nose oriented 11° below horizontal zero plane. Each experimental group received individually losartan, PD-123177 or angiotensin II (SIGMA), icv administered (7 consecutive days, 0.1µg/kg b.w.) by freehand through a plastic (silastic) cannula (Portex, 0.44 inside diameter, 0.9 mm outer diameter), stereotaxically implanted in the left cerebral ventricle at the following coordinates: 0.5 mm posterior to bregma; 1.3 mm lateral to the midline; 4.3 mm ventral to the surface of the cortex [24]. The cannula was positioned with acrylic dental cement and secured by one stainless steel screw.

After surgery the rats were isolated in separate cages and protected with large spectrum antibiotic. The sham-operated rats were injected with saline. The location of the icv cannulas in lesioned rats was verified by injecting a dye (Trypan Blue, SIGMA) through each cannula at the end of the experiment. Brains were removed and cut with a scalpel, and after the temporal lobes were removed for oxidative stress assays, the spread of the dye within the ventricles was examined. All cannulas were found to be in the right position.

Behavioral testing was performed after 7 consecutive days of treatment.

2.3. Elevated plus maze

The elevated plus maze (Coulbourn Instruments) consists of four arms, 49 cm long, 10 cm wide, and elevated 50 cm off the ground. Two arms were enclosed by walls 30 cm high and the other two arms were exposed. Rats were placed at the juncture of the open and closed arms and the amount of time spent on the open arms was recorded during a 5-min test. Time spent on the open arms is considered to be an index of anxiety [25].

2.4. Tissue collection

After the behavioral tests, all rats were anesthetized, rapidly decapitated, and the whole brain was removed. The temporal lobes were collected. Each of temporal tissue samples was weighed and homogenized with a Potter Homogenizer coupled with Cole-Parmer Servodyne Mixer in bidistilled water (1g tissue/10ml bidistilled water). Samples were centrifuged 15 min at 3000 rpm. Following centrifugation, the supernatant was separated and pipetted into tubes.

2.5. Biochemical estimations

Regarding the biochemical assessments, we decided to classically determine the main antioxidant enzymes (first line of defense in the way of free radicals) and a lipid peroxidation marker.

2.5.1. Determination of superoxide dismutase

Superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 substrate (a water soluble tetrazolium dye) and xanthine oxidase using a SOD Assay Kit (Fluka, product number: 19160) according to the manufacturer's instructions. Each endpoint assay was monitored by absorbance at 450 nm (the absorbance wavelength for the colored product of WST-1 reaction with superoxide) after 20 min of reaction time at 37°C. The percent inhibition was normalized by mg protein and presented as SOD activity units.

2.5.2. Determination of glutathione peroxidase

Glutathione peroxidase (GPx) activity was measured using the GPx cellular activity assay kit CGP-1 (Sigma Chemicals). This kit uses an indirect method, based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPx, which is then coupled with recycling GSSG back to GSH utilizing glutathione reductase (GR) and NADPH. The decrease in NADPH at 340 nm during oxidation of NADPH to NADP is indicative of GPx activity.

2.5.3. Determination of malondialdehyde

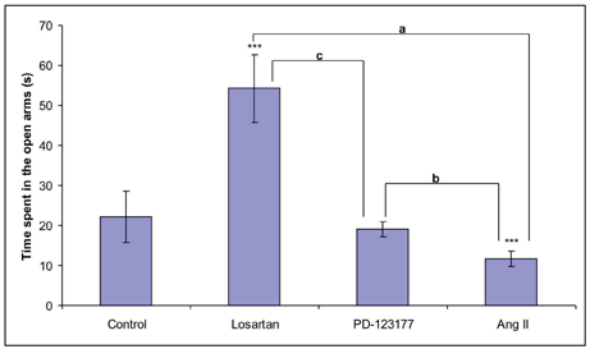
Malondialdehyde (MDA) levels were determined by thiobarbituric acid reactive substances (TBARs) assay. 200 µL of temporal lobe homogenate (supernatant) was added and briefly mixed with 1 ml of trichloroacetic acid at 50%, 0.9 ml of TRIS-HCl (pH 7.4) and 1 ml of thiobarbituric acid 0.73%. After vortex mixing, samples were maintained at 100 ºC for 20 minutes. Afterwards, samples were centrifuged at 3000 rpm for 10 min and supernatant read at 532 nm. The signal was read against an MDA standard curve, and the results were expressed as nmol/mg protein [26,27].

Total protein was measured using Bradford dye-binding method, with bovine serum albumin as standard [28].

2.6. Data Analysis

The animal's behavior in elevated plus maze was tracked and recorded using ANY-maze behavioral software (Stoelting Co., USA, version 4.5) and then statistically analyzed using one-way analysis of variance (one-way ANOVA). The results for antioxidant enzymes activity and MDA level were analyzed also using one-way ANOVA. All results are expressed as mean ± SEM. Post hoc analysis were performed using Tukey's honestly significant difference test in order to compare losartan, PD-123177 and angiotensin II groups. F values for which P<0.05 were regarded as statistically significant. Pearson's correlation coefficient and regression analysis were used to evaluate the connection between time spent in the open arms of elevated plus maze and central oxidative stress markers.

Figure 1. Effect of losartan, PD-123177 and Ang II on the time spent in the open arms of the elevated plus maze. The values are mean ± S.E.M. (n=12 animals per group).***p<0.001 vs. control group. For post-hoc analysis - a (Ang II vs. losartan): p=0.0001; b (Ang II vs. PD-123177): p=0.0001; c (losartan vs. PD-123177): p=0.0001.

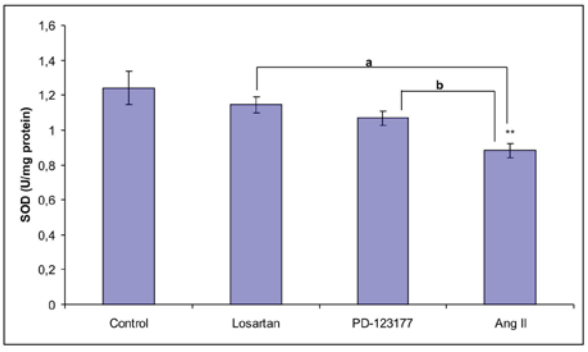


3. Results

3.1. The effects of angiotensin II and its receptor antagonists administration on anxiety state in elevated plus maze

Behaviour in the elevated plus maze is mainly used to assess exploration and anxiety status. In our experiment, the rats treated with angiotensin II spent significantly less time ($F(1,22)=19$, $p=0.0002$) in the open arms of elevated plus maze, compared to control group. On the contrary, the administration of losartan resulted in a significant increase ($F(1,22)= 79$, $p=0.0001$) of the time spent in the open arms, in comparison with control rats (Figure 1). On the other hand, the administration of PD-123177 did not resulted in any significant modifications of the time spent in open arms, compared to control group ($F(1,22)=2$, $p=0.21$). Also, *post hoc* analysis revealed significantly statistical differences between Ang II and losartan groups ($p=0.0001$), Ang II and PD-123177 groups ($p=0.0001$) and also between losartan and PD-123177 treated rats ($p=0.0001$), as seen in Figure 1.

Figure 2. Effects of losartan, PD-123177 and Ang II on SOD specific activity from the rat temporal lobe homogenates. The values are mean ± S.E.M. (n=12 animals per group). **p = 0,001 vs. control group. For post-hoc analysis - a (Ang II vs. losartan): p< 0.0001; b (Ang II vs. PD-123177): p< 0.0001.



3.2. Effects of angiotensin II and its receptor antagonists administration on oxidative stress status

Regarding the oxidative stress status, we observed a significant decrease (F(1,22)=14, p=0.001) of SOD specific activity in angiotensin II group, compared to control rats. Regarding the effects of losartan and PD-123177 administration, we did not found any significant modifications of SOD activity, in comparison with the control rats (Figure 2). Furthermore, *post hoc* analysis revealed significant differences between angiotensin II and losartan groups (p< 0.0001) and between angiotensin II and PD-123177 groups (p< 0.0001), as seen in Figure 2. Additionally, post-hoc analysis showed no significant differences between losartan and PD-123177 groups (p = 0.215).

In addition, a significant decrease (F(1,22)=5, p=0.04) of the other important antioxidant enzyme, GPx, was observed in the case of angiotensin II group, compared to control rats. Similarly to SOD, we did not found any significant modifications of GPx activity in both losartan and PD-123177 groups, compared to control rats (Figure 3). However, *post hoc* analysis revealed significant differences between angiotensin II and losartan groups (p< 0.0001) and between angiotensin II and PD-123177 groups (p< 0.0001) (Figure 3) and no significant differences between losartan and PD-123177 groups (p = 0.311).

Concerning the levels of the lipid peroxidation product MDA, we observed a significant increase (F(1,22)= 77, $p=1.18^{-8}$) of MDA concentration from the temporal lobe in angiotensin II group, compared to control rats. Also, a significant decrease of MDA levels in both losartan (F(1,22)= 66, $p=4.79^{-8}$) and PD-123177 (F(1,22)=11, p= 0.002) groups was observed, in comparison with the controls, suggesting some antioxidant effects (Figure 4).

Figure 3. Effects of losartan, PD-123177 and Ang II on GPx specific activity from the rat temporal lobe homogenates. The values are mean ± S.E.M. (n=12 animals per group). * p = 0,04 vs. control group. For post-hoc analysis - a (Ang II vs. losartan): p< 0.0001; b (Ang II vs. PD-123177): p< 0.0001.

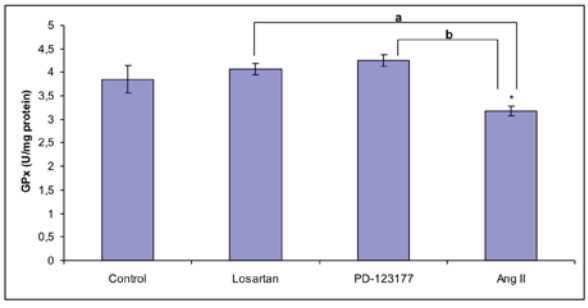
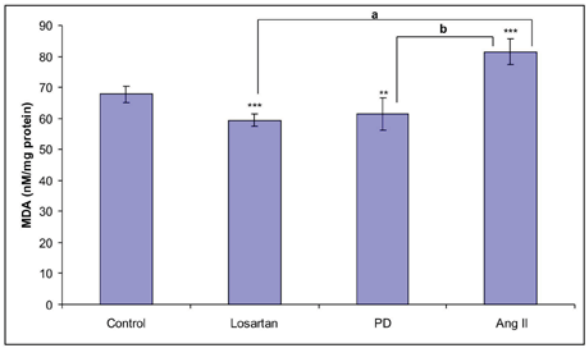


Figure 4. Effects of losartan, PD-123177 and Ang II on MDA levels from the rat temporal lobe homogenates. The values are mean ± S.E.M. (n=12 animals per group). **p = 0,002 vs. control group, ***p < 0,0001 vs. control group. For post-hoc analysis - a (Ang II vs. losartan): p< 0.0001; b (Ang II vs. PD-123177): p< 0.0001.

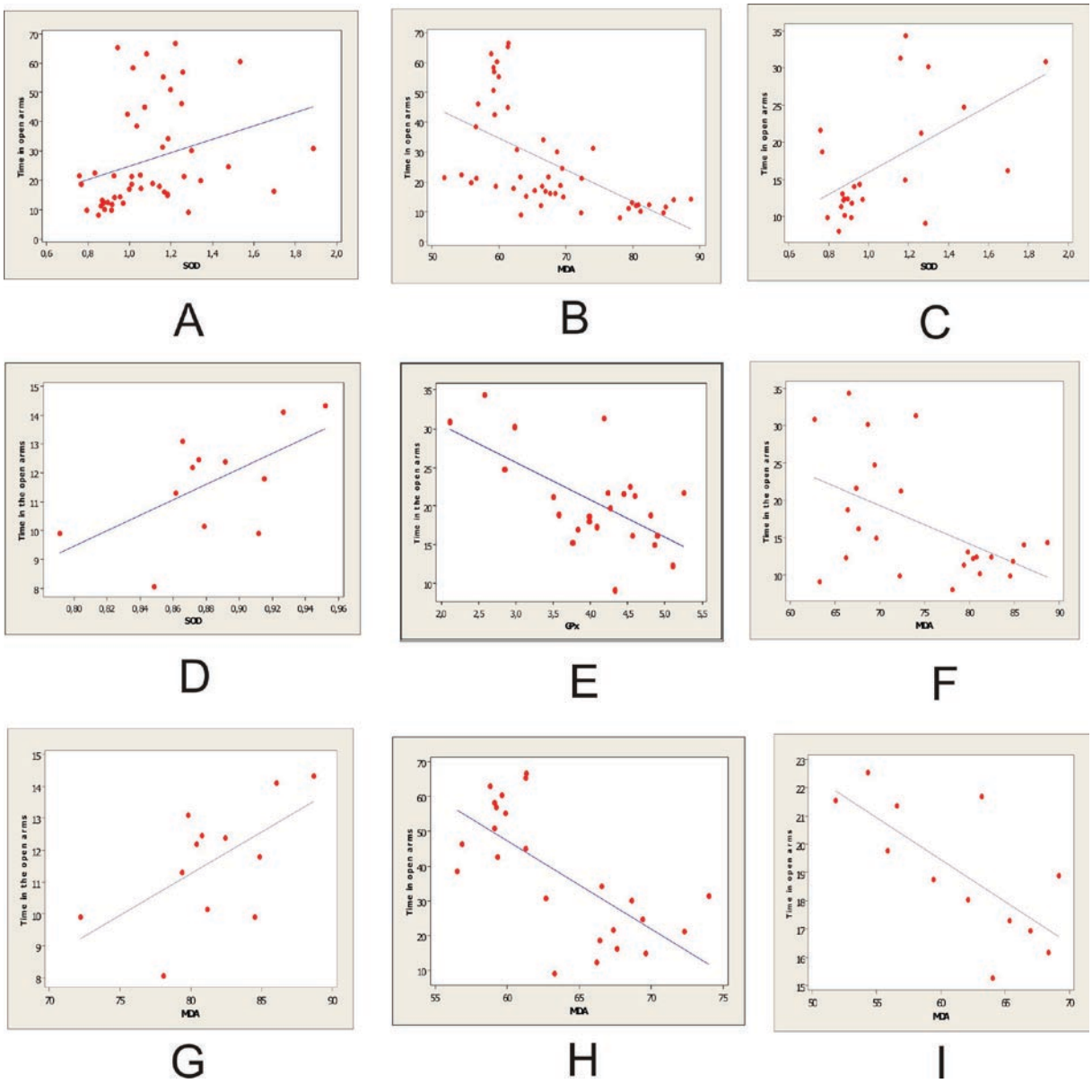


Post hoc analysis revealed also significant differences between angiotensin II and losartan groups (p< 0.0001) and between angiotensin II and PD-123177 groups (p< 0.0001) (Figure 4), while no significant differences between losartan and PD-123177 groups (p= 0.241) were found.

Interestingly, when we determined the linear regression between the time spent in the open arms of elevated plus maze vs. oxidative stress markers (for all groups), we found a significant negative correlation between time spent in the open arms vs. MDA levels (n=48, r = -0.577, p< 0.0001) (Figure 5A) and also a significant positive correlation between time spent in the open arms vs. SOD specific activity (n=48, r = 0.303, p=0.036) (Figure 5B).

When we determined this correlation individually for each group (losartan, PD-123177 and angiotensin II), together (n=24) or not (n=12) with the controls, we found a significant correlation between time spent in the open arms vs. SOD specific activity, only in the case of angiotensin II (n=24, r = 0.555, p=0.005) (Figure 5C)

Figure 5. A. Correlation between time spent in the open arms vs. MDA levels (n=48, r = -0.577, p< 0.0001); B. Correlation between time spent in the open arms vs. SOD specific activity (n=48, r = 0.303, p=0.036); C.-D. Correlation between time spent in the open arms vs. SOD specific activity, only in the case of angiotensin II (n=24, r = 0.555, p=0.005) (C), (n=12, r = 0.6, p=0.039) (D); E. Correlation between time spent in the open arms vs. GPx specific activity for PD-123177 (n=24, r = -0.634, p=0.001); F.-I. Correlation between time spent in the open arms vs. MDA levels for angiotensin II (n=24, r = -0.511, p=0.011) (F) (n=12, r = 0.6, p=0.039) (G), losartan (n=24, r = -0.689, p< 0.0001) (H) and PD-123177 (n=12, r = -0.721, p=0.07) (I).



(n=12, r = 0.6, p=0.039) (Figure 5D), but not for losartan and only a slightly connection in the case of PD-123177 (n=12, r = -0.511, p=0.05).

For the correlation between the time spent in the open arms vs. GPx specific activity, we surprisingly found some correlations for all the 3 groups analyzed separately (losartan - n=12, r = 0.5, p=0.06; PD-123177 - n=24, r = -0.634, p=0.001 (Figure 5E); and Ang II - n=12, r = -0.502, p=0.06), even though when we analyzed them altogether we failed to find any significant correlations (n=48, r = 0.184, p=0.212).

Regarding the correlations between time spent in the open arms vs. MDA levels, we manage to find strong significant correlations, especially in the case of angiotensin II (n=24, r = -0.511, p=0.011) (Figure 5F) (n=12, r = 0.6, p=0.039) (Figure 5G) and losartan (n=24, r = -0.689, p< 0.0001) (Figure 5H) (n=12, r = 0.565, p=0.05) and a slight connection in the case of PD-123177 (n=12, r = -0.721, p=0.07) (Figure 5I).

4. Discussion

The present study investigated the effects of angiotensin II and its receptor blockers icv administration on anxiety status and some oxidative stress biomarkers. Our results provide additional evidence of angiotensin II induced anxiety-like effects and increased prooxidant status. Moreover, the blockade of angiotensin II receptors by the administration of its specific antagonists, resulted in anxiolytic effects, in the case of losartan, and decreased lipid peroxidation levels, in the case of both losartan and PD-123177. In addition, we found a significant correlation between the time spent by rats in the open arms of the elevated plus maze and oxidative stress markers from the temporal lobe, which is known to be the most susceptible cortical area to reactive oxygen species [23].

Regarding the implication of RAS in anxiety-related behavior, anxiolytic properties of the AT1 receptor antagonist losartan were previously described, suggesting the anxiogenic potency of AT 1 blockade [5,10]. This was demonstrated by an increase of the time spent by experimental animals in the bright area of the light-dark aversion test or increased time spent in the open arms of elevated plus maze task [11,12]. Moreover, these effects were also observed in experimental situations which involved the administration of losartan in both Ang II-treated, as in control rats [13] or when losartan effects were compared to a positive control represented by diazepam [9,10].

However, recent knockout studies showed a reduction of exploratory activities and anxiety-like state in the case of AT 2 deficient mice, which was combined [5] or not [29,30] with locomotor deficiencies. Also, the increased anxiogenic behavioral state observed in AT 2 deficient mice was reversed by diazepam [15].

In contrast to the aforementioned results, some other studies failed to replicate these anxiolytic effects of losartan [14]. In this way, both losartan and PD-123177 were described in some reports as showing no effects in animal models of anxiety and memory [14].

These differences could be explained by different ways of administration, the strain of animal used, or the time of testing after drug administration [4,31]. In this way, if we analyze the time-dependent response, it was previously demonstrated using open-field behavior, that angiotensin II initially increases anxiety-like behavior (e.g. after 5 minutes), and then a rebound anxiolytic effect appears 15 minutes after administration [32]. This was also observed in the case of losartan, in a study combining elevated plus maze and defensive burying in mice, which revealed that losartan produced anxiolytic-like effects and Ang II anxiogenic-like responses 15 and 30 min after administration, but opposite responses during the first 15 min (e.g. losartan caused anxiogenic-like burying and Ang II suppressing burying) [33]. Moreover, the early anxiogenic-like effect of losartan was blocked by prior administration of the selective AT 2 receptor antagonist PD-123319 [4].

It seems that the peripheral administration of drugs that decrease Ang II function causes early anxiety-like behaviour, possibly AT 2 receptor mediated, and later anxiolytic-like effects.

In this way, a so called reciprocal inhibitory regulation between AT 1 and AT 2 receptors was described [34,35]. This assures modification of effects mediated from both receptor subtypes. For example, increased anxiety found in the AT 2 receptor deficient mice [15] might have been caused by unopposed AT 1 receptor rather than by lack of the AT 2 receptors [5]. In other words, considering that the anxiolytic effects of losartan are connected to the inhibition of AT 1 receptor, it seems that one possible explanation for the anxiety-like behavior in AT 2-deficient mice may be the activation of AT 1 receptors, as AT 2 receptors are absent in the brain [15].

These aspects collectively suggest that functional interactions between the two receptor types (AT 1 and AT 2) could have a key role in Ang II involvement in emotional responses.

Some authors also speculated that these aspects regarding Ang II-mediated emotional functions could be considered a possible cause of the discrepancies regarding the cognitive effects of Ang II [5], since both positive [36,37] and negative effects [38-40] of Ang II on learning and memory were reported.

In this way, the mechanisms of action for Ang II mediated effects on anxiety-like behavior are still unknown.

There is also increasing evidence that oxidative stress and consecutively the production of free radicals may play an important role in Ang II mediated effects [41]. In this way, experimental data demonstrated that increased reactive oxygen species generation through the activation of NAD(P)H oxidase is an obligatory step in Ang II effects [16,42]. It has also been reported that the inhibition of Ang II with specific receptor blockers like losartan or PD-123319, resulted in decreased oxidative stress status in the blood or glial cells [20,43]. This is generally expressed by an increase in activity of antioxidant enzymes like SOD or catalase and a decrease of some peroxidation products like malondialdehyde [20]. However, in our study we only reported a decrease of MDA levels, as a result of losartan and PD-123177 administration, while SOD and GPx specific activities did not changed significantly in comparison with the control

group. This could mainly be explained by the fact that a low oxidative stress status, as showed by decreased levels of lipid peroxidation, did not trigger any response change in the specific activity of the main antioxidant enzymes.

This could be an important aspect considering the implications of oxidative stress in different neuropsychiatric disorders and/or aging [44-46]. In this way, it was demonstrated that mice develop anxious behavior during aging [47], likely due to the accumulation of reactive oxygen species, which is a characteristic of the aging process in animals. It was also reported that the blockade of Ang II reduced anxiety and improved behavioral and motor performance in the aged rat [48] and that the administration of losartan results in a significant prolongation of life span in rats [42]. This protective effect could be related to the antioxidant action of Ang II receptor blockers and a reduced formation of reactive oxygen species.

However, there are still inconsistencies regarding the implications of Ang II specific receptors in oxidative stress, since there are reports describing no effects of PD-123319 in Ang II-induced oxidative stress [21] or consecutively DNA damage [49]. Moreover, the aforementioned effects were prevented by using AT 1 antagonists.

Also, in the present study we demonstrated a significant correlation between anxiety-related behavior and oxidative stress markers. A causal link between these two was also very recently demonstrated by several authors [50-54]. Moreover, different cell types studied in anxious mice showed a strong accumulation of intracellular ROS, in comparison to non-anxious mice, suggesting that oxidative stress is present in various cortex areas, as well as in hippocampus and cerebellum [55,56]. Thus, the existence of oxidative stress in the brain of anxious individuals could have deleterious effects and could lead to pathological anxiety, although a complete cause-effect relationship has not been established yet.

However, an important limitation of our study could be represented by the lack of some additional oxidative stress markers determinations like the glutathione reductase, reduced glutathione, xantine oxidase or carbonyl groups.

5. Conclusions

Our results showed that the inhibition of central Ang II by the icv administration of its specific antagonist receptors resulted in an alteration of anxiety state and a decreased level of lipid peroxidation in rats. Moreover, we demonstrated a significant correlation between the anxiety-related behavior and some central oxidative stress markers. These findings could raise important therapeutic aspects regarding the use of some Ang II receptor blockers in anxiety-related disorders. Additionally, oxidative stress could exert an important role in these effects.

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The authors will also like to dedicate this paper to the memory of Acad. Prof. Ion Haulica (1924-2010), the initiator or renin-angiotensin system studies in Romania and Eastern Europe.

References

[1] Von Bohlen und Halbach O, Albrecht D. The CNS rennin angiotensin system. Cell Tissue Res. 2006;326: 599- 616

[2] Haulica I, Bild W, Serban DN. Angiotensin peptides and their pleiotropic actions. J Renin Angiotensin Aldosterone Syst. 2005;6: 121-31

[3] Ciobica A, Bild W, Hritcu L, Haulica I. Brain renin-angiotensin system in cognitive function: pre-clinical findings and implications for prevention and treatment of dementia. Acta Neurol Belg. 2009;109: 171-80

[4] Gard PR. Angiotensin as a target for the treatment of Alzheimer's disease, anxiety and depression. Expert Opin Ther Targets. 2004;8: 7-14

[5] Braszko JJ, Kułakowska A, Winnicka MM. Effects of angiotensin II and its receptor antagonists on motor activity and anxiety in rats. J Physiol Pharmacol. 2003;54: 271-81

[6] Llorens-Cortes C, Mendelsohn FA. Organisation and functional role of the brain angiotensin system. J Renin Angiotensin Aldosterone Syst. 2002;3 suppl 1:39-48

[7] de Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T. International union of pharmacology. XXIII. The angiotensin II receptors. Pharmacol Rev. 2000;52: 415-72

[8] Hritcu L, Bild W, Ciobica A, Artenie V, Haulica I. Behavioral changes induced by angiotensin AT1

receptors blockade in the rat brain. Eur. Psychiatry. 2009;24 suppl 1:S859

[9] Barnes NM, Costall B, Kelly ME, Murphy DA, Naylor RJ. Anxiolytic-like action of DuP753, a non-peptide angiotensin II receptor antagonist. Neuroreport. 1990;1: 20-1

[10] Kaiser FC, Palmer GC, Wallace AV, Carr RD, Fraser-Rae L, Hallam C. Antianxiety properties of the angiotensin II antagonist, DUP 753, in the rat using the elevated plus-maze. Neuroreport. 1992;3: 922-4

[11] Cambursano PT, Haigh SJ, Keightley J, Sutcliffe MA, Gard PR. Positive effects of losartan in laboratory tests indicative of anxiolytic-like activity and the importance of animal strain. J. Pharm. Pharmacol. 1997;49 suppl. 4:64

[12] Srinivasan J, Suresh B, Ramanathan M. Differential anxiolytic effect of enalapril and losartan in normotensive and renal hypertensive rats. Physiol Behav. 2003;78: 585-91

[13] Kulakowska A, Karwowska W, Wisniewski K, Braszko JJ. Losartan influences behavioural effects of angiotensin II in rats. Pharmacol Res 1996;34: 109-115

[14] Shepherd J, Bill DJ, Dourish CT, Grewal SS, McLenachan A, Stanhope KJ. Effects of the selective angiotensin II receptor antagonists losartan and PD-123177 in animal models of anxiety and memory. Psychopharmacology (Berl). 1996;126: 206-18

[15] Okuyama S, Sakagawa T, Inagami T. Role of the angiotensin II type-2 receptor in the mouse central nervous system. Jpn J Pharmacol. 1999;81: 259-63

[16] Chabrashvili T, Kitiyakara C, Blau J, Karber A, Aslam S. Effects of ANG II type 1 and 2 receptors on oxidative stress, renal NADPH oxidase, and SOD expression. Am J Physiol Regul Integr Comp Physiol. 2003;285: R117-24

[17] Wang D, Chabrashvili T, Borrego L, Aslam S, Umans JG. Angiotensin II infusion alters vascular function in mouse resistance vessels: roles of O and endothelium. J Vasc Res. 2006;43: 109-19

[18] Laursen JB, Rajagopalan B, Galis Z, Tarpey M, Freeman BA, Harrison DG. Role of superoxide in angiotensin II induced but not catecholamine-induced hypertension. Circulation. 1997;95: 588–593

[19] Yao EH, Fukuda N, Matsumoto T, Kobayashi N, Katakawa M, Yamamoto C et al. Losartan improves the impaired function of endothelial progenitor cells in hypertension via an antioxidant effect. Hypertens Res. 2007;30: 1119-28

[20] Antelava NA, Gongadze NV, Gogolauri MI. Comparative characteristic of angiotensin-converting enzyme inhibitor-captopril and the angiotensin II receptor blokers--losartan action on the oxidative metabolism in experimental hyperlipidemia in rabbits. Georgian Med News. 2007;150: 57-60

[21] Yanagitani Y, Rakugi H, Okamura A, Moriguchi K, Takiuchi S, Ohishi M et al. Angiotensin II type 1 receptor-mediated peroxide production in human macrophages. Hypertension. 1999;33: 335-9

[22] Bouayed, J., Rammal, H., Younos, C., Soulimani, R., 2007b. Positive correlation between peripheral blood granulocyte oxidative status and level of anxiety in mice. Eur. J. Phar macol. 564, 146–149

[23] Karelson E, Bogdanovic N, Garlind A, Winblad B, Zilmer K et al. The cerebrocortical areas in normal brain aging and in Alzheimer's disease: noticeable differences in the lipid peroxidation level and in antioxidant defense. Neurochem Res. 2001;26: 353-61

[24] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 6th ed. San Diego: Academic Press Elsevier; 2006

[25] Ciobica A, Hritcu L, Padurariu M, Dobrin R, Bild V. Effects of serotonin depletion on behavior and neuronal oxidative stress status in rat: relevance for anxiety and affective disorders. Adv Med Sci. 2010;55: 289-296

[26] Ciobica A, Hritcu L, Artenie V, Stoica B, Bild V. Effects of 6-OHDA infusion into the hypothalamic paraventricular nucleus in mediating stress-induced behavioural responses and oxidative damage in rats. Acta Endocrinol. 2009;5: 425-36

[27] Hritcu L, Ciobica A, Gorgan L. Nicotine-induced memory impairment by increasing brain oxidative stress. Cent. Eur. J. Biol. 2009;4: 335-342

[28] Gurzu C, Artenie V, Hritcu L, Ciobica A. Prenatal testosterone improves the spatial learning and memory by protein synthesis in different lobes of the brain in the male and female rat. Cent Eur J Biol. 2008;3: 39-47

[29] Ichiki T, Labosky PA, Shiota C, Okuyama S, Imagawa Y, Fogo A et al. Effects on blood pressure and exploratory behaviour of mice lacking angiotensin II type-2 receptor. Nature. 1995;377: 748-50

[30] Okuyama S, Sakagawa T, Chaki S, Imagawa Y, Ichiki T, Inagami T. Anxiety-like behavior in mice lacking the angiotensin II type-2 receptor. Brain Res. 1999;821: 150-9

[31] Gard PR, Haigh SJ, Cambursano PT, Warrington CA. Strain differences in the anxiolytic effects of

losartan in the mouse. Pharmacol Biochem Behav. 2001;69: 35-40
[32] Georgiev V, Getova D, Opitz M. Mechanisms of the angiotensin II effects on the exploratory behavior of rats in open field. I. Interaction of angiotensin II with saralasin and catecholaminergic drugs. Methods Find Exp Clin Pharmacol. 1987;9: 297-301
[33] Cresswell AG, Gard PR: Behavioural evidence of a paradoxical anxiogenic effect of an angiotensin II (AT1) receptor antagonist. J. Pharm. Pharmacol. 1998;50 suppl 1:215
[34] Peng JF, Phillips MI. Opposite regulation of brain angiotensin type 1 and type 2 receptors in cold-induced hypertension. Regul Peptides. 2001;97: 91-102
[35] Gelband CH, Zhu M, Lu D, Reagan LP, Fluharty SJ, Posner P et al. Functional interactions between neuronal AT1 and AT2 receptors. Endocrinology. 1997;138: 2195-2198
[36] Braszko JJ. AT(2) but not AT(1) receptor antagonism abolishes angiotensin II increase of the acquisition of conditioned avoidance responses in rats. Behav Brain Res. 2002;131: 79-86
[37] Bild W, Hritcu L, Ciobica A, Artenie V, Haulica I. Comparative effects of captopril, losartan and PD-123319 on the memory processes in rats. Eur. Psychiatry. 2009;24 suppl 1:S860
[38] Bonini JS, Bevilaqua LR, Zinn CG, Kerr DS, Medina JH. et al. Angiotensin II disrupts inhibitory avoidance memory retrieval. Horm Behav. 2006;50: 308-13
[39] Kerr DS, Bevilaqua LR, Bonini JS, Rossato JI, Kohler CA, Medina JH. et al. Angiotensin II blocks memory consolidation through an AT2 receptor dependent mechanism. Psychopharmacology (Berl). 2005;179: 529-535
[40] Ciobica, W. Bild, I. Haulica, L. Hritcu, O. Arcan. Effects of angiotensin II, its receptor antagonists and captopril on cognitive functions and oxidative stress in rats. J. Neurol. 2009;256 suppl 2:194
[41] Ciobica A. Bild W. Hritcu L. Artenie V. Haulica I. The importance of oxidative stress in angiotensin II-mediated effects on cognitive functions. Neuropeptides. 2009;43: 420-421
[42] Basso N, Cini R, Pietrelli A, Ferder L, Terragno NA. Protective effect of long-term angiotensin II inhibition. Am J Physiol Heart Circ Physiol. 2007;293: 1351-8
[43] Holownia A, Braszko JJ. The effect of angiotensin II and IV on ERK1/2 and CREB signalling in cultured rat astroglial cells. Naunyn Schmiedebergs Arch Pharmacol. 2007;376: 157-63
[44] Padurariu M, Ciobica A, Hritcu L, Stoica B, Bild W., Stefanescu C. Changes of some oxidative stress markers in the serum of patients with mild cognitive impairment and Alzheimer's disease. Neurosci Lett. 2010;469: 6-10
[45] Padurariu M, Ciobica A, Dobrin I, Stefanescu C. Evaluation of antioxidant enzymes activities and lipid peroxidation in schizophrenic patients treated with typical and atypical antipsychotics. Neurosci Lett. 2010;479: 317-20
[46] Hritcu L, Ciobica A, Artenie V. Effects of right-unilateral 6-hydroxydopamine infusion-induced memory impairment and oxidative stress: Relevance for Parkinson's disease. Cent Eur J Biol. 2008;3: 250-257
[47] Berry A, Capone F, Giorgio M, Pelicci PG, de Kloet ER. Deletion of the life span determinant p66Shc prevents age-dependent increases in emotionality and pain sensitivity in mice. Exp Gerontol. 2007;42: 37-45
[48] Basso N, Altamirano S, Terragno NA, Ferder L, Inserra F. et al. Inhibition of the renin–angiotensin system improves spatial working memory in the senile normal rat. J Hypertens. 2002;20 suppl. 4:S134
[49] Schupp N, Schmid U, Rutkowski P, Lakner U, Kanase N, Heidland A, Stopper H. Angiotensin II-induced genomic damage in renal cells can be prevented by angiotensin II type 1 receptor blockage or radical scavenging. Am J Physiol Renal Physiol. 2007 May;292(5):F1427-34
[50] Machado-Vieira R, Salvadore G, DiazGranados N, Ibrahim L, Latov D, Wheeler-Castillo C, Baumann J, Henter ID, Zarate CA Jr. New therapeutic targets for mood disorders. ScientificWorldJournal. 2010 Apr 13;10:713-26
[51] Behl A, Swami G, Sircar SS, Bhatia MS, Banerjee BD. Relationship of possible stress-related biochemical markers to oxidative/antioxidative status in obsessive-compulsive disorder. Neuropsychobiology. 2010;61(4):210-4
[52] Matsushita M, Kumano-Go T, Suganuma N, Adachi H, Yamamura S, Morishima H, Shigedo Y, Mikami A, Takeda M, Sugita Y. Anxiety, neuroticism and oxidative stress: cross-sectional study in non-smoking college students. Psychiatry Clin Neurosci. 2010 Aug;64(4):435-41
[53] Salim S, Sarraj N, Taneja M, Saha K, Tejada-Simon MV, Chugh G. Moderate treadmill exercise prevents oxidative stress-induced anxiety-like behavior in rats. Behav Brain Res. 2010 Apr 2;208(2):545-52
[54] Gingrich JA. Oxidative stress is the new stress. Nat Med. 2005;11(12):1281-2

[55] Bouayed J, Rammal H, Soulimani R. Oxidative stress and anxiety: Relationship and cellular pathways. Oxid Med Cell Longev. 2009;2(2):63-7

[56] Rammal H, Bouayed J, Younos C, Soulimani R. Evidence that oxidative stress is linked to anxiety-related behaviour in mice. Brain Behav Immun. 2008;22(8):1156-9