

The effects of two types of renin-angiotensin system inhibitors on the hypertension induced by new pressor protein associated with beta-factor XIIa in rats

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Abstract

Background and purpose: New pressor protein (NPP) is a human plasma enzyme, which is structurally related to the beta-fragment of activated factor XII. The present study aimed to compare the effects of angiotensin converting enzyme inhibitors (captopril) and angiotensin type 1 receptor blocker (losartan) on the hypertension induced by NPP injection in normal (sham-2NX) and bilaterally nephrectomized rats (2NX).

Experimental approach: In total, 60 male Wistar rats were sham operated or bilaterally nephrectomized under anesthesia. After 24 h of anesthesia with Inactin[®] (100 mg/kg), their systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured before and after the intravenous administration of captopril, losartan, and NPP.

Findings / Results: In the sham-2NX group, after NPP injection, changes were observed in SBP (145.99 ± 3.6 mmHg), DBP (93.9 ± 3.87 mmHg), and HR (400.29 ± 12.78 bpm). In the captopril group, SBP and DBP had no significant changes, while HR increased significantly ($P = 0.001$). In the losartan group, SBP and DBP decreased ($P = 0.001$ and $P = 0.000$, respectively), while HR had no significant changes. In the 2NX group, after NPP injection, changes were denoted in SBP (127.89 ± 9.03 mmHg), DBP (65.86 ± 5.69 mmHg), and HR (333.35 ± 11.47 bpm). In addition, captopril injection increased DBP ($P = 0.016$) and HR ($P = 0.036$) in response to NPP injection, while losartan injection had no significant effects in this regard.

Conclusion and implications: It could be concluded that losartan could improve hypertension in normal rats, while captopril deteriorated hypertension in bilaterally nephrectomized rats in this hypertension model.

Keywords: Captopril; Factor XII; Hypertension; Losartan; New pressor protein.

INTRODUCTION

According to the literature, 26% of the world population have hypertension, the prevalence of which is expected to increase to 29% in developed countries in 2025 (1). The high prevalence of hypertension is a tremendous health concern as it is the primary cause of cardiovascular diseases and stroke, which are the first and third leading cause of death across the world, respectively (2,3).

New pressor protein (NPP) is an extrarenal enzyme derived from the trypsin-activated

plasma of normal humans and rats. NPP has a strong homology with the heavy chain of the beta-fragment of the activated human coagulation factor XII (β -FXIIa). NPP is an anionic enzyme that potentially elevates blood pressure (BP), heart rate (HR), and sympathoadrenal catecholamine release in bioassay rats. Plasma catecholamine levels rise concurrently, which is predominantly of an adrenal medullary origin, with the epinephrine levels rising more than the norepinephrine levels (4-6).

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Intravenous physiological bolus doses (10-20 μ L plasma equivalent/ \sim 300 g) of impure human or rat NPP have been reported to cause biphasic blood pressure responses in rats. Furthermore, they have been associated with a brief initial depressor phase, followed by a significantly prominent and prolonged pressor phase (10-15 min) accompanied by a marked elevation in heart rate. The cardiovascular effects of NPP largely depend on catecholamines, as observed after adrenergic blockade with phentolamine and propranolol (5). Presumably *via* a peptide-mediated sympathoadrenal pathway.

Hypertension is a common condition in hemodialysis patients. Reports have suggested that the FXIIa/kinin-mediated system is involved in hypertension induction in rats and humans with chronic kidney disease. In this regard, previous laboratory studies have indicated high NPP activity in hypertensive dialysis patients, which changes with SBP and body fluid volume and plays a key role in the incidence of hypertension in anephric hemodialysis patients (7). Renin-angiotensin system (RAS) inhibitors are commonly used in the treatment of hypertension (8,9). However, there are controversies regarding the capability of the two RAS classes of angiotensin converting enzyme (ACE) inhibitor or angiotensin AT1 receptor blockers (ARBs) in the treatment of hypertension (10-12). The present study aimed to compare the effects of ACE and ARB inhibitors in the NPP-induced hypertension model in normal (sham-2NX) and bilaterally nephrectomized (2NX) rats.

MATERIALS AND METHODS

Materials

Inactin (Promonta, Hamburg, Germany) and captopril (Sigma-Aldrich C4042, St Louis, Mo, USA) were dissolved in 0.9% saline. Losartan (Sigma-Aldrich C4042, St Louis, Mo, USA) was dissolved in ethanol and 0.9% saline. Moreover, atropine sulphate was obtained from Ingram and Bell (Toronto, ON, Canada).

Statement of ethical guidelines

The animals in the current research were used in accordance with the principles and guidelines to the ethical and legal requirements

under the Animals for Research Act (R.S.O. 1990, CHAPTER A.22) as outlined by the Canadian Council on Animal Care. All the experimental protocols were approved by the Animal Care Committees of the School of Medicine at the University of Toronto, Canada.

Surgery and instrumentation

Male Wistar rats (n = 60) weighing 250-300 g were obtained from Canadian Biobreeding Laboratories. The animals were exposed to sham operation or nephrectomy as are described below. Under the anesthesia induced by a combination of halothane and nitrous oxide in pure oxygen, the hairs of the animals backed at the level of the last rib, and the flank areas were shaved. Afterwards, the shaved areas were antisepticated with 70% ethanol. A midline skin incision was made above the spinal cord. Through the incision, the surrounding skin was separated laterally with a blunt dissection. Following that, the incision was pulled to right and left. In addition, an incision was made in the muscular layers of the flank areas, and the kidneys were exposed. The adrenal vessels were tied using a 1-0 silk thread temporarily, and the kidneys were decapsulated and removed in order to preserve the adrenal gland. The sham-operated rats were subjected to a similar operation, while the kidneys were not removed. The muscular incision was sutured with a 3-0 silk thread, and the midline skin incision was closed using a nine-millimeter stainless steel autoclip.

The animals recovered from anesthesia and were kept in cages with free access to water and rodent chow. After 24 h, the animals were anesthetized with Inactin (100 mg/kg), and the right carotid arteries were cannulated using PE-50 polyethylene catheters for the measurement of arterial systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) using Statham DC pressure transducers (Hato Rey, USA). The transducers were attached to a Mac Lab/8 data acquisition system (AD Instruments and Lamont Scientific, Toronto, Canada), which was connected to a Power Macintosh 7200/1200 PC compatible computer and driven by Mac Lab Chart software version 3.5.6.

Only the rats with sustained minimal tissue trauma and blood loss and stable BP (SBP/DBP

of ~80/40 mmHg) and HR (~350 beats/min) were used in the experiments, and the other subjects were excluded from further evaluation. The animals received a single subcutaneous injection of atropine sulphate (2.4 mg/kg) during the surgery.

Experiment design and protocol

The rats were divided into two series; the first series included 2NX rats (three groups of 8-10 rats) receiving vehicle (control), captopril (2.5 mg/kg, i.v.), and losartan (10 mg/kg, i.v.). The second series included sham-operated rats divided into three groups of 8-10 rats each in a similar manner as described above (2NX rats). In addition, captopril (2.5 mg/kg) and losartan (10 mg/kg; i.v.) were administered intravenously 40 min before the recording of SBP, DBP, and HR. The animals in each group received single intravenous NPP injections (20 µL plasma equivalent), and the changes in SBP, DBP, and HR were measured at the peak of the blood pressure responses (13).

Human plasmas, which were considered normal but unsuitable for transfusion purposes, were obtained from the Canadian Services (formerly the Canadian Red Cross Society Toronto Center, Ontario, Canada). The plasma bags thawed in cold tap water, and the aliquots were used immediately or frozen at the temperature of -20 °C in capped polystyrene tubes for further assessments. The plasma was

activated with trypsin in a controlled reactions described earlier. In this process, trypsin (type III, bovine, T-8253; Sigma Chemical Co., St Louis, Missouri, USA) was prepared as a stock solution in HCl (0.002 mol/L) and added to the plasma, with mixing at 3-10% v/v to achieve a final trypsin concentration (mg/mL) to ensure the minimal plasma dilution. After incubation for 10 min at the temperature of 23 °C, the reaction was terminated by rapid freezing on dry ice. The activated plasma preparation was administered to the rats at the dose of 20 µL/300 g of body weight and expressed in terms of the plasma equivalence.

Statistical analysis

Data analysis was performed using the Graphpad (prism 5), R 3.5.1, and plot design using Mann-Whitney U test and Wilcoxon test for the comparisons. The obtained data were expressed as mean ± standard error of the mean (SEM). In all the statistical analyses, $P \leq 0.05$ considered significant.

RESULTS

Effects of NPP injection on SBP, DBP, and HR in sham-operated control rats

The injections of physiological saline and normal human inactivated plasma caused no changes in the SBP and HR of the rats pretreated with captopril (Fig. 1A).

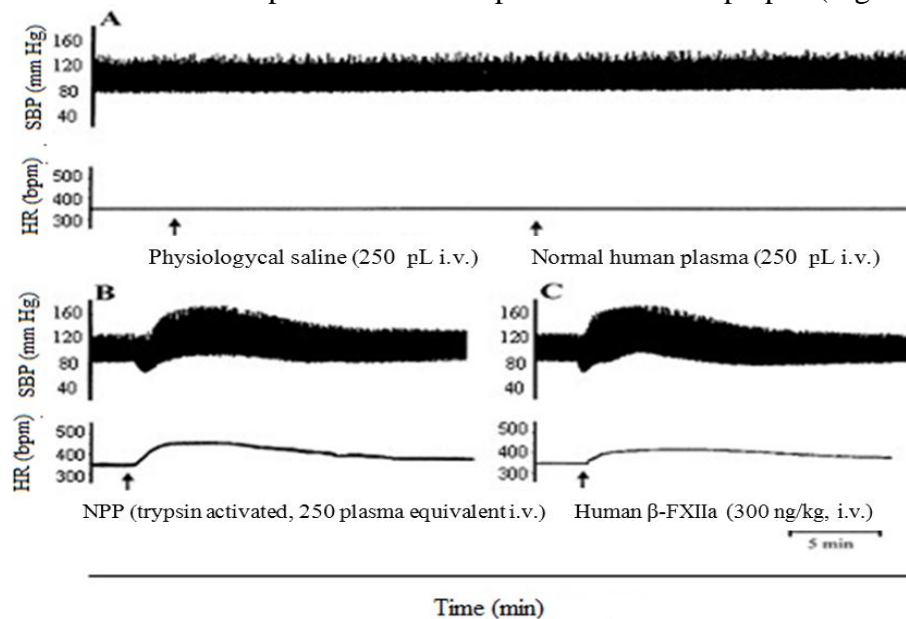


Fig. 1. (A) Representative SBP (mmHg) and HR (bpm) responses to physiological saline and normal inactivated human plasma; (B) NPP (20 µL plasma-activated equivalent, i.v.); and (C) coagulation β-FXIIa (300 ng/kg, i.v.); producing comparable sbp and hr responses in bioassay rats (~15 min). SBP, Systolic blood pressure; HR, heart rate; bpm; beats per minute.

However, NPP and purified human coagulation β -FXIIa exerted biphasic effects on SBP, including an initial, insignificant reduction followed by a more significant increase, followed by an increase in the HR, which lasted for approximately 15 min (Fig 1. B and C) (13).

Effects of captopril and losartan injection on SBP, DBP, and HR in sham-2NX rats

In the sham-2NX control rats, SBP (126.09 ± 4.01 mmHg), DBP (81.64 ± 4.38 mmHg), and HR (357.80 ± 13.78 bpm) were considered as the baseline parameters. According to the findings, losartan injection significantly

decreased SBP ($P = 0.002$) and DBP ($P = 0.000$). However, captopril injection only decreased DBP ($P = 0.038$) (Fig. 2A-C).

Effects of captopril and losartan injection on DBP, DBP, and HR in 2NX rats

In the 2NX rats, SBP (105.6 ± 8.21 mmHg), DBP (46.87 ± 5.69 mmHg), and HR (267 ± 4.16 bpm) were considered as the baseline parameters. According to the obtained results, losartan injection had no significant effects on these parameters, while captopril injection increased SBP ($P = 0.019$) and DBP ($P = 0.007$) (Fig. 3A-C).

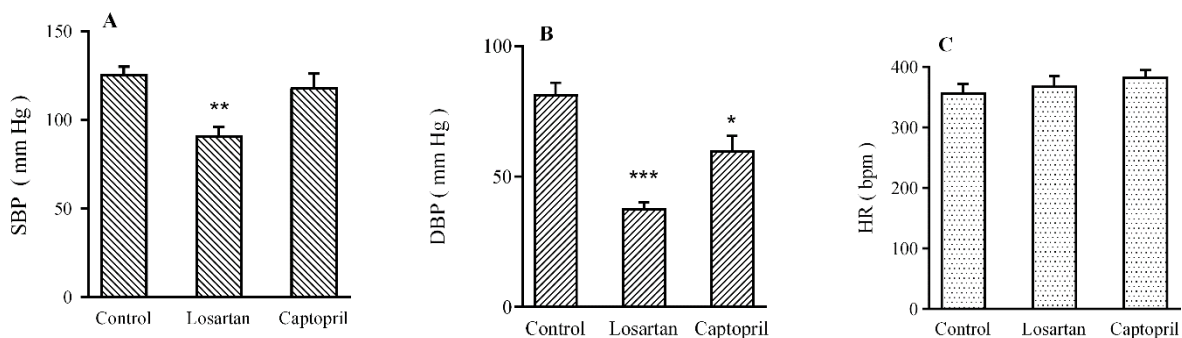


Fig. 2. Effects of losartan (10 mg/kg, i.v.) and captopril (2.5 mg/kg, i.v.) injection on (A) SBP (mmHg), (B) DBP (mmHg), and (C) HR (bpm) in sham-operated rats (significant differences between baseline and treatment groups. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ indicate significant differences in comparison with control group. Data are presented as mean \pm SEM; $n = 8-10$. SBP, Systolic blood pressure; DBP, diastolic blood pressure; HR, hart rate; bpm; beats per minute.

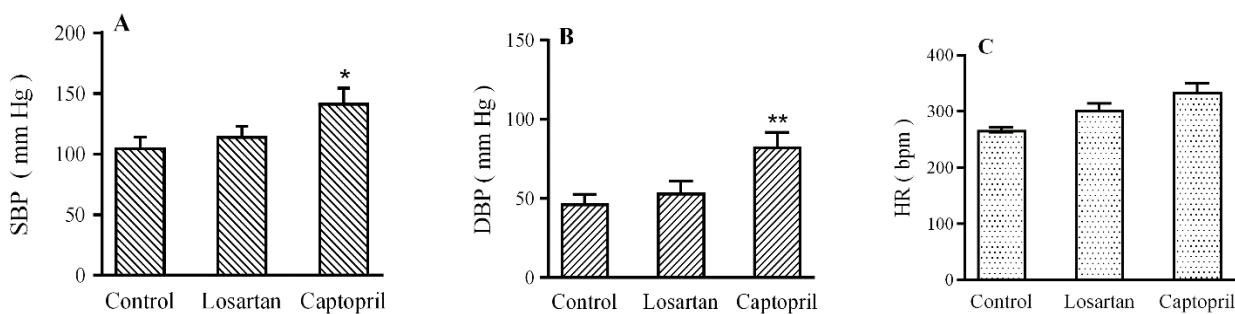


Fig. 3. Effects of losartan (10 mg/kg, i.v.) and captopril (2.5 mg/kg, i.v.) injection on (A) SBP (mmHg), (B) DBP (mmHg), and (C) HR (bpm) in 2NX rats. * $P < 0.05$, ** $P < 0.01$ indicate significant differences in comparison with control group. Data are presented as mean \pm SEM; $n = 8-10$. SBP, Systolic blood pressure; DBP, diastolic blood pressure; HR, hart rate; 2NX rats, bilaterally nephrectomized rats; bpm; beats per minute.

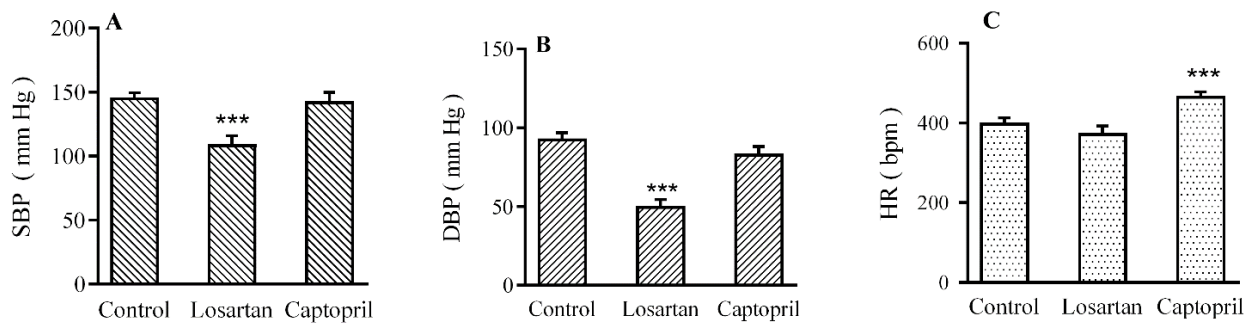


Fig. 4. Effects of human NPP (20 μ L plasma-activated equivalent, i.v.) on (A) SBP (mmHg), (B) DBP (mmHg), and (C) HR (bpm) in sham-2NX rats; captopril and losartan have been used, as pretreatments i.v. injection, at 2.5 and 10 mg/kg, respectively. Data are presented as mean \pm SEM; n = 8-10. *** P < 0.001 indicate significant differences in comparison with control group NPP, New pressor protein; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, hart rate; 2NX rats, bilaterally nephrectomized rats; bpm; beats per minute.

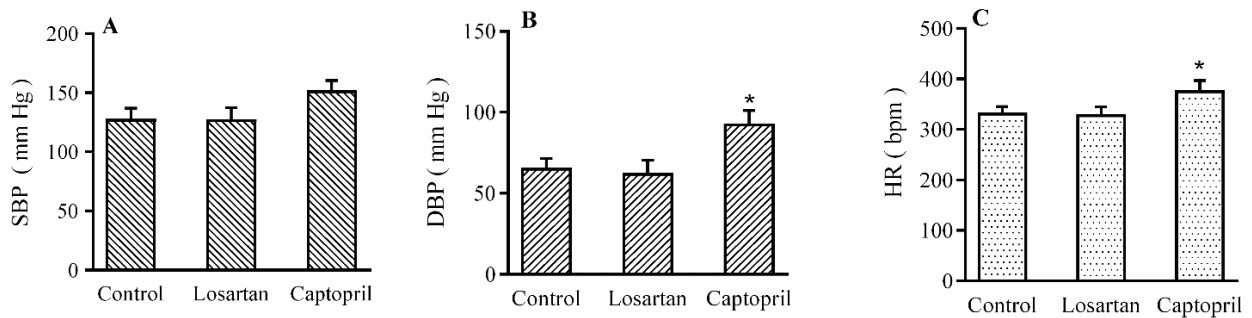


Fig. 5. Effects of human NPP (20 μ L plasma-activated equivalent, i.v.) on (A) SBP (mmHg), (B) DBP (mmHg), and (C) HR (bpm) in 2NX rats; captopril and losartan have been used, as pretreatments i.v. injection, at 2.5 and 10 mg/kg, respectively. Data are presented as mean \pm SEM; n = 8-10. * P < 0.05 indicate significant differences in comparison with control group NPP, New pressor protein; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, hart rate; 2NX rats, bilaterally nephrectomized rats; bpm; beats per minute.

Effects of captopril and losartan pretreatment on SBP, DBP, and HR responses to NPP injection in sham-2NX rats

In sham-2NX control rats, NPP injection was observed to increase SBP (145.99 \pm 3.6 mmHg), DBP (93.09 \pm 3.87 mmHg), and HR (400.29 \pm 12.78 bpm). Moreover, pretreatment with losartan caused a significant reduction in the SBP (P = 0.001) and DBP responses (P = 0.000) to NPP in the sham-operated rats. However, pretreatment with captopril only increased the HR response to NPP injection (P = 0.001) (Fig. 4A-C).

Effects of captopril and losartan pretreatment on SBP, DBP, and HR responses to NPP injection in 2NX rats

In the 2NXgroup, NPP injection was observed to increase SBP (127.89 \pm 9.03

mmHg), DBP (65.86 \pm 5.69 mmHg), and HR (333.35 \pm 11.47). However, pretreatment with losartan had no significant effects on the SBP, DBP, and HR responses to NPP in the 2NX rats, while pretreatment with captopril increased the DBP (P = 0.016) and HR (P = 0.036) responses to NPP injection (Fig. 5A-C).

DISCUSSION

Hypertension is a serious complication in chronic kidney disease and anephric patients. Several classes of drugs have been recommended for the treatment of hypertension. Use of renin-angiotensin inhibitors is considered to be a treatment option for blood pressure control. These agents may act through the direct inhibition of rennin, ACE or ARB. However, there is a controversy

regarding the effectiveness of ACE inhibitors and ARB in the treatment of hypertension and cardiovascular events. The present study aimed to compare the effectiveness of two renin angiotensin system inhibitors (captopril and losartan) in the NPP-induced hypertension model associated with FXIIa.

The intravenous injection of activated coagulation FXII, which is referred to as a novel NPP, has been reported to increase SBP, DBP, and HR in rats. Therefore, it could be inferred that it affects the heart and peripheral vasculature (4,5). Moreover, plasma catecholamines significantly increase when measured at the peak pressure response to NPP. The ratio of catecholamines also increases significantly in favor of adrenaline.

In the present study, the basal blood pressure was initially assessed in the sham-2NX and 2NX rats, and the effects of pretreatment with captopril and losartan were also investigated in the mentioned groups in baseline conditions. The baseline blood pressure in the sham-2NX rats was estimated at 126.09/81.64 mmHg, and the HR was calculated to be 357.8 bpm. As is depicted in Fig. 2, pretreatment with losartan significantly decreased SBP and DBP, while pretreatment with captopril only reduced DBP less significantly. However, this pretreatment had no effect on HR. Therefore, it could be concluded that RAS plays a key role in maintaining the blood pressure and vascular tone, and the role of losartan in reducing baseline blood pressure was found to be more significant compared to captopril.

In the current research, the baseline blood pressure in the 2NX rats was estimated at 105.6/46.89, and HR was determined 267 bpm. Hypotension in these rats could be attributed to various causes, especially the removal of the renal RAS, which plays a pivotal role in maintaining the blood pressure and shows the importance of renal renin, as well as the origin of the ACE in maintaining the blood pressure (14).

As is illustrated in Fig. 3, pretreatment with losartan had no significant effects on the blood pressure and HR, while pretreatment with captopril significantly increased SBP and DBP. Therefore, it could be inferred that in bilateral nephrectomy, captopril administration

exacerbates the blood pressure, further complicating the condition. The effect of captopril in this regard could be due to the increased concentrations of blood pressure peptides, which are normally destroyed by ACE or neutral endo peptidase (15). In this group of rats, neither losartan nor captopril caused significant change in the HR. These results indicate that after kidney removal, basal blood pressure and HR reduce, emphasizing on the importance of renal RAS in maintaining the vascular tone and blood pressure in a normal state.

In the present study, NPP injection was used to develop a hypertension model since in inflammatory or precoagulant conditions, the production of endogenous NPP/FXIIa increases (7,16). As it is depicted in Fig. 4, the NPP injection in the sham-2NX rats increased the blood pressure (145.99/93.09) and HR. The previous studies in this regard have denoted that peptides (e.g., bradykinin and pituitary adenylate cyclase activating polypeptide) may be involved in these effects through releasing adrenal catecholamines (17-19). In this group of rats, pretreatment with losartan significantly reduced SBP and DBP, while it had no effect on the HR. On the other hand, pretreatment with captopril only increased the HR significantly without affecting the blood pressure. Therefore, it could be concluded that pretreatment with losartan in the sham-2NX rats in baseline and NPP-mediated hypertension conditions could significantly decrease the blood pressure without affecting the HR, while captopril administration increased the HR without affecting the blood pressure.

In the 2NX rats in the current research, NPP injection led to the increased blood pressure (127.89/65.86). The previous findings in this regard have also denoted that NPP is involved in hypertensive and renal-impaired hemodialysis patients (7). Furthermore, the activation of factor XII plays a key role in the patients with chronic kidney transplantation (6) though increasing total peripheral resistance and releasing adrenal and non-adrenal catecholamines (4). In this group of rats, losartan had no effects on the blood pressure and HR, while captopril administration increased DBP and HR. Therefore, the results

of the present study in this regard indicated that in the 2NX rats, losartan had no effects on the blood pressure or heart rate. On the other hand, captopril increased DBP, which may deteriorate the condition. Recent studies have indicated that use of ACE inhibitors may increase the risk of lung cancer compared to ARB (20,21). Most of the previous data regarding NPP mechanisms have been based on the studies conducted on rats administered with ganglion blocker agents, while in the current research, we examined the effects of NPP on rats in non-ganglion blocking conditions since this condition is closer to the natural state of hypertensive rats, and use of ganglion blocker agents is not the routine method for the treatment of hypertension. Therefore, there are discrepancies between the preliminary data proposed by the previous studies in this regard and our findings (13).

CONCLUSION

According to the results, losartan administration in the sham-2NX rats could improve the effects of NPP-mediated hypertension. Therefore, it is considered to be a viable option for the treatment of hypertension in these animals although this agent had no effect on NPP-induced hypertension in the bilateral 2NX rats. On the other hand, captopril administration in the bilaterally nephrectomized rats was observed to deteriorate the condition. Therefore, it seems that new drugs should be found for the treatment of this type of hypertension in nephrectomized rats due to the fact that many chronic kidney disease (CKD) patients with no kidneys are not able to benefit from these drugs.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest for this study.

AUTHORS' CONTRIBUTION

A. Pejhan and M.I. Mustafa designed and performed experiments, P.C. Papageorgiou supervised the research, M.H. Rakhshani analyzed data, A. Gohari analyzed data and co-wrote the paper, and R. Gomohammadi designed experiments and co-wrote the paper.

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