Effect of Subchronic Administration of Captopril on α1-Adrenoceptor Agonist-Induced Contraction of Isolated Aorta in Rat

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ABSTRACT

Angiotensin II is a major endocrine hormone that affects directly both vascular smooth muscle and endothelial cells. Since vascular reactivity to angiotensin II changes in more physiological and pathophysiological conditions, the present study was performed to investigate the effect of intraperitoneal administration of angiotensin-converting enzyme inhibitor and captopril (30 and 50 mg kg^{-1}, once daily for 8 weeks) on contractile response of rat aorta. After 8 weeks, the treated rats were anesthetized, their thoracic aortas were excised and placed in a Petri dish filled with Krebs solution for recording of contraction and relaxation response. The obtained results showed that captopril did not modify body weight gain and food or water intake but contractile response of aortic rings to phenylephrine in treated rats with 30 and 50 mg kg^{-1} captopril, in the presence of endothelium, decreases about 11-22% and 29-32% ($P<0.05$-$P<0.01$), respectively, when compared to the controls. Denuded aortic rings from 30 and 50 mg kg^{-1} captopril-treated rats showed 11-21% and 7-11% decrease in contractile response, respectively. There was a marked endothelium-dependent relaxation response to acetylcholine in 50 mg kg^{-1} captopril-treated rats compared to the controls ($P<0.05$). Endothelium-independent relaxation response to isosorbide dinitrate showed no significant difference in all groups. According to these results, it is suggested that captopril exerts its relaxant effect directly and/or indirectly through endothelium by production and releasing of endothelium-derived relaxing factors. *Iran. Biomed. J. 8 (4): 193-198, 2004*

INTRODUCTION

Angiotensin converting enzyme (ACE) is a dipeptidyl carboxipeptidase which cleaves angiotensin I (AngI) to angiotensin II (AngII), a potent vasoconstrictor agent. It is well established that AngI produced by renin has no direct vasoconstrictor effect but it is converted into AngII by circulating and endothelial ACE [1-2]. This mechanism has a major importance in the regulation of vascular tone. However, the existing evidence suggest that the presence of a renin-angiotensin system in the vessel wall, the presence of renin in smooth muscle and endothelial cells [3] and angiotensinogen mRNA in the rat aorta adventitia and periaortic brown adipose tissue have

previously been reported [4]. It has also been suggested that the local production of AngII could play an autocrine or paracrine role on vascular smooth muscle cells [5]. The influence of AngII on vascular structure has widely been studied. AngII has been shown to stimulate DNA and protein synthesis in cardiovascular tissue and induced hypertrophy without cellular proliferation in cultured rat aortic smooth cells [6]. Overexpression of ACE has been shown in experimental models of atherosclerosis mechanical injury [7] and hyper-cholesterolemia [8] and human subjects [9-10]. The role of ACE in atherosclerosis is not limited to increased formation of AngII. This enzyme catalyses the breakdown of antiathero-sclerotic kinins-bradykinin and substance P [11-12]. The

multiple atherogenic effects of AngII can be diminished by the ACE inhibitors and AT1-receptor antagonists [13]. ACE activities in vascular tissues and the heart may be important targets in terms of the ability of ACE inhibitors to lower blood pressure or inhibit cardiac hypertrophy, respectively [14].

In addition, various substances containing thiol groups (-SH), such as glutathione, acetylcysteine or ACE inhibitors (captopril) can inhibit the free-radical production in atherogenesis [15-16] and have antioxidant properties [17]. However, the effect of ACE inhibitors on α1-adrenoceptor agonist-induced contraction of vascular smooth muscle is uncertain. The aim of this study was to assess the aortic contractile response after subchronic administration of two doses of captopril as ACE inhibitor containing thiol group in normal rat.

MATERIALS AND METHODS

Animals. Male Wistar rats (Animal House of Iran University of Medical Sciences), weighing 220-250 g, were used in this study. All groups of rats were maintained under standard housing conditions for a period of 8 weeks with free access to food and water. Body weight of all the rats was determined before and 8 weeks after intraperitoneal injection of saline or captopril. In this study, totally four groups of rats (n = 8) were used:

Group A) Control rats for obtaining the concerned normal values.

Group B) Vehicle-treated (VT) rats received saline (0.5 ml, once daily) intraperitoneally for 8 weeks.

Group C) Captopril-treated (CT) rats received captopril (30 mg kg^{-1}, once daily) intraperitoneally for 8 weeks.

Group D) CT rats received captopril (50 mg kg^{-1}, once daily) intraperitoneally for 8 weeks.

Preparation of aortic rings. Eight weeks after injection, the animals were weighed and anesthetized with diethyl ether followed by decapitation. Thoracic aortas were excised and trimmed free of adhering fat and connective tissues, then the aortic tissues were placed in a Petri dish filled with Krebs solution with the following composition (in mM): NaCl, 118.5; KCl, 4.74;

CaCl$_2$, 2.5; MgSO$_4$, 1.18; KH$_2$PO$_4$, 1.18; NaHCO$_3$, 24.9 and glucose, 10.0. The dissected aorta was cut transversely into rings of 3-4 mm in length. One ring of each pair was left intact and in the other ring, endothelium was mechanically removed. The rings with or without endothelium were mounted in an organ bath of 50 ml capacity filled with Krebs solution which was kept at 37°C and continuously bubbled with a 95% O$_2$ and 5% CO$_2$ gas mixture. Preparation was allowed to equilibrate for 60 min under a resting tension of 2 g. During the equilibration period, the solution of tissue bath was replaced every 30 min. Successful removal of the endothelium was confirmed by loss of acetylcholine (10^{-5} M)-induced relaxation in preconstricted rings by phenylephrine (10^{-6} M) [18]. To evaluate isosorbide dinitrate and acetylcholine-induced vasodilation, the rings with or without endothelium were preconstricted to their EC$_{80}$ value with phenylephrine to obtain a stable plateau and then the cumulative dose-response curve to isosorbide dinitrate and acetylcholine was obtained [19]. EC$_{80}$ values were calculated from the cumulative doses of phenylephrine that produced 80% of its maximal response for each aorta preparation with or without endothelium. Consecutive dose-response curves were taken at minimum 30-min intervals, during which the Krebs solution was changed at least three times. The responses of the aortic rings were recorded on a physiological recorder (Physiograph, MK-IV-P. Narco-Biosystems) using isometric transducer(F-60 myograph, Narco-Biosystem, INS. Houston, Texas).

Drugs. The following pharmacological agents were used: phenylephrine hydrochloride, isosorbide dinitrate, acetylcholine hydrochloride that were purchased from Sigma (St. Louis, Mo, USA) and captopril was supplied from Arastou company (Tehran, Iran)

Data analysis. The contractile responses of aortic rings to phenylephrine with or without endothelium were expressed as grams of tension per milligram of tissue and relaxation responses for acetylcholine and isosorbide dinitrate were expressed as a percentage decrease of the maximum contractile response induced by phenylephrine. The sensitivity to the agonist was evaluated as EC$_{50}$, which is the negative logarithm of the concentration of the drug required to produce 50% of maximum response. All values were given as means ±S.E.M. Statistical significance was indicated by a *P*<0.05, which was obtained from paired and unpaired students's *t*-test.

RESULTS

Body weight, serum glucose and cross-sectional area. No marked alteration in body weight, food or water intake was observed following two-month administration of captopril (30 and 50 mg kg^{-1}) compared to vehicle-treated and control groups. Body weight has been shown in Table 1.

Table 1. Body weight of vehicle- and captopril-treated rats.

| Groups	Week 0	Week +8
Vehicle-treated	205.0 ± 7.5	247.6 ± 4.4
Captopril-treated (30 mg kg^{-1})	201.7 ± 6.3	278.3 ± 6.8
Captopril-treated (50 mg kg^{-1})	217.8 ± 5.1	261.4 ± 4.8

Data are represented as mean $\pm$ SEM.

Vascular reactivity. Cumulative dose-response curves to phenyephrine (10^{-9} -10^{-4} M) from aortic rings with or without endothelium have been shown in Figures 1A and 1B, respectively. The treatment with 30 and 50 mg kg^{-1} captopril for a period of 8 weeks caused a 11-22% and 29-32% reduction in the maximum contractile response to phenylephrine in aortic rings with endothelium compared to controls, respectively (Fig. 1A). Denuded aortic rings from rats treated with 30 and 50 mg kg^{-1} captopril for 8 weeks showed a 11-21% and 7-11% decrease in contractile response, respectively when compared to the controls (Fig. 1B). Aortic rings with intact endothelium from captopril-treated group (50 mg kg^{-1}) showed a significant decrease ($P<0.05$) in contractile response to PE only at concentrations higher than 10^{-5} when compared to vehicle-treated group (Fig. 1A).

Endothelium-dependent relaxation response. Cumulative dose-response curve to acetylcholine (10^{-9} -10^{-4} M) from aortic rings with endothelium has been shown in Figure 2A. Endothelium-dependent relaxation response of aortic rings from rats treated with 30 and 50 mg kg^{-1} captopril for 8 weeks increased about 3-5% and 5-8%, respectively when compared to the controls.

Although there was a marked and greater relaxation response to acetylcholine in captopril-treated group, especially at a dose of 50 mg kg^{-1} for captopril, as compared to vehicle-treated ones, the existing difference did not reach a significant level.

Endothelium-independent relaxation response. Cumulative dose-response curve to isosorbide dinitrate (10^{-9} -10^{-4} M) from aortic rings without

(A)

(B)
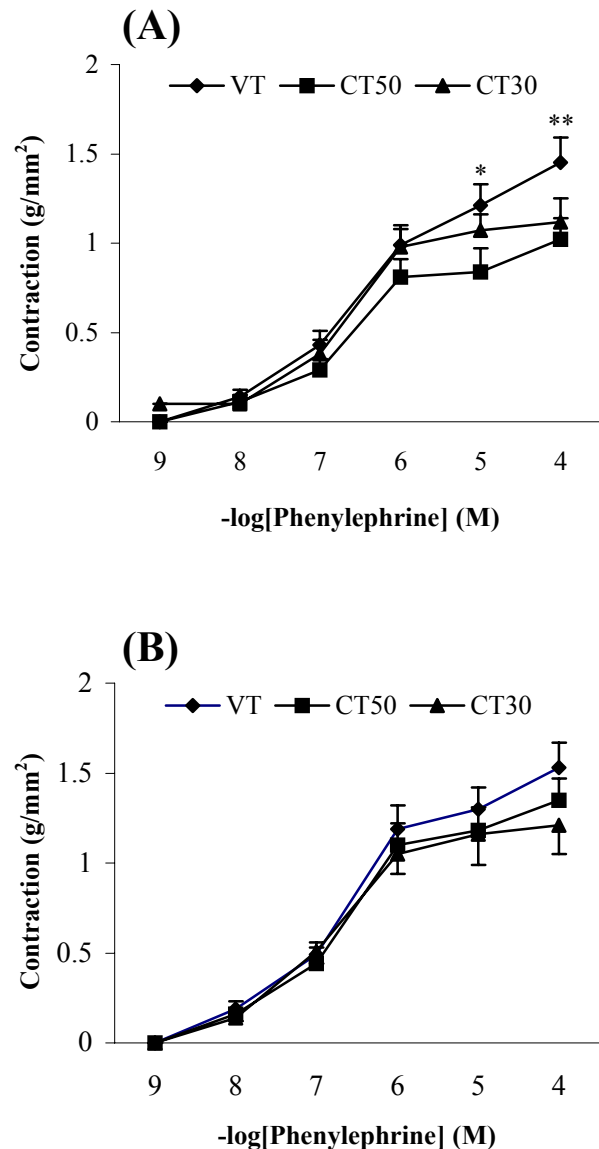
Fig. 1. Cumulative concentration-response curves for phenylephrine in aortic preparations two months after treatment with captopril. Contractile responses are expressed as grams of tension per cross-sectional area (mm^2). Captopril has been administered at a dose of 30 and 50 mg kg^{-1} in the presence **(A)** and absense **(B)** of endothelium. Data are shown as means $\pm$ SEM. VT, vehicle-treated; CT, captopril-treated; $^*P<0.05$, $^{**}P<0.01$ (Compared to VT).

endothelium has been shown in Figure 2B. Endothelium-independent relaxation response of aortic rings showed no considerable difference in all groups.

Furthermore, maximum responses (E$_{max}$) of aortic rings from control and captopril-treated rats (captopril at a dose of 50 mg kg^{-1}) and their sensitivity expressed as pD$_2$, to above-mentioned vasoactive agents have been shown in Table 2.

(A)

(B)
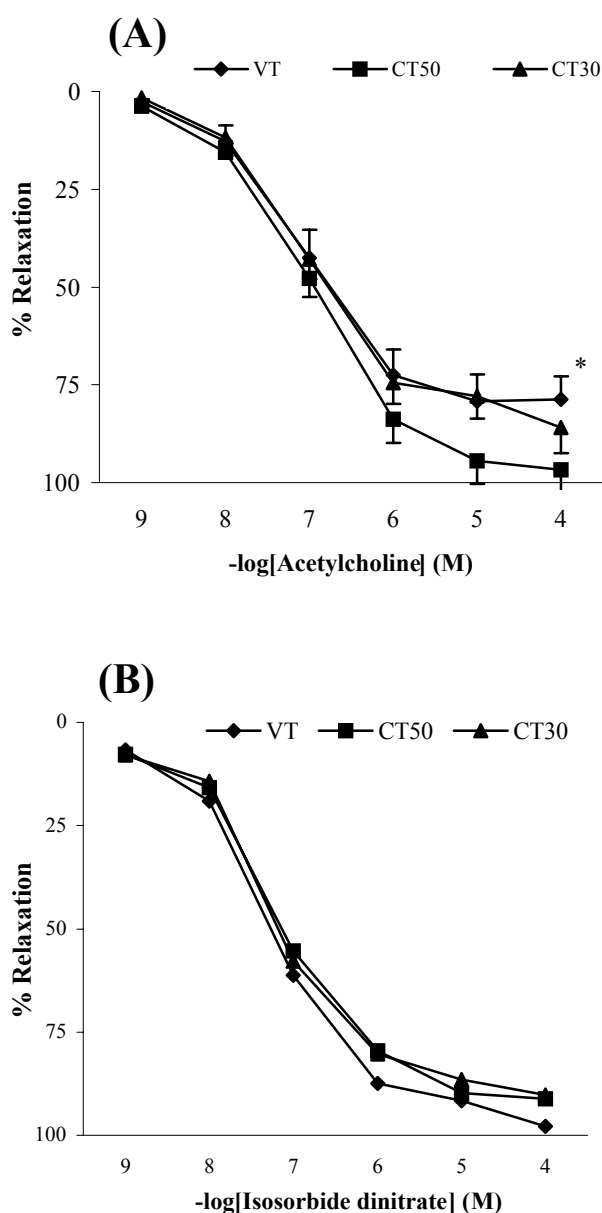
Fig. 2. Cumulative concentration-response curves for acetylcholine and isosorbide dinitrate in aortic rings precontracted with phenylephrine. **(A)**, Relaxation responses to acetylcholine and **(B)**, isosorbide dinitrate two months after the treatment with 30 and 50 mg kg^{-1} captopril.Relaxation responses are expressed as a percentage. Data are shown as means ± SEM. VT, vehicle-treated; CT, captopril-treated; *$P<0.05$ (Compared to VT).

In this respect, there was no significant difference among the groups regarding pD_2.

Dose-dependent effect of captopril on vascular contractile and relaxation response. The results showed that the attenuating effect of captopril on contractile response of aorta was dose-dependent, so that the percent of decrease of $\alpha 1$-adrenoceptor agonist-induced contraction response of aorta rings in 50 mg kg^{-1} captopril-treated rats was more than 30 mg kg^{-1} captopril-treated rats. On the other hand, there was no significant difference in endothelium-dependent and independent relaxation response of aortic rings to acetylcholine and isosorbide from rats treated with 30 and 50 mg kg^{-1} captopril (Figs. 1 and 2).

DISCUSSION

The goal of the present study was to investigate the effect of subchronic administration of different doses of captopril on $\alpha 1$-adrenoceptor agonist-induced contraction of isolated aorta in an *in vivo* model. The results showed that captopril reduces phenylephrine-induced contraction re-sponse of isolated aorta and accentuates its endo-thelium-dependent relaxation response to acetyl-choline. Furthermore, the attenuating effect of captopril on phenylephrine-induced contraction response was dose-dependent.

ACE inhibitors in addition to their hemodynamic activities have advantageous vascular structural and functional effects. Several mechanisms could explain the beneficial effects of captopril on the functional reactivity of vascular system. It has been shown that captopril not only decreases the AngII level, but also causes synthesis and releasing of variety vasodilators through endothelium such as prostacyclin, bradykinin and endothelium-derived nitric oxide [20, 21]. Moreover, captopril increases plasma level of prostaglandin E2 in human by stimulation of phospholipase A2 [22]. A possible mechanism by which captopril administration can

Table 2. Maximum responses (E_{max}) and pD_2 values of agonists in aortic rings from vehicle- and captopril-treated (50 mg kg^{-1}) rats.

Groups	pD_2			E_{max}		
	Phenylephrine	Acetylcholine		Phenylephrine	Acetylcholine	
	E +	E -		E +	E -	
Vehicle-treated	6.48 ± 0.28	6.63 ± 0.23	6.89 ± 0.12	1.45 ± 0.17	1.53 ± 0.17	81.6 ± 7.9
Captopril-treated (50 mg kg^{-1})	6.61 ± 0.32	6.54 ± 0.19	7.23 ± 0.21	1.02 ± 0.16**	1.35 ± 0.19	87.9 ± 8.6*

Data are expressed as mean ± SEM. For phenylephrine and acetylcholine, maximum responses (E_{max}) are presented as g/mm^2 and percentage decrease of the maximum contractile response induced by agonists, respectively. E+, (mean) with endothelium; E- (mean) without endothelium. *$P<0.05$, **$P<0.01$.

improve the vascular reactivity may be dependent on inhibiting the oxidative stress [17]. Consistent with this idea, it has been shown that ACE inhibitors could inhibit lipid peroxidation in sera and aorta of rabbits in diet-induced hyper-cholesterolemia [23]. In addition, captopril treatment increases anti-oxidant enzymes (superoxide dismutases), non-enzymatic antioxidant defenses and glutathione content in several mouse tissues [24]. However, whether the lipid peroxi-dation-lowering effect of captopril results from its direct superoxide scavenging properties or indirectly increasing NO synthesis is controversial, since NO has also antioxidant activity per se and the presence of thiol groups in captopril structure potentiates its scavenging properties [25]. In addition, the ameliorating effect of captopril on the vascular responsiveness may closely be related to its anti-hypertensive activity. One of the mechanisms by which captopril lowers the blood pressure may be due to its ability to modulate the levels of G-proteins and adenylyl cyclase activity [26] or through a stiffness-decreasing and dilated effect on resistance arterioles [27]. It is also believed that captopril may exert a direct nitrovasodilatory effect on smooth muscles [28]. Moreover, since captopril could exert a part of its relaxant effect in the absence of endothelium [29] therefore, it is possible that anti-hypertensive action of captopril is mediated through a direct relaxant effect on smooth muscle or by interfering with the production and/or release of vasodilators or vasoconstrictors derived from smooth muscle. In conclusion, the obtained findings clearly showed that captopril could attenuate the α_1-agonist-induced contraction of rat aorta and produce a direct or an indirect vasorelaxant effect. Since it can reduce the risky factors of some cardiovascular disorders including hypertension, therefore, it is highly recommended that captopril may be administered as a complementary therapeutic regimen for patients with cardiovascular abnormalities. Further studies are to be undertaken to investigate the possible beneficial effect of captopril administration and the related mechanisms for its efficacy.

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