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EFFECTS OF LOSARTAN INFUSED INTO HIPPOCAMPAL CA1 AREA ON EXPLORATORY BEHAVIOUR OF RATS

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Abstract

The effects of the specific antagonist of angiotensin II (Ang II) type I (AT1) receptors losartan, infused uni- or bilaterally into the hippocampal CA1 area of male Wistar rats, on exploratory behaviour were examined. Microinjected bilaterally at a dose of 100 μg, losartan significantly decreased the horizontal and vertical movements (Opto Varimex apparatus). Microinjections of losartan into the left CA1 area suppressed the exploratory activity, while right-side losartan administration did not affect it, compared to the respective controls. The effect was more pronounced when losartan was infused into the left-side as compared to the right hippocampal CA1 area. The main finding was the presence of hippocampal asymmetry in exploratory behaviour to unilateral microinjections of losartan depending on the microinjected hemisphere, suggesting different distribution of AT1 receptors in left and right CA1 hippocampal area.

Key words: losartan, AT1 receptors, hippocampus, exploratory behaviour, rat

Introduction. The brain renin-angiotensin system (RAS) exists independently from the peripheral system. Recently, it has been generally accepted that the brain RAS with its bioactive peptides, which mainly include angiotensin II (Ang II), is involved not only in cardiovascular functions and body fluid homeostasis [1], but also in the control of some superior functions involving the regulation of cognitive functions (learning and memory processes) [2, 3] emotional responses [3], and also nociception [4]. These effects are mediated by specific angiotensin receptors. Four receptor subtypes have been proposed within the RAS: the Ang

II type 1 and 2 receptors (AT1, AT2), an Ang IV-specific receptor (AT4), and a putative Ang-(1–7)-selective receptor. The heptapeptide Ang-(1–7) can be generated from Ang I through angiotensin converting enzyme (ACE) independent pathways. There are three recognised angiotensin receptor subtypes [5]: two structurally similar and a third that is different. The AT1 and AT2 subtypes are G-protein coupled receptors. In contrast, the AT4 subtype is a much larger protein insensitive to guanine nucleotides, suggesting that it is not G-protein-linked [6].

Ang II receptors (AT1, AT2 and AT4) may be involved in mediating the effects of Ang II in brain. It is known that the concentration of Ang II and the expression of its different receptor subtypes are particularly high in the hippocampus [7, 8], a subcortical structure involved in the consolidation and retrieval of different types of memories [9–11].

In the CA1 region of the hippocampus, Ang II directly excites pyramidal neurons [12]. Ang II is full agonist at the AT1 and AT2 receptor subtypes in accordance with the nomenclature (Guide to Receptors and Channels).

Recently, it has been reported that orally administered losartan (specific antagonist of the Ang II type I (AT1) receptors) can suppress the enhancing effect of voluntary running on cell proliferation in the rat hippocampus [13].

Our previous studies showed behavioural asymmetries in locomotor-exploratory activity, anxiety, learning, and memory, following unilateral infusions of Ang II into the CA1 hippocampal area [14, 15]. Having in mind the different and asymmetric effects on the exploratory behaviour of rats, observed after unilateral microinjection of Ang II in the hippocampus CA1 area [14, 15], of particular interest to us was whether the AT1 receptor is involved in this lateralisation.

The aim of the present study was to examine the involvement of AT1 receptors in exploratory behaviour after unilateral and bilateral topical application into hippocampal CA1 area in rats. In the experiments, we used the specific angiotensin II type 1 (AT1) receptor antagonist losartan, infused uni- or bilaterally into the hippocampal CA1 area.

Materials and methods. ***Animals.*** The experiments were carried out on male Wistar rats (200–240 g at the time of surgery). The experiments were performed according to the *"Rules for care and experiments on laboratory animals"* of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences.

Stereotaxic implantation and drug injection into the CA1 hippocampal area. After anaesthesia (Calypsol 50 mg/kg i.p.), the rats were placed in a stereotaxic apparatus (Stoelting, USA) and guide cannulas (right and left) were implanted into the CA1 area, according to the coordinates of the stereotaxic atlas of Pellegrino and Cushman [16] ($P = 4.3$ mm, $L = \pm 2.0$ mm, $h = -3.0$ mm). After surgery, the animals were allowed 7 days to recover before the behavioural studies. During the recovery period, the rats were handled daily.

Losartan (Sigma) was dissolved in saline and 1 µl of the losartan solution (pH 7.4) was microinjected into the CA1 hippocampal area at a dose of 100 µg. Losartan or saline were injected through injection cannula over a period of 1 min and it was left in place for additional 30 s. The rats were injected into the CA1 area every third day either with losartan or with 1 µl saline (randomly selected), i.e. each rat received 6 microinjections. Prior to sacrificing, the animals were injected through the injection cannula with 1 µl 2% Fastgreen dye. Injection sites were histologically verified post-mortem in 25 µm coronal brain sections. Animals with misplaced or asymmetrical cannulae and dye-diffusion beyond the CA1 area were excluded.

Exploratory behaviour. Exploratory activity was recorded in an Opto Varimex apparatus (Columbus Instruments, USA) with experimental chamber 50 cm × 50 cm × 25 cm. This apparatus records the number of photo-beam interruptions during the movements of the animal. It provides selective counting of the number of horizontal and vertical movements in arbitrary units (AU). The information obtained was recorded automatically every minute (for 5 min) at one and the same time (between 10:00 a.m. and 1:00 p.m.). The rats were placed in the central quadrant of the activity monitor 15 min after the microinjection of losartan.

Statistical analysis. Behavioural data were analysed by analysis of variance (ANOVA). Separate three-way repeated ANOVA was used to process the data obtained for horizontal and for vertical movements during 5-min period between subject factors: drug (two levels: losartan and saline), side of injections (three levels: bilateral, left and right) and time (five levels: 1st, 2nd, 3rd, 4th and 5th min). Separate two-way ANOVA was used to process the data obtained for the total number of horizontal and vertical movements during the whole 5-min period of observation. ANOVA data were further analysed by post-hoc Student–Newman–Keuls (SNK) test.

Results. *Effects of losartan microinjected into hippocampal CA1 area on the number of horizontal movements.* Repeated three-factor ANOVA analysis of the Opto Varimex data about the horizontal movements after bilateral and unilateral (left or right) microinjections of losartan into the hippocampal CA1 area demonstrated a significant effect for three factors: "drug" ($F_{1,174} = 231.552$; $P \leq 0.001$), "side" ($F_{2,174} = 29.365$; $P \leq 0.001$) and "time" ($F_{4,174} = 42.417$; $P \leq 0.001$). There was also significant interaction between "drug" × "side" × "time" ($F_{8,174} = 12.9189$; $P \leq 0.01$).

Post-hoc SNK comparisons showed that bilateral microinjections of losartan into hippocampal CA1 areas significantly decreased number of horizontal movements compared to bilateral saline microinjections at the 1st ($P \leq 0.005$), 2nd ($P \leq 0.04$), 3rd ($P \leq 0.005$), 4th ($P \leq 0.02$) and 5th min ($P \leq 0.02$) (Fig. 1).

Unilateral microinjections of losartan into the left-side CA1 area significantly decreased the number of horizontal movements as compared to left-side saline

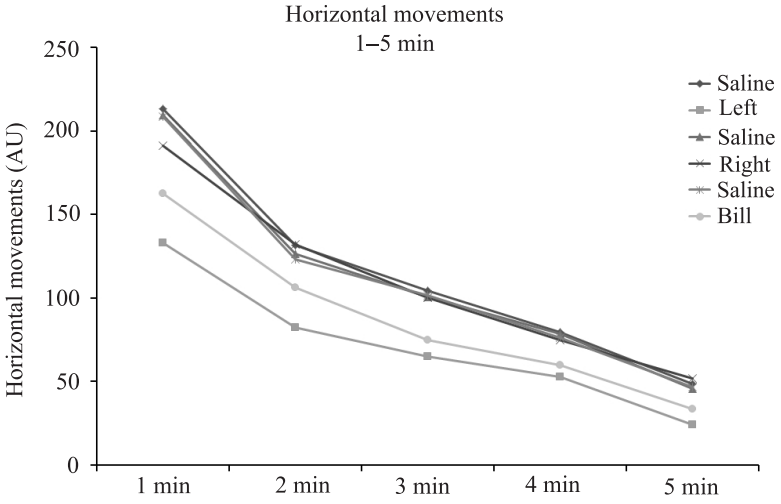

Fig. 1. Effect of losartan on the number of horizontal movements every minute after uni- or bilateral microinjections into the hippocampal CA1 area. $n = 8$. Means (± S.E.M.) are presented. Asterisks depict – drug treated vs. respective saline-treated. $^{***}P \leq 0.001$. Circles depict – left-side vs. right-side $^{\circ\circ\circ}P \leq 0.001$

microinjections at 1st ($P \leq 0.001$), 2nd ($P \leq 0.003$), 3rd ($P \leq 0.001$), 4th ($P \leq 0.001$), and 5th min ($P \leq 0.001$) (Fig. 1). Right-side infusion of losartan induced no significant changes in the number of horizontal movements, as compared to the respective controls (Fig. 1).

Post-hoc test demonstrated significant differences between left-side and right-side on 1st ($P \leq 0.001$), 2nd ($P \leq 0.001$), 3rd ($P \leq 0.001$), 4th ($P \leq 0.001$), and 5th min ($P \leq 0.001$) (Fig. 1).

The analysis of the changes in the number of horizontal movements every minute after uni- or bilateral microinjections of losartan showed that habituation was not disturbed.

Repeated two-factor ANOVA analysis of the horizontal activity for the whole period of observation (5 min) showed a significant effect for "drug" ($F_{1,30} = 233.093$; $P \leq 0.001$) and "side" ($F_{2,30} = 29.122$; $P \leq 0.001$). There were significant "drug" × "side" interaction ($F_{2,30} = 53.683$; $P \leq 0.001$).

Post-hoc comparisons demonstrated that bilateral and left-side microinjections of losartan significantly decreased the total number of horizontal movements as compared to saline treated controls ($P \leq 0.001$; $P \leq 0.001$, respectively). This effect was more pronounced after microinjection of losartan into the left CA1 hippocampal area. Losartan infused unilaterally into right-side induced no significant changes in horizontal activity, compared to the controls (Fig. 2). Injections of losartan into the left CA1 area significantly decreased the total number of horizontal movements compared to the right-side injections ($P \leq 0.001$) (Fig. 2).

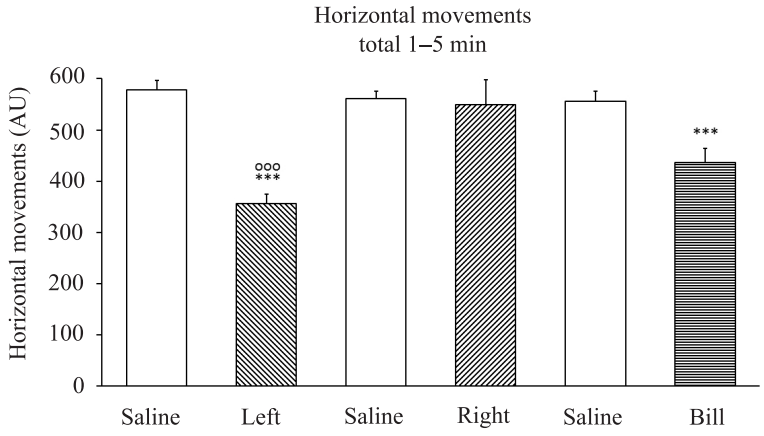

Fig. 2. Effect of losartan microinjected uni- and bilaterally into the hippocampal CA1 area on the total number of horizontal movements for the whole period of observation (5 min). $n = 8$. Means (± S.E.M.) are presented. Asterisks depict – drug treated vs. respective saline-treated. $^{***}P \leq 0.001$. Circles depict – left-side vs. right-side $^{\circ\circ\circ}P \leq 0.001$

Effects of losartan microinjected into hippocampal CA1 area on the number of vertical movements. Repeated three-factor ANOVA was used to analyse the effect of losartan, microinjected into the CA1 area, on the number of vertical movements at every minute for 5 min. The analysis demonstrated

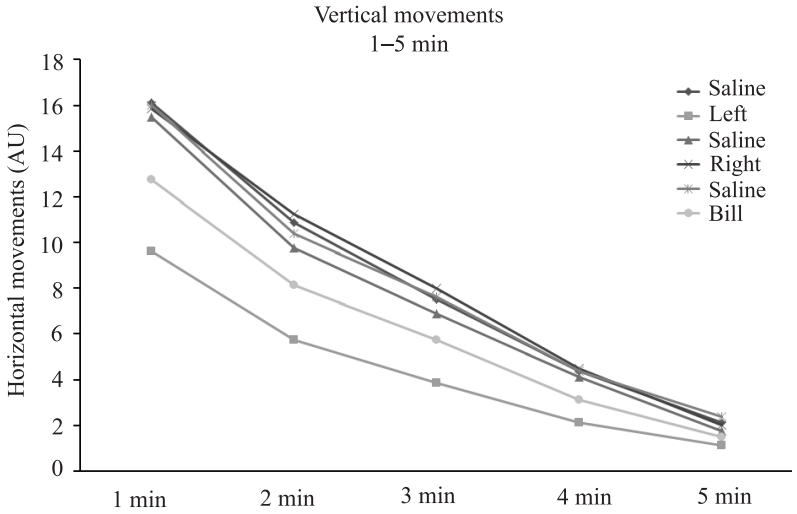

Fig. 3. Effect of losartan on the number of vertical movements every minute after uni- or bilateral microinjections into the hippocampal CA1 area. $n = 8$. Means (± S.E.M.) are presented. Asterisks depict – drug treated vs. respective saline-treated. $^{***}P \leq 0.001$. Circles depict – left-side vs. right-side $^{\circ\circ\circ}P \leq 0.001$

significant effects for factors "drug" ($F_{1,174} = 51.789$; $P \leq 0.001$), "side" ($F_{2,174} = 31.623$; $P \leq 0.001$) and "time" ($F_{4,174} = 78.025$; $P \leq 0.001$).

Post-hoc comparisons demonstrated that losartan microinjected into both CA1 hippocampal areas decreased the number of vertical movements as compared to the saline microinjections on the 1st ($P \leq 0.05$), 2nd ($P \leq 0.01$), 3rd ($P \leq 0.05$), and 4th min ($P \leq 0.05$) (Fig. 3). Left-side microinjections of losartan decreased significantly the number of vertical movements, as compared to the respective controls: 1st ($P \leq 0.001$), 2nd ($P \leq 0.004$), 3rd ($P \leq 0.002$), 4th ($P \leq 0.001$), and 5th min ($P \leq 0.004$) (Fig. 3). Right-side infusion of losartan did not affect significantly the number of horizontal movements, compared to the saline treated controls (Fig. 3). Left-side losartan administration decreased the number of vertical movements at every minute during the whole 5-min period, as compared with the injections into the right CA1 area (Fig. 3).

Repeated two-factor ANOVA of the total number of vertical movements for the whole 5-minute period of observation showed a significant effect on the vertical movements for the factor "drug" ($F_{1,30} = 51.789$; $P \leq 0.001$) and for the factor "side" ($F_{2,30} = 27.462$; $P \leq 0.001$).

Follow-up post-hoc SNK comparisons indicated that the effect of losartan was significantly smaller when it was injected into the left ($P \leq 0.001$) and into the both ($P \leq 0.01$) CA1 hippocampal areas, as compared to the respective saline-treated controls, while right-side application did not affect significantly the total number of vertical movements (Fig. 4).

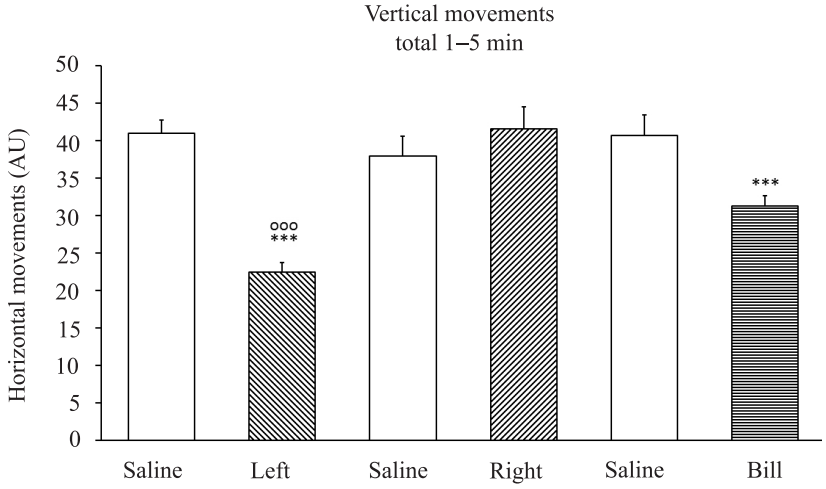

Fig. 4. Effect of losartan microinjected uni- and bilaterally into the hippocampal CA1 area on on the total number of vertical movements for the whole period of observation (5 min). $n = 8$. Means (± S.E.M.) are presented. Asterisks depict – drug treated vs. respective saline-treated. $^{***}P \leq 0.001$. Circles depict – left-side vs. right-side $^{\circ\circ\circ}P \leq 0.001$

Infusion of losartan into the left CA1 area produced a significant decrease in the total number of vertical movements, compared to the right-side infusion ($P \leq 0.001$) (Fig. 4).

Discussion. The present results supported the important role of the hippocampal AT1 receptors in mediating the effect of exploratory behaviour and extended understanding about the asymmetric behavioural effects of Ang II, microinjected in discrete brain structures.

It was found that microinjections of losartan into hippocampal CA1 area affect exploratory activity as reflected in both horizontal and vertical movements. Bilateral administration of losartan into CA1 areas decreased exploratory activity.

Of particular interest was the question of whether or not equal doses of losartan infused into left-side or right-side hippocampal CA1 area would induce the same behaviour changes. It was found that unilaterally microinjected losartan evoked asymmetric behavioural effects, i.e. influenced the exploratory activity of rats in a different manner. Thus, the number of horizontal and vertical movements after losartan application into the left-side was decreased, while losartan into the right-side did not affect it, as compared to the respective control. The present study provided for the first time information on the inhibitory effect of losartan on exploratory behaviour, when infused bilaterally or unilaterally into the left CA1 hippocampal area. The most important finding is that inhibition of AT1 receptors by losartan exerts a differential and asymmetric effect on exploratory activity. The gradual decrease in the number of movements in losartan-treated rats suggests that there is no disturbance of habituation. Habituation is characterised by the gradual waning of a behavioural response to repeated stimulation and is considered the simplest form of learning.

The present findings are also in agreement with studies showing asymmetric effects on exploratory behaviour after microinjection of Ang II into the CA1 hippocampal area. Belcheva et al. [14] have shown that only left-side Ang II injections affected exploratory behaviour, while Ang II administered into the right CA1 area had no effect on the exploratory activity.

Some authors [17, 18] have suggested that the influence of Ang II on exploratory behaviour is carried out in a non-specific mechanism, while others [14, 15, 19, 20] believe that this is accomplished by the action of various AT receptors and the involvement of other transmitter systems. On the basis of our results and the data of other authors, we suggest that AT1 receptors, widely distributed in the hippocampus, are involved in the exploratory behaviour.

Conclusion. For the first time this study provides information about asymmetric effects of losartan on the exploratory behaviour. Asymmetric behavioural responses could be evoked by inhibition of CA1 hippocampal AT1 receptors. This finding suggests that there may be a differential distribution of AT1 receptors in the brain hemispheres, as well as that these receptors may be involved in learning and memory processes and/or in movement disorders.

REFERENCES

[1] Haulica I., W. Bild, D. N. Serban. J. Renin Angiotensin Aldosterone Syst., **6**, 2005, No 3, 121–131.

[2] Ciobica A., W. Bild, L. Hritcu, I. Haulica. Acta Neurol. Belg., **109**, 2009, No 3, 171–180.

[3] Ciobica A., V. Bild, L. Hritcu, M. Padurariu, W. Bild. Central European Journal of Medicine, **6**, 2011, No 3, 331–340.

[4] Marques-Lopes J., D. Pinho, A. Albino-Teixeira, I. Tavares. Brain Res., **1325**, 2010, 41–52.

[5] de Gasparo M., A. Husain, W. Alexander, K. J. Catt, A. T. Chiu, M. Drew et al. Hypertension, **25**, 1995, No 5, 924–927.

[6] de Gasparo M., K. J. Catt, T. Inagami, J. W. Wright, T. Unger. Pharmacol. Rev., **52**, 2000, No 3, 415–472.

[7] Sirett N. E., J. J. Bray, J. I. Hubbard. Brain Res., **217**, 1981, No 2, 405–411.

[8] von Bohlen und Halbach O., D. Albrecht. Cell Tissue Res., **326**, 2006, No 2, 599–616.

[9] Holscher C., W. Jacob, H. A. Mallot. Behav. Brain Res., **142**, 2003, Nos 1–2, 181–191.

[10] Moita M. A., S. Rosis, Y. Zhou, J. E. LeDoux, H. T. Blair. Neuron, **37**, 2003, No 3, 485–497.

[11] Izquierdo I., J. H. Medina. Neurobiol. Learn. Mem., **68**, 1997, No 3, 285–316.

[12] Haas H. L., D. Felix, M. R. Celio, T. Inagami. Experientia, **36**, 1980, No 12, 1394–1395.

[13] Mukuda T., H. Sugiyama. Neurosci. Res., **58**, 2007, No 2, 140–144.

[14] Belcheva I., M. Chobanova, V. Georgiev. Regul. Pept., **74**, 1998, Nos 2–3, 67–71.

[15] Belcheva I., V. Georgiev, M. Chobanova, C. Hadjiivanova. Neuropeptides, **31**, 1997, No 1, 60–64.

[16] Pellegrino L., A. Cushman. A stereotaxic atlas of the rat brain. New York, Appleton-Century-Crofts, 1967.

[17] Braszko J. J., K. Wisniewski. Peptides, **9**, 1988, No 3, 475–479.

[18] Braszko J. J., K. Wisniewski, G. Kupryszewski, B. Witczuk. Behav. Brain Res., **25**, 1987, No 3, 195–203.

[19] Georgiev V., D. Getova, M. Opitz. Methods Find. Exp. Clin. Pharmacol., **13**, 1991, No 1, 5–9.

[20] Georgiev V., M. Opitz. Methods Find. Exp. Clin. Pharmacol., **11**, 1989, No 12, 743–745.

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