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Rapid communication

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HYPOTENSIVE EFFECT OF ANGIOTENSIN II AFTER AT_1-RECEPTOR BLOCKADE WITH LOSARTAN

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Recent data suggest that hypotensive effect of losartan may not be attributed solely to AT_1-receptor blockade, but also to excessive AT_2 or other receptors stimulation by elevated angiotensin II and its derivative peptides. Therefore in the present study we examined the effect of angiotensin II on mean blood pressure after AT_1-receptor blockade with losartan. Male Wistar rats were anaesthetised and received injection of either losartan (30 mg/kg, 1 ml/kg, i.v.) or saline (the same volume and route) followed by bolus injection of angiotensin II (100, 300 or 1000 ng/kg; 1 ml/kg, i.v.) or 1-hour infusion of angiotensin II (200 ng/kg/min; 2.5 ml/kg/h, i.v.). Control animals received saline instead. Angiotensin II, given either as the injection or the infusion, caused an evident increase in mean blood pressure (p ranged from 0.05 to 0.001 depending on the experimental group). Losartan caused a rapid drop in mean blood pressure and blunted the hypertensive effect of angiotensin II ($p < 0.01$). Moreover, in the losartan-pretreated animals the hypotensive phase was enhanced by the infusion, but not single injection of angiotensin II, which was most evident from the 30 th minute of observation ($p < 0.05$ vs control). In conclusion, hypotensive effect of losartan may be amplified by simultaneous increase in angiotensin II level, the situation observed during chronic AT_1-receptor blockade.

Key words: *Angiotensin II, losartan, AT_2*-receptors, rats.

INTRODUCTION

The renin – angiotensin system is one of the most important mechanisms regulating blood pressure and electrolyte/blood volume homeostasis (1). The main substance of this system, angiotensin II, exerts many pharmacological actions leading to blood pressure elevation, including peripheral vasoconstriction, aldosterone secretion, catecholamines and endothelin release as well as renal sodium reabsorption (1). The actions of angiotensin II are mediated by two distinct classes of receptors, AT_1 and AT_2, the former being responsible for most of the known physiological effects of this octapeptide (2, 3).

Losartan is the prototype and the first introduced member of a new class of antihypertensive drugs, which selectively block the AT_1 receptor (4), thus,

among others, blunting the hypertensive action of angiotensin II. However, in the course of treatment with losartan, removal of angiotensin II negative feedback on renin secretion leads to increased plasma renin activity and in consequence to the increase in angiotensin II plasma level (5).

Although blockade of AT_1 receptor seems to be the most important mechanism of lowering the blood pressure by AT_1 receptor antagonists, the question arises if excessive stimulation of non — AT_1 receptors in conditions of angiotensin II and its derivatives abundance may be involved in the mechanism of action of these drugs. Hence, the aim of this study was to test the above hypothesis by an administration of exogenous angiotensin II in conditions of AT_1 — receptor blockade with losartan.

MATERIALS AND METHODS

Animals

Male Wistar rats 220—340 g in weight were used throughout the study. The animals were housed in a room with a 12-h light/dark cycle, in group cages as appropriate, were given tap water and fed a standard rat chow. 24 hours before the experiments the animals were deprived of food but had free access to water.

Procedures involving the animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and Guidelines for the Use of Animals in Biomedical Research (Thromb Haemost 1987; 58: 1078—84).

Drugs

Losartan (DuP 753, DuPont Merck Pharmaceutical Co., USA), angiotensin II (Sigma Chemical, St. Louis, USA), heparin (Heparinum, Polfa, Warsaw, Poland) and pentobarbital (Vetbutal, Polfa, Poland) were used in the study.

Experimental protocol

The rats were anaesthetised with pentobarbital (45 mg/kg i.p.). A needle was placed in the left femoral vein for drug administration. The mean blood pressure (MBP) was measured directly through a cannula filled with heparin solution (150 U/ml), placed in the left common carotid artery and connected to the pressure transducer (Gould P23ID) and a monitor Trendoscope 8031 (SandW Vickers Ltd., Białystok, Poland). Tracheotomy was performed to secure airway patency during the experiment. After 15 min to allow for blood pressure stabilisation the drugs were administered as described below.

Losartan (LOS) was dissolved in 0.9% NaCl to a concentration of 30 mg/ml. Angiotensin II (Ang II) was dissolved in 0.9% NaCl to a concentration of 0.1 mg/ml and stored at —20°C. Each day a fresh aliquot was dissolved with 0.9% NaCl to concentrations of 100,300 and 1000 ng/ml for bolus injections and 4800 ng/ml for infusions. The volume of intravenous injections (1 ml/kg for bolus injection and 2.5 ml/kg/h for infusions) was chosen to maintain euvolemia.

In the first experimental protocol the rats were randomly divided into groups and received initial injection of LOS (30mg/kg) or saline (VEH), that was after 10 minutes followed by bolus injection of Ang II (100, 300 or 1000 ng/kg) or VEH (the same volume). In the second experimental protocol after the initial injection of LOS or VEH as described above, the animals were given

a 1-hour infusion of Ang II (200 ng/kg/min) or VEH. Each of the experimental groups (eight groups in the first protocol and four groups in the second protocol) consisted of 5 animals.

The mean blood pressure was recorded before and 1, 5 and 10 minutes after LOS or VEH administration and then in 10 seconds intervals after bolus injection of Ang II or VEH (the first experimental protocol) or in 5 minutes intervals after the beginning of the infusion of Ang II or VEH (the second experimental protocol).

Statistical analysis

The data are shown as mean ± SEM. The changes in mean blood pressure for each time period were compared between groups by analysis of variance (ANOVA) and within the group vs time 0 by means of the Mann-Whitney U test. The p values less than 0.05 were considered significant.

RESULTS

The initial mean blood pressure was 131.3 ± 4.2 mm Hg and it was comparable in all groups of animals (data not shown). The initial bolus of VEH did not significantly change this parameter. The bolus injections of Ang II caused a dose- dependent rise in MBP reaching maximum after 20—40 seconds and lasting for 140—180 second (*Fig. 1*, upper curves). In the animals given intravenous LOS injection a rapid drop in MBP reaching plateau between the

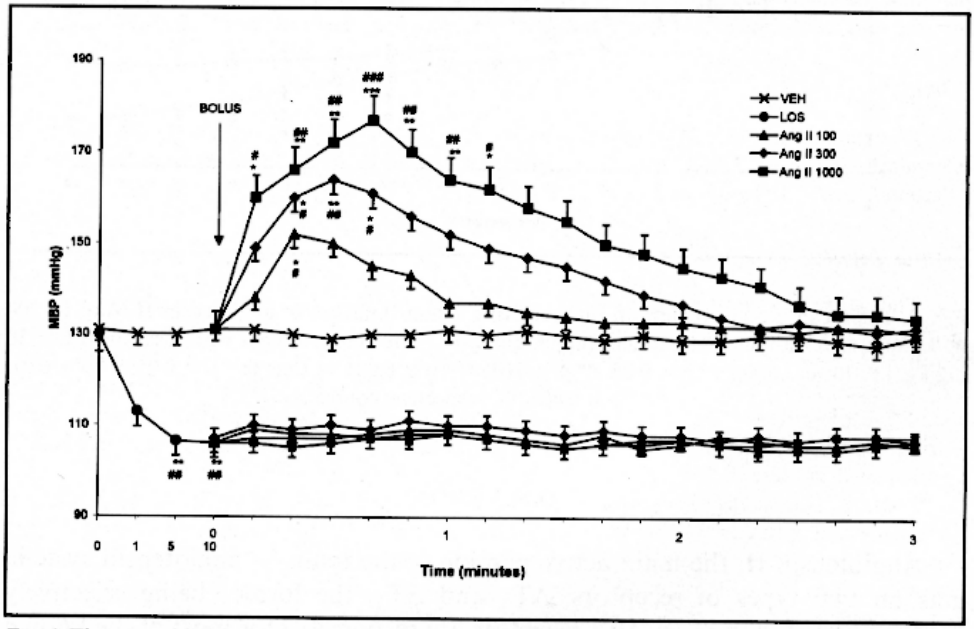

Fig. 1. The mean blood pressure in rats receiving bolus injections of angiotensin II (Ang II; 100, 300 and 1000 ng/kg, i.v.) or saline (VEH) without pretreatment (upper curves) or after pretreatment with losartan (LOS; 30 mg/kg, i.v.; lower curves). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs time 0; # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$ vs respective control.

5th and 10th minute was observed (133.4±6.6 to 107.3±6.2, n = 30; $p<0.01$). Bolus injections of Ang II did not significantly change the MBP regardless of the dose administered (*Fig. 1*, lower curves).

The 1-hour infusion of Ang II, given after initial VEH bolus injection, caused an evident increase in MBP enlarging during the course of experiment (*Fig. 2*, upper curve). In the animals pre-treated with losartan and infused with VEH a slow decrease in MBP in the course of the 1-hour observation period was observed. The infusion of Ang II enhanced the hypotensive effect of losartan, which was significant beginning from the 30th minute of observation ($p<0.05$) (*Fig. 2*, lower curves).

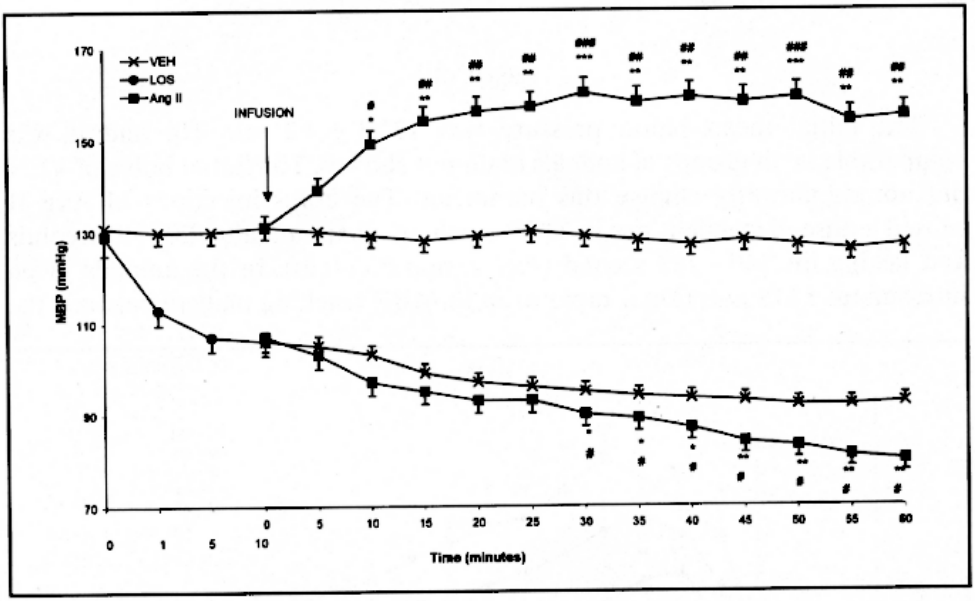

Fig. 2. The mean blood pressure in rats receiving 1-hour infusion of angiotensin II (Ang II; 200 ng/kg/min, i.v.) without pretreatment (upper curves) or after pretreatment with losartan (LOS; 30 mg/kg, i.v.; lower curves). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs time 0; # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$ vs respective control.

DISCUSSION

Angiotensin II, the main active peptide of the renin — angiotensin system, acts on two types of receptors: AT_1 and AT_2, the former being selectively blocked by losartan (2, 3). AT_1 receptors are responsible for most of the known physiological actions of angiotensin II including vasoconstriction (6), while the function of AT_2 receptors remains poorly defined. Ichiki et al. (7) have shown that mice lacking AT_2 receptor have higher blood pressure and increased

vasopressor responses to angiotensin II. Other studies show that AT_2 receptor stimulation increases aortic cyclic GMP level, which possesses vasodilator properties (8). Thus, it may be postulated that AT_1 and AT_2 receptors exert opposite haemodynamic effects.

In this study we have shown that hypotensive effect of losartan is amplified by simultaneous increase in angiotensin II level and that this effect is visible only in animals in which angiotensin II is administered for at least 30 minutes.

Our results are not in line with experiments of Scheuer and Perrone (9), who observed a biphasic pressure response to angiotensin II. In their study losartan administration completely inhibited the vasopressor phase and unmasked a strong vasodepressor phase in response to bolus injections of angiotensin II, which in turn was eliminated by AT_2 receptor antagonist, PD 123319. In our study losartan eliminated the vasopressor effect of angiotensin II, but no vasodepressor phase was present either in animals given angiotensin II bolus injections alone or after AT_1 receptor blockade with losartan. The discrepancy between these results remains to be explained. However, it cannot be excluded that the hypotensive effect of angiotensin II in our experiments may be due to excessive stimulation of AT_2 receptors in conditions of AT_1 receptors blockade.

As mentioned above, treatment with AT_1 receptor antagonists results in an increase in angiotensin II plasma level and subsequent increase in its derivative peptides levels, due to the removal of negative feedback on renin secretion (5). Angiotensin- (1—7), being a product of removal of the C-terminal phenylalanine from the moiety of angiotensin II by specific peptidases, has been shown to release nitric oxide (10) and prostaglandins (11). Studies by Benter et al. have demonstrated hypotensive effect of this heptapeptide in pithed rats (12) and spontaneously hypertensive rats (13). The product of further degradation of angiotensin II and angiotensin- (1—7) by dipeptidases and aminopeptidases, angiotensin IV, has also been shown to possess biological functions (14). Recent data also suggest that angiotensin- (1—7) itself as well as a product of its degradation angiotensin- (1—5) are able to inhibit angiotensin- converting enzyme (15). Therefore, it may be postulated that hypotensive effect of angiotensin II observed in our study, besides of stimulation of AT_2 receptors, could be due to its conversion to other active peptides, which could explain why this effect did not occur after acute angiotensin II administration. However, our data is only preliminary and it demands further investigation whether this effect is mediated by angiotensin II acting on AT_2 receptors or by other angiotensins acting on other specific receptors.

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