THE EFFECTS OF CHRONIC LOSARTAN PRETREATMENT ON REstraint
STRESS-INDUCED CHANGES IN MOTOR ACTIVITY, NOCICEPTION AND PENTYLENETETRAZOL GENERALIZED SEIZURES IN RATS

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ABSTRACT
Accumulated evidence has shown that renin-angiotensin system has a pivotal role in stress responses.
Aim: to assess the participation of AT_1 receptor in stress-induced modulation of motor activity, nociception and seizure susceptibility in male Wistar rats.
Material and methods: AT_1 receptor antagonist losartan was administered subcutaneously to rats for 10 days at a dose of 10 mg/kg either alone or as a pretreatment before chronic restraint stress applied for 10 days. Locomotor and exploratory activity (open field test), the nociception (paw-pressure test) and the seizure susceptibility (pentylenetetrazol seizure test) were analysed.
Results: Chronic restraint stress decreased motor activity and increased anxiety-like behaviour (grooming) while losartan pretreatment alleviated anxiety-like behaviour. Chronic restraint stress had an antinociceptive effect in paw-pressure test and losartan pretreatment abolished stress-induced antinociception. Both chronic restraint stress and losartan showed anticonvulsant activity in pentylenetetrazol seizure test. However, drug pretreatment attenuated this effect in chronically-stressed rats.
Conclusions: Our findings suggest that the AT_1 receptor is involved in the mechanism of stress-induced changes in anxiety-like behaviour, nociception and seizure susceptibility in rats.

Key words: losartan, restraint stress, open field, nociception, PTZ generalized seizures, rats

INTRODUCTION
Accumulated evidence supports the viewpoint that renin-angiotensin system (RAS) and stress phenomenon are interrelated. Stress is accompanied by an increase in the plasma renin activity and the circulation of angiotensin (Ang) II thereby contributing to the modulation of hypothalamic pituitary adrenal axis (HPA) and cardiovascular response to stress.\(^1\) Ang II receptors and, in particular AT_1 receptor subtype, are widely spread throughout the brain and can be found in areas responsible for the regulation of stress responses.\(^2\) Various studies have shown a distinct involvement of angiotensin receptors in the effects of Ang II in restraint stress.\(^3-6\) Restraint stress (RS) can increase the expression of AT_{1A} mRNA receptors and AT_1 receptor binding in a variety of brain areas.\(^7\) It is known that brain RAS, and particularly AT_1 receptors, are involved in acute RS-induced analgesia and impaired retention.\(^8\) Different stressful manipulations, including RS, can induce anticonvulsant effects in rodents.\(^9\) A putative role of Ang peptides in the seizure phenomenon and the nociception has been suggested.\(^10-13\) Therefore, in the present study, we studied the effect of chronic pretreatment with AT_1 receptor antagonist losartan on stress-induced changes in the motor and anxiety-like behaviour, the pain and seizure susceptibility in rats.

MATERIAL AND METHODS
Animals
Male Wistar rats (180–200 g) were kept under standard laboratory conditions under a 12 h light/dark cycle (lights 07:00-19:00 h) with food and water provided ad libitum. The experiments were carried out in accordance with the guidance and general recommendations of Local Ethics Committee of Institute of Neurobiology, Bulgarian Academy of Sciences on the use of laboratory animals.
TREATMENT

Chronically stressed rats were restrained (60 min per day during 10 days) in plastic transparent tubes (6 cm inner diameter and 16 cm long). Non-stressed rats were left undisturbed and handled for 10 days. Four main groups (n=10 each) were used: non-stressed + chronic vehicle (control); non-stressed + chronic losartan (ChronLos); chronic vehicle + chronic restrain stress (CRS); chronic losartan + chronic restrain stress (ChronLos + CRS). Subcutaneous (s.c.) injections of 10 mg/kg (1 ml/kg) Losartan potassium (Merck & Co, Inc) freshly dissolved in saline, or vehicle (saline, 1 ml/kg) were given to corresponding groups thirty minutes before every CRS procedure. All behavioural tests were carried out during the autumn between 09:00 and 12:00 a.m. 15 minutes after the last CRS or losartan administration.

OPEN FIELD TEST

The procedure consisted of subjecting an animal for 5 min in the centre of an exploratory box (100 x 100 cm, divided at 25 equal squares). The total number of squares crossed and the time of grooming (in sec) were recorded.

PAW-PRESSURE TEST

The pain threshold was determined with an analgesimeter (Ugo Basile). The mechanical pressure (in grams) on the right hind paw eliciting pain responses such as withdrawal or struggle was established as mechanical pain threshold.

PENTYLENETETRAZOL (PTZ) GENERALIZED SEIZURE TEST

PTZ (90 mg/kg, s.c.) (Sigma, St. Luis, Mo., USA), freshly dissolved in saline, was used to evaluate the latency (in seconds) of the generalized clonic and tonic-clonic seizures (GTCS). The animals were observed individually in plexiglass cages for 60 min.

STATISTICS

All data are expressed as mean ± SEM. Two-way ANOVA with factors (drug and stress) with subsequent post-hoc Bonferroni test was applied. Differences were considered significant at p < 0.05.

RESULTS

OPEN FIELD TEST

Two-way ANOVA revealed a significant interaction between factors drug and stress [F(1,32) = 14.67, p = 0.0006 - number of crossing; F(1,32) = 7.518, p = 0.01 - number of rears; F(1,32) = 17.24, p = 0.0002 - grooming]. Both chronic restraint stress and losartan per se diminished the horizontal (t = 3.843, p = 0.001 – CRS; t = 5.981, p = 0.0001 –ChronLos) and vertical (t = 3.259, p = 0.005 – CRS; t = 3.771, p = 0.002 –ChronLos) activity (Figs 1A, B). Chronic RS increased the incidence of the grooming (t = 5.465, p < 0.001) whereas chronic losartan pretreatment alleviated this anxiety-like behaviour (Fig. 1C).

PAW PRESSURE TEST

Two-way ANOVA revealed a main effect of the factor stress [F(1,36) = 14.35, p < 0.0006], and drug [F(1,36) = 5.352, p < 0.03] without interaction

Figure 1. Effects of chronic restraint stress (CRS), chronic losartan (ChronLos, 10 mg/kg daily during 10 days) and chronic losartan pretreatment before RS on the ambulation (A), rearings (B) and grooming (C) behaviour in rats.
between the factors. Chronic RS induced antinociception ($t = 4.061, p < 0.001$). Chronic losartan treatment did not change the pain threshold per se. Chronic losartan administration before every RS exposure abolished stress-induced antinociception ($t = 2.406, p = 0.03$) (Fig. 2).

**PentyleneTetrazol-Induced Generalized Seizures**

ANOVA showed a significant interaction between chronic RS and drug on the latencies for appearance of seizures [$F(1,35) = 48.22, p < 0.0001$]. Post-hoc test showed a significant increase in the latency for clonic and GTCS in both stressed and drug-treated groups ($p < 0.05$) (Table 1). However, anticonvulsant effect was abolished after losartan pretreatment in stressed animals.

**DISCUSSION**

Chronic restraint stress induced a hypomobility in Wistar rats in accordance with previous data. It was suggested that stress-induced hypoactivity could represent a diminished motivation to interact with the environment which would explain the decreased exploratory behaviour after chronic restraint stress in our study. This is also supported by the finding that RS of rats increased grooming behaviour which may be considered a behavioural response that follows changes elicited by anxiogenic stimuli. The reversion of stress-induced anxiety by chronic losartan pretreatment in the present study agrees with previous data showing that chronic blockade of AT$_1$ receptor alleviated stress-induced responses.

Acute RS induces an opioid-dependent and catecholamine-mediated analgesia in different experimental models of nociception. We found that chronic RS also induced antinociception in paw pressure test in rats. There are data considering the role of Ang II and its receptors in the modulation of nociception. Moreover, participation of AT1 receptors in the mechanism of stress-induced analgesia is strongly supported.

The present study showed that chronic losartan treatment did not change the pain threshold in paw pressure test but it abolished the antinociception induced by chronic RS. So far, the effect of chronic blockade of AT$_1$ receptors on the stress-induced antinociception has not been considered. Pretreatment with AT$_1$ receptor antagonist abolished stress-induced increase in AT$_1$ receptor binding in paraventricular nucleus and even decreased it under control level. These biochemical data may explain the opposite effect of losartan pretreatment on RS-induced antinociception.

Chronic treatment with losartan exerted seizure-protective effect in rats which agrees with previous data. Similarly, Ang II-induced increase in the PTZ threshold in nonstressed mice is fully reversed to a decrease for the three seizure phases in stressed mice. JM Saavedra et al. postulated that vulnerability to stress might result from an activated Ang II system or from a failure in the feedback mechanisms controlling the brain Ang II stimulation during stress.

In summary, the selective AT$_1$ receptor antagonist losartan modulates the stress-induced changes in motor activity and anxiety-like behaviour. Furthermore, losartan reverses antinociceptive and anticonvulsant effects of chronic stress.

| Table 1. Effects of chronic losartan (ChronLos), chronic restraint stress (CRS) and combination ChronLos + CRS on the latency (in seconds) to generalized clonic and tonic-clonic seizures |
|---------------------------------|-----------------|-----------------|
| Treatment                      | Latency to clonic seizures | Latency to tonic-clonic seizures |
| Controls                       | 287.3 ± 22.5     | 544.7 ± 38.4    |
| ChronLos                       | 753.8 ± 3*       | 755.67 ± 4.4*   |
| CRS                            | 784.7 ± 3.7*     | 786.7 ± 3.4*    |
| Losartan+stress                | 270.8 ± 45.66*+  | 376.9 ± 70.9*+  |

* $p < 0.05$ vs control; $^a$ $p < 0.05$ vs ChronLos - treated group; $^+$ vs CRS-treated group.
Based on our results we can suggest participation of AT₁ receptors in the feedback compensatory mechanism at exposure to chronic stress against overactivated RAS.

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REFERENCES

ЭФФЕКТЫ ХРОНИЧЕСКОГО ИНЪЕЦИРОВАНИЯ ЛОСАРТАНОМ НА ВЫЗВАННЫЕ ИММОБИЛИЗАЦИОННЫМ СТРЕССОМ ИЗМЕНЕНИЯ ДВИГАТЕЛЬНОЙ АКТИВНОСТИ, БОЛЕВОЙ ЧУВСТВИТЕЛЬНОСТИ И ПЕНТИЛЕНТЕТРАЗОЛ-ГЕНЕРАЛИЗОВАННЫХ СУДОРОГ У КРЫС

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РЕЗЮМЕ

ВВЕДЕНИЕ: В последнее время увеличиваются данные, показывающие, что ренин-ангиотензиновая система играет ключевую роль в стресс-индуцированных реакциях.

ЦЕЛЬ: Авторы ставят себе целью исследовать участие AT1-рецепторов в стресс-индуцированных изменениях двигательной активности, болевой и судорожно-приступной чувствительности у мужских крыс породы Wistar.

МАТЕРИАЛ И МЕТОДЫ: AT1-рецепторный антагонист лосартан применяли на крысах подкожно в течение 10 дней (доза – 10 мг/кг) самостоятельно или как предварительное инъектирование до хронического иммобилизованного стресса в течение 10 дней. Исследованы двигательная и прочувствованная активности (т.е. открытое поле), ноцицепция (т.е. механическое давление на лапу) и судорожная чувствительность (т.е. пентилентетразол-индукционные судороги).

РЕЗУЛЬТАТЫ: Хронический иммобилизированный стресс уменьшает двигательную активность и повышает состояние тревожности, в то время как лосартан облегчает поведение, связанное с тревожностью. Применен самостоятельно, хронический иммобилизированный стресс вызывает антиноцицептивный эффект при тесте «надавливание на лапу», в то время как лосартан, применен до стрессогена, устраняет стресс-индукционную антиноцицепцию. Как хроническая иммобилизация, так и хроническое введение лосартана показали антиконвульсивную активность, но AT1-рецепторный антагонист устраняет этот эффект у крыс, подвергнутых стрессу.

ВЫВОДЫ: Полученные результаты показывают, что AT1-рецептор участвует в механизме стресс-индуцированных изменений в состоянии тревожности, ноцицепции и судорожной чувствительности у крыс.