INTRODUCTION

The blood pressure control systems interact with pain regulatory systems and the brain regions related to these systems overlap (1, 2). Mechanisms which determine adaptive relationship between blood pressure and nociception are complex with substantial role of baroreceptor-related processes, endogenous opioid activity and noradrenergic activity (1). Arterial hypertension is often associated with behavioral hypoalgesia (3). Spontaneously hypertensive rats (SHRs), which are a validated animal model of human genetic hypertension, demonstrate abnormal nociception (4). They are hypoalgesic in the hot plate test, normal to hyperalgesic in the tail flick test and show exaggerated pain responses in models of inflammatory pain (5-8). However, hot plate hypoalgesia is no longer observed after the habituation of SHR to the experimental environment (9, 10). In addition, hot plate hypoalgesia in SHR is abolished after administration of angiotensin converting enzyme (ACE), paw pressure test for the determination of pain threshold and Rotarod test to study motor coordination were used. Chronic treatment was administered to the SHR with the AT_{1} receptor antagonist losartan (10 mg/kg/day, s.c.) for 14 days. SHR showed lower pain threshold and smaller day-night variations of nociception as compared to Wistar rats. Chronic losartan decreased the ABP and produced an inverted diurnal pattern of nociception in SHR, increasing the pain threshold at 03:00 h. Neither strain differences nor changes in motor coordination after losartan treatment were observed in SHR. Our results suggest that SHR have disturbances in diurnal variation in nociception and that the AT_{1} receptor plays a role in the regulation of the circadian rhythm of mechanical pain threshold in SHR.

SUMMARY

Angiotensin (AT) II plays a key role in the regulation of blood pressure and water-salt balance and modulates nociception. Peptides based on AT influence central functions through the activation of AT_{1}, AT_{2} or AT_{4} receptors. The aim of this study was to elucidate the role of AT_{1} receptors in diurnal variation in nociception in spontaneously hypertensive rats (SHR). Male Wistar rats (16 weeks-old) and SHR were caged individually and exposed to light from 08:00 to 20:00 h. The tail cuff method for noninvasive measurement of arterial blood pressure (ABP), paw pressure test for the determination of pain threshold and Rotarod test to study motor coordination were used. Chronic treatment was administered to the SHR with the AT_{1} receptor antagonist losartan (10 mg/kg/day, s.c.) for 14 days.

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An increased angiotensin II (Ang II)-like immuno-reactivity in numerous brain structures is characteristic of SHR in comparison with normotensive controls (14). High concentrations of angiotensin receptors are established in the suprachiasmatic nucleus that implements a role of the biological clock (15). Losartan, a selective AT_{1} receptor antagonist, not only reduces blood pressure but also normalizes the shifted SHR’s circadian blood pressure rhythm (16). Losartan reverses Ang II-induced and stress-induced antinociception in normotensive rats as well as hot plate hypoalgesia in SHR but the effect of this AT_{1} receptor antagonist on the circadian rhythm of nociception is not known (11, 17).

The aim of the present study was to investigate the diurnal pattern of mechanical nociception and the role of angiotensin AT_{1} receptor subtype in 24-hour variations of the nociception in SHRs.
MATERIALS AND METHODS

Animals and drug treatment

Adult male Wistar (WIS, Bulgarian Academy of Sciences Breeding House) and spontaneously hypertensive rats (SHR, Medical University Sofia) were used. All rats were 16-weeks old at the beginning of the study. The animals were housed individually in metabolic cages in a separate room under standardized laboratory conditions: temperature 22 ± 1°C, humidity 60% ± 10% and an artificial 12h/12h (light [08:00-20:00 h]/ dark [20:00-08:00 h]) cycle with light intensity of about 250 lux at the front of the cages. Standard rodent diet and tap water were provided ad libitum except during the measurements. Rats were allowed 2 weeks of habituation to the laboratory conditions before the start of the experiments. Losartan potassium (kindly gifted by Merck & Co, Inc.) was dissolved in sterile isotonic saline and administered s.c. at a dose of 10 mg/kg/day for 14 days through osmotic minipumps (Alzet, model 2002), which delivered at 0.5 ml/h for 14 days. The pumps were inserted s.c. under pentobarbital anesthesia (Nembutal, Abbott, 35 mg/kg, i.p.) between the scapulae in a small pocket formed using a hemostat, in accordance with manufacturer’s instructions. Control groups were implanted with saline-filled pumps. All pumps were removed under pentobarbital anesthesia upon completion of their delivery rate (15th day after the implantation). All experiments were approved by the appropriate ethics committees of approved institutions.

Experimental design

The rats were divided into 3 groups: control group 1 - saline-infused Wistar rats (n = 10) with an average body weight (b.w.) of 315.6 ± 5.4 g; experimental group 1 - losartan-infused WIS rats with an average b.w. 309.6 ± 5.2 g; control group 2 - saline-infused SHR (n = 10) with an average b.w. of 278 ± 5.1 g; experimental group 2 - losartan-infused SHR with an average b.w. of 251.5 ± 4.4 g. Blood pressure was measured between 09:00 and 11:00 h before and 13 days after chronic losartan and saline administration. The animals were weighed every day during habituation period and during infusion. The time points of mechanical paw-pressure threshold measurements were chosen depending on the light/dark regimen. For each experimental group, test was carried out on the 12th day of drug or saline treatment with first time point 1 h after light onset (09:00 h) and subsequent tests in 3-hours interval. Rotarod test was carried out during the 12th day of drug or saline treatment, in intervals of 6 h (09:00, 15:00, 21:00 and 03:00 h). The dark period measurements were taken in dim red light (25-W red bulb). All experiments were carried out during the autumn (October-November).

Measurement of arterial blood pressure

Systolic arterial blood pressure (ABP) was measured noninvasively in conscious unrestrained rats by the tail cuff method (Ugo Basile blood pressure recorder 5800) (18). The animals were adapted to measurement procedures including prewarming in a thermostatic chamber (29-30 °C) and accustomed to tail cuff and pulse transducer. The ABP value for each rat was obtained as a mean of three consecutive measurements.

Paw pressure test

The paw pressure withdrawal reflex was determined with an analgesimeter (Ugo Basile). The mechanical pressure (in grams) required to elicit nociceptive responses (such as withdrawal/struggle) was established as the mechanical nociceptive threshold. The mechanical nociceptive threshold testing was optimized by single training of the animals 1 day before the experiments (19).

Rotarod test

Motor coordination was measured by a “rotarod” test according to the procedure described elsewhere (20). The apparatus consisted of a horizontal rod (6 cm in diameter, 11 cm long) bordered with discs (30 cm diameter). It was programmed to rotate at a constant rate of 8 rpm. The animals were placed on the rotating rod with head directed against the direction of the rotation so that the animals had to progress forward to maintain equilibrium. Animals were trained 1 h before the start of the testing. The length of time the animal remained on the rotating cylinder was recorded in sec, up to 180 sec.

Statistical analysis

All data are expressed as mean ± SEM. Statistics were performed by Sigma Stat three way ANOVA using strain, time and drug as factors followed by Bonferroni post test for multiple comparisons. P values of less than 0.05 were considered to be statistically significant.

RESULTS

Arterial blood pressure

Normotensive control WIS rats showed no differences in ABP values before and after infusion (118.64 ± 1.8 mm Hg before and 121.36 ± 1.66 mm Hg after infusion, P > 0.05). Control SHR showed slightly higher ABP (185.76 ± 1.58 mm Hg) before infusion compared to the value after saline infusion (180 ± 1.05 mm Hg, P < 0.05). Control SHR showed significantly higher ABP compared to WIS controls (F (1, 19) = 523.18, P < 0.001). Chronic infusion of losartan (10 mg/kg/day, 14 days) diminished significantly ABP in SHR to 148.33 ± 3.93 mm Hg (F (1, 19) = 60.82, P < 0.001) vs. SHR controls.

Diurnal variations of mechanical paw pressure threshold in WIS and SHR

The strain differences between WIS and SHR and the effects of chronic losartan treatment in the diurnal patterns of mechanical nociception are presented in Figures 1 and 2. Three-way ANOVA showed significant interaction between drug, strain and time factors (F (1, 323) = 2.828, P < 0.005). Bonferroni post test showed that vehicle treated SHRs had significantly lower nociceptive thresholds at 09:00 h compared to the vehicle treated WIS (t = 4.804, P < 0.001; t = 4.393, P < 0.001). One-way ANOVA showed that normotensive WIS rats presented significant variations in paw-pressure withdrawal reactions related to the time of day-and-night cycle (F (8, 164) = 6.89, P = 8.43 E-08). They bore highest pain thresholds at the beginning of light phase (09:00 h) and the lowest between 03:00-06:00 h (Fig 1). Saline treated control SHR showed insignificant diurnal variations in pain thresholds as measured at particular time points.
Effect of losartan on the diurnal variation in nociception in WIS rats and SHR

Chronic treatment with losartan (10 mg/kg/day, 13 days) caused antinociception at 12:00 (t = 3.394; P < 0.01) and 06:00 h (t = 3.561; P < 0.01) in WIS rats, thereby diminishing the typical daily variation of the controls (Fig 2). SHR chronically treated with losartan (10 mg/kg/day, 13 days) displayed significant diurnal fluctuations in paw pressure thresholds (F (8, 85) = 4.71, P < 0.001) with a peak at 03:00 h, corresponding to the lowest threshold of saline treated SHR and WIS (Fig 2). Post hoc analysis showed that chronic treatment with losartan significantly increased the pain threshold at 03:00 h in comparison with SHR controls (t = 4.33, P < 0.001). Chronic losartan
treated SHR showed lower paw pressure threshold over almost the whole 24-h period ($t = 5.192, P < 0.001$; $t = 6.772, P < 0.001$; $t = 3.238, P < 0.05$; $t = 3.155, P < 0.05$; $t = 3.499, P < 0.01$; $t = 5.696, P < 0.001$; $t = 4.306, P < 0.001$) except at 24:00 and 03:00 h.

**Twenty four-hour pattern of motor coordination (rotarod test) in WIS and SHR**

Saline-infused control WIS and SHR did not show any significant differences related to motor coordination at chosen time points of light/dark cycle. Chronic losartan (10 mg/kg/day, 13 days) treatment did not change the motor coordination in WIS and SHR (Fig 3).

**DISCUSSION**

The 16-weeks old SHR used in this study had developed hypertension as compared to normotensive WIS. The small decrease of ABP in vehicle infused SHR may be due to the adaptation of the animals to the investigator in terms of prolonged handling. Chronic subcutaneous administration of the AT1 receptor antagonist losartan significantly decreased arterial blood pressure. These data are consistent with previous reports on the antihypertensive effects of losartan after systemic administration in SHR and hypertensive persons (16, 21, 22). The SHR circadian blood pressure rhythm was characterized by a pattern that peaked during the rats’ active (light-off or dark) phase but the peak time was a little closer to the resting (light-on) phase compared with that for WKY rats (13). We have used losartan at a dose that was reported both to reduce blood pressure and to restore the shifted circadian blood pressure rhythm (16).

Present results demonstrated that SHR have no disturbances in motor coordination when compared to WIS, as was established previously thereby rejecting the possibility of motor disturbances influence on the pain-induced withdrawal reactions (23). No published data are available about diurnal variation in mechanical nociception in SHR. Our study showed small, insignificant fluctuations in SHR’s pain threshold at particular time points in the frameworks of the 24-h cycle. Moreover, SHR demonstrated lower pain threshold compared with normotensive WIS at the beginning of the light phase although both WIS and SHR have similar diurnal rhythm of nociception with lowest thresholds at the middle of the dark phase. It is well known that SHR were characterized not only with raised ABP but also with increased motor activity, decreased nociception in hot plate test and “normal” nociception in tail flick test (6). Despite hypoalgesic reactions with regard to phasic pain, SHR displayed hyperalgesic reactions in other tests with prolonged stimulation compared to normotensive rats (7). Recently it was found that even well defined hypoalgesia in hot plate test disappeared after adaptation of SHR to an experimental environment without pain stimulation (5, 9, 24). The authors suggest that the changes in nociception may result from a cognitive function disorder in SHR. The latter supplies ground for the use of SHR as a model of hyperactivity with attention deficit (25). Their hyperactivity may explain shortened latency of two way active avoidance in comparison with WKY (26).

Keeping in mind that our tests were carried out after preliminary learning procedure and after multiple measuring, established lower pain threshold in SHR may be due in part to an adaptation to test and inherent hyperactivity (19).

Our results obtained after chronic losartan treatment in SHR (10 mg/kg, 13 days, s.c.) showed an inverted diurnal rhythm of mechanical pain threshold with peak value at the middle of the dark phase and low thresholds during the light phase. The miniosmotic pumps used in the present experiments provided a constant concentration of substances during the treatment. Time of day effects of losartan on the mechanical pain threshold may be due to the circadian fluc-
tutions of endogenous renin-angiotensin system activity related to AT1 and/or AT2 receptor subtypes. Activation of these receptors relates with different, often opposite modulation of physiological functions such as blood pressure, cognition and nociception (27-30). Our previous study showed that exogenous Ang II exerted an antinociceptive effect in WIS rats only at time points, characterized with naturally low pain threshold (31). Taken together these data showed that activation of Ang II receptors or their selective blocking had no equivalent effect at different time points of day and night cycle. Chronic systemic treatment with losartan doses that normalize circadian rhythm of blood pressure resulted in the appearance of an inverted diurnal pattern of mechanical nociception in SHR in this study (32).

We summarize that AT1 receptors take part in the regulation of diurnal variations of pain threshold through a mechanism that is probably different from that regulating blood pressure rhythms.

ACKNOWLEDGMENTS

This work was supported by the Medical Science Council, Medical University, Sofia, Bulgaria, contract No. 35/2006. Merck and Du Pont kindly provided Losartan.

DISCLOSURES

The authors state no conflicts of interest.

REFERENCES


