ROLE OF THE ANGIOTENSIN II RECEPTORS IN THE STRESS-INDUCED VISCERAL ANTINOCICEPTION

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ABSTRACT

Introduction: Angiotensin II (ANG II) is defined as stress-related neuropeptide, with a significant role in maintaining of blood pressure and water-salt balance but several studies showed that it participates also in stress-induced changes in nociception. We aimed to study the effects of ANG II and its selective AT_1 and AT_2 receptor antagonists on the restraint stress-induced changes in nociception.

Materials and methods: Visceral model writhing test was usedin miceexposed to 2 hoursrestraintstress (RS).Visceral pain reactions (writhes)were induced by intraperitoneally injected 1 % acetic acid.ANG II, AT_1 receptor antagonist losartan and AT_2 receptor antagonist PD123319 were injected intracerebroventricularly.

Results: Exposure to acuteRS decreased significantly the number of writhes. Injection of ANG II, AT_1 receptor antagonist losartan or AT_2 receptor antagonist PD123319 alone were not able to change stress-induced antinociception (SIA). The antinociceptive effect of RS was augmented significantly by ANG II only when AT_2 receptors were blocked.

Conclusion: These data supported the assumption that brain angiotensin receptors take a part in the processing of information from visceral nociceptors. In spite of significant role of AT_2 receptors in the regulation of nociception under normal condition, our data showed an impact of AT_1 receptor activation on the nociception in stress conditions.

Key words*: restraint stress, visceral nociception, mice, angiotensin receptors, losartan*

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Introduction

Life exists by maintaining a complex dynamic equilibriumor homeostasis that is constantly challenged byintrinsic or extrinsic adverse forces, the stressors [7]. Pacák and Palkovits (2001) define stress as a state of threatened homeostasis [21]. During stress, a wide range of behavioral and physiological adaptive responses of the organism is activated to sustain homeostasis. These compensatory adaptive stress responses in all vertebrates are the activation of the hypothalamic-pituitary-adrenal and sympatho-medullo-adrenal axis. Acute restraint stress (RS) induces anopioid-dependent and catecholamine-mediated antinociception in different experimental models [18].

Considerable evidence supports an important role for the brain renin–angiotensin system (RAS) in the control of stress-induced physiologies [2, 35]. The decapeptide ANG I formed by the protease renin acting upon the amino terminal of angiotensinogen, serves as a substrate for angiotensin converting enzyme to form ANG II [16]. ANG II is an active ligand and performs its functions by the AT_1 and AT_2 receptor subtypes [1, 9, 12, 19, 28, 29].

Although the main effects of ANG II are related to the well-known central actions as maintenance of salt and volume homeostasis, blood pressure control, release of pituitary hormones, and control of behavioural responses like thirst and drinking [33, 34], ANG II is also defined as stress-related neuropeptide with expressed effects on the nociception. Several studies showed that it exerted antinociceptive effects in different experimental pain models [13, 28] and participates in stress-induced

changes in nociception [23]. ANG II injected intracerebroventricularly (icv) showed an opioid-dependent antinociception [17]. Recently, we have shown that selective AT_1 receptor blocking was able to diminished chronic restraint stress-induced antinociception (SIA) [23].

Although the AT_1 receptor antagonists, in particular losartan, are widely used as antihypertensive drugs, no attention has been directed to the interaction between restraint stress, ANG II and nociceptive abdominal constriction test. Therefore, the aim of our study was to study the effects of ANG and its selective AT_1 and AT_2 receptor antagonists, losartan and PD123319, on the restraint stress-induced changes in nociception.

Material and methods

Animals and housing: The experiments were carried out on male albino mice ICR strain (18–20 g) bred in an air-conditioned room at a temperature of 24 ± 1^{o}C with food and water available ad libitum except during the experiments. All tests were conducted between 09:00 and 12:00 h.

The experimental procedures were approved by local ethic committee of Institute of Neurobiology, Bulgarian Academy of Sciences which are fully in accordance with EC Directive 2010/63/EU for animal experiments.

Drugs and treatment: Acetic acid diluted with distilled water to a concentration of 1% was administered intraperitoneally (ip). Selective AT_1 receptor antagonist losartan was kindly gifted by Merck & Co. Inc. AT_2 receptor antagonist PD123319 and ANG II were obtained by Sigma-Aldrich, dissolved in saline and injected (icv) in a total volume of 5 µl/mouse (2.5 µl/per side in combination of two drugs). The injections were given free hand directly into the lateral cerebral ventricle of conscious mice [16] using Hamilton microsyringe with total volume of 10 µl. The injection coordinates were 3 mm caudal to the right coronary suture and 2.5 mm lateral to the midline into a depth of 3 mm from the scalp. The equivalent volume of vehicle was administered to the control groups.

Animals were randomly divided into seven groups: 1. Control group injected with saline; 2. RS group injected with saline; 3.ANG II (0.1 µg/mouse) injected 15 minutes before RS; 4. Losartan (50 µg/mouse) injected 15 minutes before RS; 5.PD123319 (10 µg/mouse) injected 30 minutes before RS; 6. Losartan injected 5 min before ANG II and 20 minutes before RS; 7. PD123319injected 15 minutes before ANG II and30 minutes before RS. Each group consisted of 10 to 12 mice.

Acute model of restraint stress (RS): The mice were placed for 2 h in plastic tubes (25 mm inner diameter and 10 cm long) with suitable ventilation at one end and with the other side closed off by plastic tape so that they were unable to move.

Acetic acid-induced abdominal constriction test: Immediately after injection of the 1%acetic acid (10 ml/kg, ip), the mice were placed in individual cages and the number of specific abdominal constrictions (writhes) of each mouse was countedat 5-min intervals for 30 minutes. The mice with decreased number of writhes were considered protected by the test agent [8].

Statistical analysis: The data were analyzed by a multifactor analysis ofvariance (ANOVA), followed by the Bonfferoni post hoc test. All values are presented as mean ± standard error of the mean (SEM). Differences between the experimental groups were considered to be significant if $P \leq 0.05$.

Results

Exposure to 2h RS produced a significant antinociceptive effect (SIA) on visceral reactions during the whole period of observation [$t = 14.822$, $p < 0.001$] vs control group (Fig. 1, 2,3). Icv infused ANG II 15 min before RS only temporary diminished SIA on 10thmin after the injection of the irritant [$t = 2.098$, $p < 0.05$], while AT_1 receptor antagonist losartan showed significant and more pronounced

anti-SIA effect [t = 5.261, p <0.001] (Fig. 1).

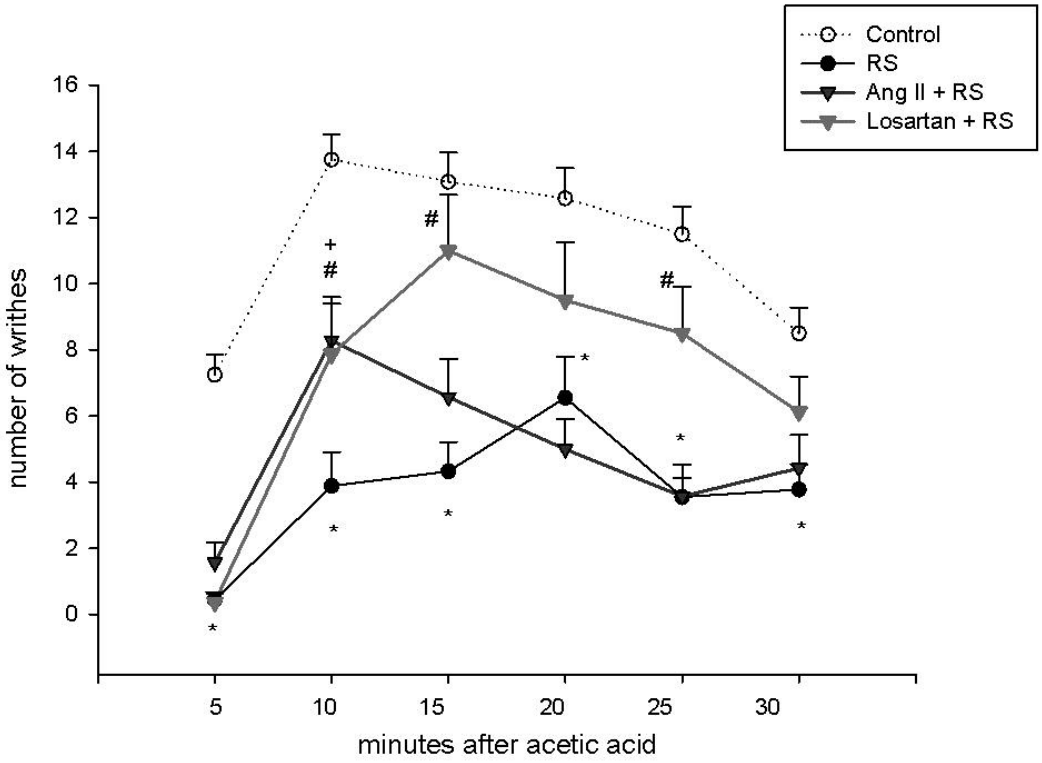

*Fig. 1. Time course for the effects of 2 hours restraint stress (2h RS), ANG II (0.1 µg/mouse, icv) and losartan (50 µg/mouse, icv) on the writhing responses induced by 1% aceticacid (1 ml/kg, ip). The number of abdominal constrictions (mean ± SEM) was measured for 30 min. *P < 0.05 vs. control; #P < 0.05 losartan + RS vs RS; P < 0.05 of + ANGII + RS vs RS.*

AT_2 receptor antagonist PD 123319 injected 30 min before RS produced a short-term anti-SIA effect at the beginning of the observation (first 15 min) and further did not influencedsignificantly the effect of restraint stress [t = 1.519, p > 0.05] (Fig.2).

Pretreatment with a selective AT_1 receptor antagonist losartan augmented significantly the anti-SIA effect of ANG II [t = 4.390, p<0.001], while pretreatment with selective AT_2 receptor antagonist PD 123319 did not influenced significantly the ANG II-induced SIA diminishment [t = 1.510, p > 0.05]. PD even abolished ANG II-induced effect on 10^{th}min after the beginning of observation (Fig.3).

Discussion

Stress-induced antinociception or SIA is an in-built mammalian pain-suppression response that occurs during or following exposure to a stressful or fearful stimulus [5]. Several factors have been reported to induce analgesia due to stress. One of them is immobilization which evokes the orchestrated interplay of several neurotransmitter systems in the brain underlies the characteristic phenomenology of behavioral, endocrine, autonomic and immuneresponses to stress. Among these transmitters are corticotropin-releasing hormone (CRH), arginine-vasopressin (AV), adrenocorticotrophin hormone (ACTH), opioid peptides, catecholamines (dopamine, adrenaline, noradrenaline) [10, 30]. The present

results emphasized the impact of acute restraint stress, similarly to another type of stressful stimuli, on the peripheral visceral nociception [17].

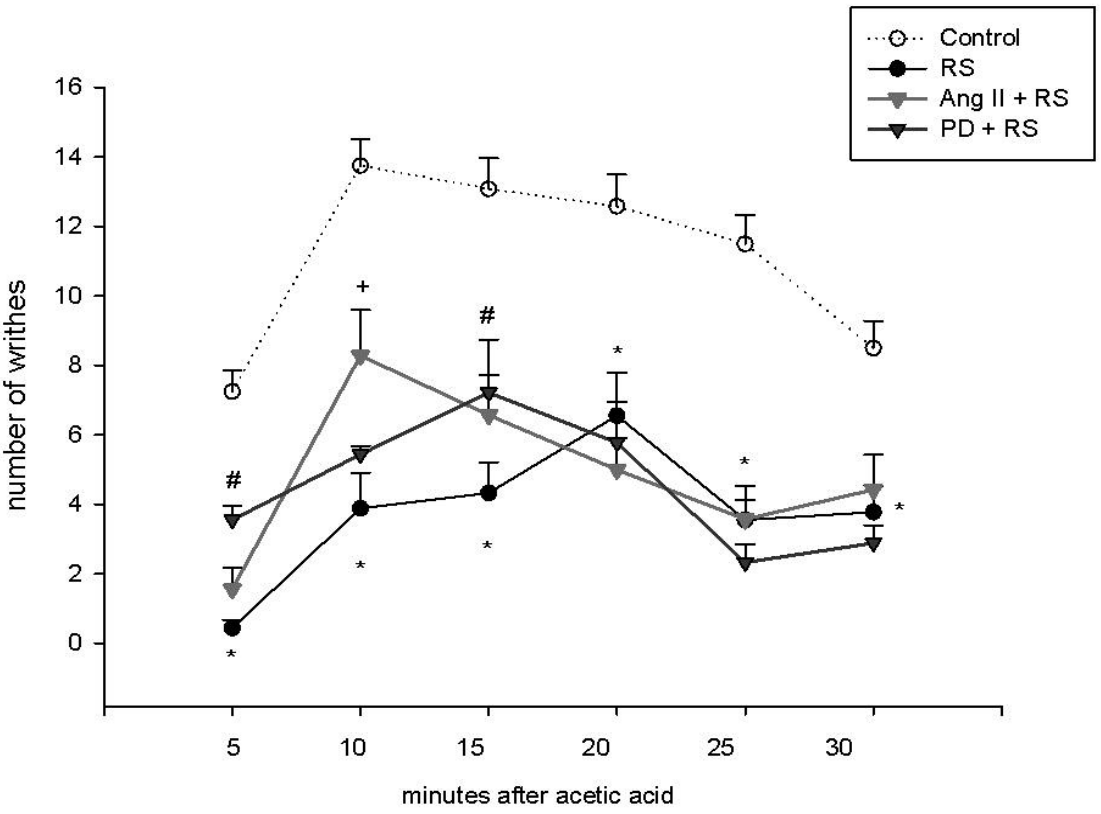

*Fig. 2. Time course for the effects of 2 hours restraint stress (2h RS), ANG II (0.1 µg/mouse, icv) and PD 123319 (10 µg/mouse, icv) on the writhing responses induced by 1% aceticacid (1 ml/kg, ip). The number of abdominal constrictions (mean ± SEM) was measured for 30 min. *P < 0.05 vs. control; #P < 0.05 of PD + RS vs RS; + P < 0.05 of ANGII + RS vs RS.*

Considerable evidence supports an important role of the brain RAS in the control of stress-induced physiologies. The application of stressors elevated circulating and brain levels of renin and ANG II [26, 35]. Stress also up-regulates the expression of AT_1 receptors both within the PVN where CRH cell bodies are located [6, 31], and in the anterior pituitary [17]. During the stress, synthesized within the anterior pituitary ANG II facilitates release of ACTH [11]. Thus, stress-induced upregulation of PVN AT_1 receptors appears to provoke CRH synthesis that precludes the facilitation of ACTH release and elevated adrenal corticoid secretion. Short periods of isolation stress have been shown to elevate AT_1 receptor expression in the PVN, along with correlated elevations in pituitary ACTH, adrenal corticosterone, catecholamines, and aldosterone. Nishimura et al. (2000) have shown that peripheral treatment with the AT_1 receptor antagonist, candesartan, decreased AT_1 receptor binding following isolation both in the anterior pituitary and adrenal glands, and in the PVN. This treatment also interfered with expected elevations of pituitary ACTH and adrenal corticosterone [21].

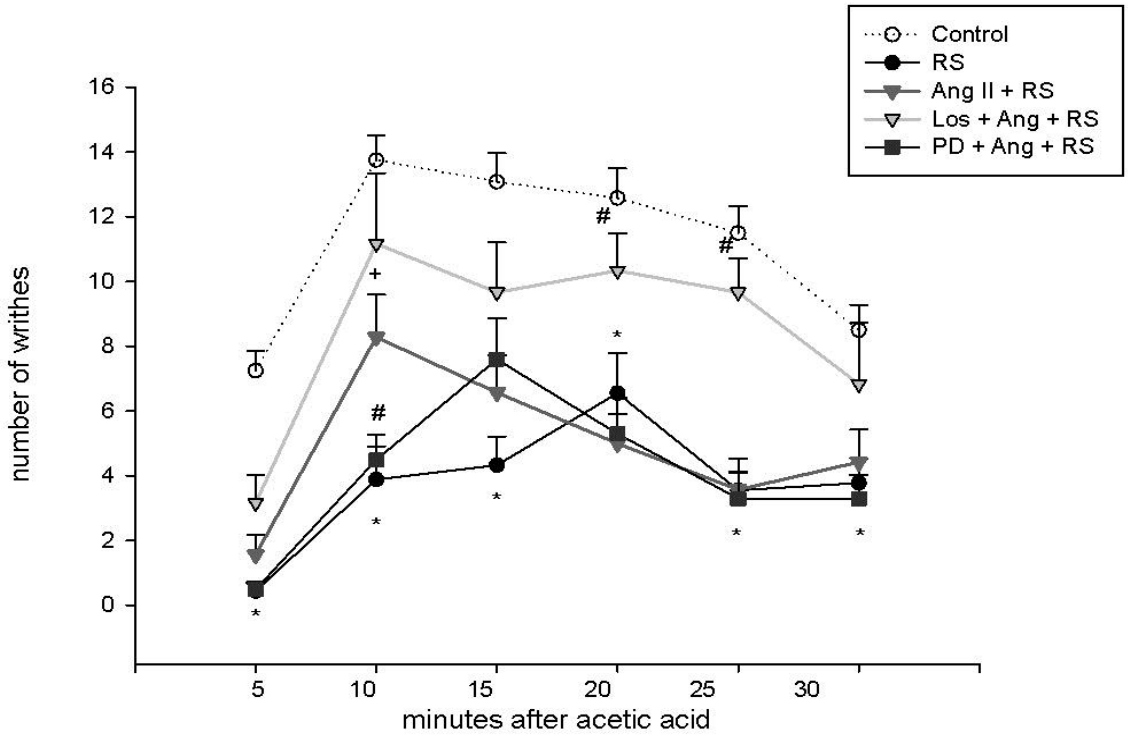

*Fig. 3. Time course for the effects of 2 hours restraint stress (2h RS), combinations of Losartan + ANG II and PD 123319 + ANG II on the writhing responses induced by 1% aceticacid (1 ml/kg, ip). The number of abdominal constrictions (mean ± SEM) was measured for 30 min. *P < 0.05 vs. control; #P < 0.05 vs ANG+RS.*

Another putative mechanism by which ANG II participate in SIA is opioidergic. The antinociceptive effect of both ANG and RS was successfully abolished by the systemic injection of AT_1 receptor antagonist losartan and opioid antagonist naloxone [27]. Recently, we have shown that chronic RS-induced antinociception also is diminished by systemically injected losartan in model of phasic pain [23]. Our present data support the theory that the octapeptide Ang II takes a part in visceral stress-induced antinociception and its effect is related mainly to activation of AT_1 receptor type. This statement is supported by the demonstrated anti-SIA effects of losartan which was augmented with additional injection of exogenous Ang II. The selective blocking of AT_2 receptor by PD 123319 had negligible effect on SIA both, when it was injected alone and also in combination with Ang II.

Our previous data showed that ANG II exert significant antinociceptive effect in writhing test, that was due to activation of AT_2 receptor subtype and blocking of AT_1 receptors additionally enhance this effect perhaps by removal of the inhibitory interactions between two receptors [13]. These data were further enriched by established antinociceptive effect of a selective AT_2 receptor agonist CGP 42112A in another acute pain model [24]. We can explain different action of AT_1 and AT_2 receptor subtypes with their different distribution in brain regions, and their different intracellular mechanism of action often related to opposite physiological effects. Taken together, we might suggest that brain ANG II plays a complex role in the regulation of visceral pain, and modulates different systems responsible for SIA.

Comparing the present data with our previous studies, we can assume that under normal conditions AT_2 receptors are predominantly important for visceral nociception. However the acute physical stress activated other mechanisms in which the AT_1 receptors have a greater role.

Acknowledgements: This work was supported by European Commission through the COPERNICUS programme, contact No. CIPA CT 94-0239, and by Grant VFP-L-6 from the National Fund Scientific Research at the Bulgarian Ministry of Education and Science.

References

1. Allen, A.M., B.J. Oldfield, M.E. Giles, G. Paxinos, M.J. McKinley, F.A. Mendelsohn, 2000. Localization of angiotensin receptors in the nervous system. In: Quirion, R., A. Bjorklund, T. Hodfelt, (Eds.), Handbook of Chemical Neuroanatomy. Elsevier, Amsterdam, pp. 79–124.
2. Armando, I., A. Seltzer, C. Bregonzio, J.M. Saavedra, 2003. Stress and angiotensin II: novel therapeutic opportunities. Curr Drug Targets - CNS Neurol Dis, 2, 413-419.
3. Armario, A., 2006. The hypothalamic-pituitary-adrenal axis: what can it tell us about stressors?CNS Neurol Disord Drug Targets, 5(5), 485-501.
4. Bocheva, A., E. Dzambazova, R.Hadjiolova, L.Traikov, R.Mincheva, I. Bivolarski, 2008.Effect of Tyr-MIF-1 peptides on blood ACTH and corticosterone concentration induced by three experimental models of stress. Auton Autacoid Pharmacol, 28(4), 117-123.
5. Butler RK, Finn DP (2009). Stress-induced analgesia. Prog Neurobiol, 88(3), 184-202.
6. Castren, E., Saavedra, J.M., 1988. Repeated stress increases the density of angiotensin II binding sites in rat paraventricular nucleus and subfornical organ. Endocrinology, 122, 370–372.
7. Chrousos, G.P., P.W. Gold, 1992. The concepts of stress system disorders:overview of behavioral and physical homeostasis. JAMA, J Am Med Assoc, 267, 1244-1252.
8. Collier, H.O.J., L.C. Dinneen, C.A. Johanson, C. Schneider, 1968. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmacol Chemother, 32, 295-310.
9. deGasparo, M., K.J. Catt, T. Inagami, J.W. Wright, T. Unger, 2000. International Union of Pharmacology. XXIII. The angiotensin II receptors. Pharmacol Rev, 52, 415–472.
10. Dzhambazova, E., B. Landzhov, A. Bocheva, A. Bozhilova-Pastirova, 2011. Effects of D-kyotorphinon nociception and NADPH-d neurons in rat's periaqueductal gray after immobilization stress. Amino acids, 41(4), 937-944.
11. Ganong, W.F., K. Murakami, 1987. The role of angiotensin II in the regulation of ACTH secretion. Ann N Y Acad Sci, 512, 176–186.
12. Gard, P.R., 2002. The role of angiotensin II in cognition and behaviour. Eur J Pharmacol, 438, 1-14.
13. Georgieva, D., V.Georgiev, 1999. The role of angiotensin II and of its receptor subtypes in the acetic acid-induced abdominal constriction test. Pharmacol Biochem Behav, 62(2), 229-232.
14. Haley, T.J., W.G.McCormick, 1957. Pharmacological effects produced by intrace-rebral injection of drugs in the conscious mouse. Br J Pharmacol Chemother, 12(1), 12-15.
15. Haulica, I., C. Neamtu, A. Stratone, G. Petrescu, D. Branisteanu, V. Rosca, S. Slatineanu,1986. Evidence for the involvement of cerebral renin–angiotensin system (RAS) in stress analgesia. Pain, 27, 237-245.
16. Johnston, C.I., 1990. Biochemistry and pharmacology of the renin–angiotensin system. Drugs, 39,

21–31.
17. Leong, D.S., J.A. Terron, A. Falcon-Neri, I. Armando, T. Ito, O. Johren, L.H. Tonelli, K.L. Hoe, J.M. Saavedra, 2002. Restraint stress modulates brain, pituitary and adrenal expression of angiotensin II $AT_{(1A)}$, $AT_{(1B)}$ and $AT_{(2)}$ receptors. Neuroendocrinology, 75, 227-240.
18. Lewis, J.W., J.T. Cannon, J.C. Liebeskind, 1980. Opioid and non-opioid mechanisms of stress analgesia. Science, 203, 623-625.
19. McKinley, M.J., A.L. Albiston, A.M. Allen, M.L. Mathai, C.N. May, R.M. McAllen, B.J. Oldfield, F.A.O. Mendelsohn, S.Y. Chai, 2003. The brain renin-angiotensin system: location and physiological roles. Int J Biochem Cell Biol, 35, 901-918.
20. Menendez L, F. Andrés-Trelles, A. Hidalgo, A. Baamonde, 1994. Gender and test dependence of a type of kappa mediated stress induced analgesia in mice. Gen Pharmacol, 25(5), 903-908.
21. Nishimura, Y., T. Ito, K.L. Hoe, J.M. Saavedra, 2000. Chronic peripheral administration of the angiotensin II AT(1) receptor antagonist candesartan blocks brain AT(1) receptors. Brain Res, 871, 29-38.
22. Pacák, K., M. Palkovits, 2001. Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. Endocr Rev, 22(4), 502-548.
23. Pechlivanova, D.M., A.G. Stoynev, J.D. Tchekalarova, 2011. The effects of chronic losartan pretreatment on restraint stress-induced changes in motor activity, nociception and pentylenetetrazol generalized seizures in rats. Folia Med (Plovdiv), 53(2), 69-73.
24. Pechlivanova, D.M., P.P. Markova, D. Popov, A.G. Stoynev, 2013. The role of the angiotensin AT2 receptor on the diurnal variations of nociception and motor coordination in rats. Peptides, 39, 152-156.
25. Pelegrini-da-Silva, A., A.R. Martins, W.A. Prado, 2005. A new role for the rennin-angiotensin system in the rat periaqueductal gray matter: angiotensin receptor-mediated modulation of nociception. Neuroscience, 132, 453-463.
26. Peng, J., M.I. Phillips, 2001. Opposite regulation of brain angiotensin type 1 and type 2 receptors in cold-induced hypertension. Regul Pept, 97, 91–102.
27. Raghavendra V., K. Chopra, S.K. Kulkarni, 1999. Brain renin angiotensin system (RAS) in stress-induced analgesia and impaired retention. Peptides, 20, 335–342.
28. Speth, R.C., S.M. Thompson, S.J. Johns, 1995. Angiotensin II receptors: structural and functional considerations. In: Mukhopadhyay, A.K., Raizada, M.K. (Eds.), Current Concepts: Tissue Rennin Angiotensin Systems as Local Regulators in Reproductive and Endocrine Organs. Plenum Press, New York, pp. 169-192.
29. Thomas, W.G., F.A.O. Mendelsohn, 2003. Molecules in focus: angiotensin receptors: form and function and distribution. Int J Biochem Cell Biol, 35,774-779.
30. Tsigos, C., G.P. Chrousos, 2002. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J Psychosom Res, 53(4), 865-871.
31. Wright, J.W., B.J. Yamamoto, J.W. Harding, 2008. Angiotensin receptor subtype mediated physiologies and behaviors: new discoveries and clinical targets. Prog Neurobiol, 84(2), 157-81.
32. Wright, J.W., J.W. Harding, 1992. Regulatory role of brain angiotensins in the control of physiological and behavioral responses. Brain Res Rev, 17, 227-262.
33. Wright, J.W., J.W. Harding, 1995. Brain angiotensin receptor subtypes AT_1, AT_2 and AT_4 and their functions. Regul Pept, 59, 269-295.

34. Wright, J.W., J.W. Harding, 2004. The brain angiotensin system and extracellular matrix molecules in neural plasticity, learning, and memory. Prog Neurobiol, 72(4), 263-293.
35. Yang, G., Y. Wan, Y. Zhu, 1996. Angiotensin II - an important stress hormone. Biol Signals 5, 1-8.