B2-receptor modulation of the reactivity to phenylephrine and angiotensin II in the carotid artery of normotensive rats after trandolapril treatment

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Abstract

This study was designed to study the effects of angiotensin converting enzyme inhibitors (ACEI) following treatment with trandolapril (0.3 mg kg⁻¹ day⁻¹) on carotid arterial responsiveness in normotensive Wistar rats. Carotid arteries were obtained from control or trandolapril-treated animals and mounted in an isolated organ bath. Reactivity to angiotensin II (Ang II), phenylephrine (Phe) and KCl was studied. Agonist concentration-response curves were constructed in either the absence or presence of the endothelium or after incubation with L-NAME (10⁻⁴ M), HOE140 (10⁻⁷ M) or indomethacin (10⁻⁵ M). Trandolapril treatment decreased the Ang II and Phe potencies in carotid arteries, but did not affect the maximal response. The KCl responses (potency and Emax) were similar in both control and trandolapril-treated arteries. The absence of endothelium increased the response to both agonists in control and trandolapril-treated arteries; however, the inhibitory component from the endothelial layer of the Phe response was greater in trandolapril-treated animals than in control animals. The presence of L-NAME or HOE140 abolished the changes in the potency values of trandolapril-treated animals. The presence of indomethacin did not change the effect of trandolapril on the potency values of both agonists. We conclude that trandolapril treatment decreased the carotid arterial reactivity in normotensive rats and that this effect is endothelium-dependent. Furthermore, the involvement of B2-receptors and NO production, but not of prostaglandins, is suggested in this mechanism.

Key words: angiotensin II, bradykinin, carotid, phenylephrine, trandolapril

Introduction

Ang II plays a fundamental role in the control of the functional and structural integrity of the arterial wall and may be important for physiological processes regulating blood pressure and the
pathological mechanisms underlying vascular disease (Timmermans et al., 1993). The control of vascular tone is achieved mainly by sympathetic nervous input (Calzada and Artinano, 2001). However, Ang II can modulate sympathetic neural function by facilitating peripheral sympathetic function through multiple mechanisms, which include increase in catecholamine biosynthesis and release from adrenal medulla, stimulation of ganglionic cells and attenuation of pre-junctional catecholamine reuptake (Timmermans et al., 1993).

ACEI have been widely used in the treatment of hypertension, myocardial infarction and congestive heart failure. They also retard the development of chronic renal failure, diabetic nephropathy and atherosclerosis in experimental model and may be advantageous in the improvement of endothelial dysfunction in patients with coronary artery disease (Chobanian et al., 1990; Mancini et al., 1996; Omata et al., 1996). ACEI exert numerous actions by reducing both Ang II generation and bradykinin (BK) breakdown (Tschope et al., 2002), however, the mechanisms underlying their pharmacological effects are not fully understood.

ACEI can also affect the reactivity of vessels. ACEI increase BK-induced relaxation (Gohlkeet al., 1995), increase the release of endothelium-derived nitric oxide (NO) (Auch-Schwelk et al., 1993), decrease the production of endothelin-1 (Itoh et al., 2002) and can have a free radical scavenging action (Chopra et al., 1992). The acute and chronic administration of ACEI can attenuate the contraction responses of aortic rings of renal hypertensive rats to alpha-adrenergic agonists in vitro (Kikta et al., 1983) and prevent functional changes in vascular reactivity in diabetic rats (Baluchnejadmoharad et al., 2004).

Trandolapril is a non-sulphhydryl ACEI and acts as a peripheral vasodilator, causing a long lasting decrease in blood pressure with decreased vascular resistance, mainly in renal and splenic vessels, without affecting cardiac output (Richer et al., 1987). The present study was undertaken to assess whether trandolapril treatment has an effect on adrenergic and Ang II-mediated responses in the carotid artery of normotensive rats.

**Materials and methods**

Male Wistar rats of 18-21 weeks of age were used in this study. The animals were fed a commercial diet, had water ad libitum and were kept under a 12 h light:12 h darkness schedule (light from 06:00 to 18:00h) at a room temperature of 25°C. All experimental procedures conformed to the International Guidelines and Ethical Animal Committee of the Ribeirão Preto Campus, University of São Paulo, Brazil.

Trandolapril (treated group), or the vehicle alone (control group), was administered (i.p.) for a period of 6 days to ensure that it had reached a steady state in the plasma. This period was selected on the basis of the study by Bjornsson et al. (1997) who concluded that the plasma concentration steady state of any drug is reached after 5 times the t1/2 for a single daily dose and taking into consideration that the t1/2 of tradaloprilat (the active component of trandolapril) is 24 hours (Conen and Brunner, 1993; Wiseman and McTavish, 1994). The trandolapril was dissolved in ethanol:water (1:10, v/v) and injected at 0.3 mg kg⁻¹ day⁻¹ (determined after measurement of the ACE activity). Both plasma and tissue ACE activities were measured by a method previously described by Ryan et al. (1977), utilizing Hip-His-Leu as the substrate.
Briefly, under general anesthesia blood samples were collected for measuring serum ACE activity and the rats were then sacrificed.

A segment of carotid was rapidly removed, cleaned of fat and connective tissue, blotted dry and then weighed. Sodium borate buffer (0.4 M, pH 8.3), containing [glycine-1-^14^C] - hippuryl-L-hisitidy1-leucine and hippuryl-L-hisitidy1-leucine (Amersham-England) as substrate, was added to the samples (plasma and tissue), which were then incubated at 37°C for 30 min with constant shaking. The reaction was stopped by addition of 1N HCl and water-saturated ethyl acetate and the samples then centrifuged at 2,000 rpm for 5 min. The supernatant was removed and 5 ml of a mixture of 67% toluene, 33% triton 100, 0.2% 2,5-diphenyloxazole (PPO) and 0.05% 1.4-bis(5-phenyloxasol-2-y1)benzene (POPOP) was added. The amount of [^14^C] Hip-His-Leu liberated from the substrate was analyzed in a Scintillation Analyzer (1500 Tri-Carb, Packard) and the results were expressed as nmol Hip-His-Leu/ml plasma or nmol Hip-His-Leu/mg of tissue.

For vascular studies, both trandolapril or vehicle treated animals were anaesthetized with tribromoethanol (2.5 mg kg^-1, _i.p._) and sacrificed. The carotid arteries were removed and immediately placed in Krebs-Henseleit solution, which was composed of the following (mmol/L): NaCl 118.4; KCl 4.7; CaCl_2 2.5; KH_2PO_4 1.2; MgSO_4.7H_2O 1.2; NaHCO_3 25; C_6H_12O_6 11.6; pH 7.4, at 37°C. Carotid artery rings (4 mm long) were mounted in organ chambers, bubbled with a mixture of 95% O_2 and 5% CO_2, then connected to force transducers (Letica, Spain). In some preparations, the endothelium was mechanically removed. Rings were stretched in a stepwise fashion to the optimal point of their tension curves, previously determined as being 1 g (data not shown) and the arteries were allowed to equilibrate for 60 min. The absence of endothelium was confirmed by the lack of relaxation to ACh (10^-6 M) when the artery was pre-contracted by phenylephrine (Phe) (10^-7 M).

Concentration-response curves were obtained for Phe (10^-10-10^-5 M), Ang II (10^-11-10^-6 M) or KCl (4.7-120 mM). In certain experiments the preparations were incubated with indomethacin (an inhibitor of prostanoids synthesis) at a concentration of 10^-5 M for 45 min, N-nitro-L-arginine methyl ester (L-NAME; an inhibitor of NO synthesis) at a concentration of 10^-6 M for 30 min or D-arginyl-L-arginyl-L-propyl-trans-4-hydroxy-L-propylglycyl-3-(2-thienyl)-L-alanyl-L-seryl-D-1,2,3,4-tetrahydro-3-isoquionolinecarbonyl-L-(2a, 3b, 7aβ)-octahydro-1H-indole-2-carbonyl-L-arginine (HOE140; a bradykinin receptor antagonist) at a concentration of 10^-7 M for 60 min. These concentrations are reported as the final molar concentration in the organ chamber solution. Results were expressed as g of tension and they were plotted as the mean ± SEM of observations from 5-14 animals.

Estimates of EC_50 and the maximum effect (Emax) were determined from each concentration-response curve using the Prism software package (version 2.0, Graph Pad Software Inc., USA). The pD_2 values (-log EC_50) were used because their distribution was normal and represent the agonist potency (Kenakin, 1996). Results are expressed as the mean ± S.E.M. For these results, mean values were compared statistically using the unpaired "student-t" test (version 2.0, Graph Pad Software Inc., USA).

Drugs used were: Trandolapril (Knoll, USA), Phe (Sigma, USA), Ang II (Sigma, USA), L-NAME (Calbiochem, USA), indomethacin (Calbiochem, USA), HOE140 (Sigma, USA).
Results

Effect of trandolapril treatment on ACE activity

Figure 1 demonstrates the ACE activity in both the plasma and the carotid artery following trandolapril treatment. Trandolapril, administered at 0.3 mg kg⁻¹day⁻¹ for a period of 6 days, decreased the activity in both the plasma and the carotid artery.

Effect of trandolapril treatment on muscular contraction

To analyse whether the trandolapril treatment had affected the contraction of the muscular layer, the KCl effect was studied on the vessels without endothelium from both control or trandolapril-treated animals. KCl (4.7–120 mM) induced a concentration-dependent contraction in the carotid arteries from both groups (Fig. 2). No difference in potencies (−1.39 ± 0.03 and −1.40 ± 0.03, control and trandolapril-treated group, respectively) or Emax values (0.37 ± 0.02 g and 0.34 ± 0.02 g, control and trandolapril-treated group, respectively) was observed in either group.

Effect of trandolapril treatment on the arterial responsiveness to Phe or Ang II

Chronic treatment with trandolapril in vivo caused a parallel rightward displacement of the contraction-response curves for both Phe and Ang II in isolated carotid arteries with endothelium. The trandolapril treatment decreased the pD₂ values for both Phe and Ang II, but it did not affect the maximal response to either agonist (Figs. 3A and B; Tables 1 and 2). Hill's slopes were not different from unity.

Effect of the absence of the endothelium on the arterial responsiveness to either Phe or Ang II after trandolapril treatment

To determine the involvement of endothelium-derived relaxing factors in the response to either Phe or Ang II in carotid arteries from trandolapril-treated animals, the endothelium was mechanically removed from the arterial rings before the experiments commenced. In both the arteries from the control and trandolapril-treated groups in the absence of endothelium, there was an increase in the pD₂ values for Phe compared with arteries with an intact endothelium.
Role of bradykinin in carotid responsiveness

Fig. 2. Concentration–dependent contraction curves for KCl in isolated carotid arteries without endothelium from either control or trandolapril-treated animals. Values are expressed as the mean ± SEM and represent 6–10 animals.

Fig. 3. Concentration–dependent contraction curves for Phe (A) and Ang II (B) in isolated carotid arteries with intact endothelium from either control or trandolapril-treated animals. Values are expressed as the mean ± SEM and represent 6–10 animals.

from both control and trandolapril-treated groups (Fig. 4A, Table 1). However, the contribution of the endothelium was different between control and trandolapril-treated groups. The endothelial inhibitory component of the response to Phe (defined as the difference in areas under the concentration–dependent curves obtained in preparations with or without endothelium) was greater in the trandolapril-treated preparations than those of the control artery (0.18 vs 0.09 arbitrary units, respectively). Ang II potency (pD2 value) was increased after
endothelium removal only in arteries from trandolapril-treated animals (Fig. 4B, Table 1). The Emax for both Phe and Ang II were also increased following removal of the endothelium (Figs. 4A and B; Table 2). The Hill’s slopes were not different from unity.

**Effect of indomethacin on the arterial responsiveness to Phe or Ang II after trandolapril treatment**

The presence of indomethacin (10⁻⁵ M), an inhibitor of prostanoids synthesis, decreased the Phe pD₂ value in control arteries with endothelium, but did not influence the potency in trandolapril-treated arteries with endothelium (Fig. 5A, Table 1). There was no difference in the Emax values for Phe between the two groups (Fig. 5A, Table 2). Indomethacin also decreased both the potency and Emax for Ang II in control arteries, but it increased the pD₂ value for Ang II in arteries from treated animals (Figs. 5A and B; Tables 1 and 2). Hill’s slopes were not different from unity.

**Table 1.** pD₂ values for both Phe and Ang II in isolated carotid artery preparations following trandolapril treatment

<table>
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<tr>
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<th>Phe</th>
<th>Ang II</th>
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<tr>
<td></td>
<td>Control</td>
<td>Trandolapril</td>
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<tr>
<td><strong>pD₂ values</strong></td>
<td></td>
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<tr>
<td>Without endothelium</td>
<td>8.64 ± 0.19 (10)</td>
<td>6.55 ± 0.13* (10)</td>
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<tr>
<td>pD₂ values</td>
<td>7.50 ± 0.20** (9)</td>
<td>7.07 ± 0.10** (6)</td>
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<tr>
<td>With indomethacin (10⁻⁵ M)</td>
<td>7.58 ± 0.12** (14)</td>
<td>6.90 ± 0.11 (8)</td>
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<tr>
<td>pD₂ values</td>
<td>8.36 ± 0.51 (11)</td>
<td>7.87 ± 0.22** (8)</td>
</tr>
<tr>
<td>With HOE140 (10⁻⁷ M)</td>
<td>7.87 ± 0.28** (9)</td>
<td>8.36 ± 0.22** (7)</td>
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* as respective control group, ** vs respective control or trandolapril group. Unpaired “Student-t” test (P<0.05). The numbers in parenthesis are the number of animals used in the experiments.

**Table 2.** Emax values (g) for both Phe and Ang II in isolated carotid artery preparations following trandolapril treatment

<table>
<thead>
<tr>
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<th>Phe</th>
<th>Ang II</th>
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<td></td>
<td>Control</td>
<td>Trandolapril</td>
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<tr>
<td><strong>Emax (g)</strong></td>
<td></td>
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<tr>
<td>Without endothelium</td>
<td>0.29 ± 0.01 (10)</td>
<td>0.33 ± 0.02 (10)</td>
</tr>
<tr>
<td>Emax (g)</td>
<td>0.47 ± 0.04* (9)</td>
<td>0.59 ± 0.05* (6)</td>
</tr>
<tr>
<td>With indomethacin (10⁻⁵ M)</td>
<td>0.33 ± 0.02 (14)</td>
<td>0.32 ± 0.02 (8)</td>
</tr>
<tr>
<td>Emax (g)</td>
<td>0.34 ± 0.03 (11)</td>
<td>0.45 ± 0.01* (8)</td>
</tr>
<tr>
<td>With HOE140 (10⁻⁷ M)</td>
<td>0.32 ± 0.04 (9)</td>
<td>0.33 ± 0.03 (7)</td>
</tr>
</tbody>
</table>

* as respective control or trandolapril group. Unpaired “Student-t” test (P<0.05). The numbers in parenthesis are the number of animals used in the experiments.
Role of bradykinin in carotid responsiveness

Fig. 4. Concentration–dependent contraction curves for Phe (A) and Ang II (B) in isolated carotid arteries without endothelium from either control or trandolapril-treated animals. Values are expressed as the mean ± SEM and represent 6–12 animals.

Fig. 5. Concentration–dependent contraction curves for Phe (A) and Ang II (B) in isolated carotid arteries with intact endothelium and indomethacin (10⁻⁵ M) from either control or trandolapril-treated animals. Values are expressed as the mean ± SEM and represent 5–14 animals.

Effect of L-NAME on the arterial responsiveness to Phe or Ang II following trandolapril treatment

Inhibition of NO synthesis in vitro, using L-NAME, increased the Emax only for Phe and Ang II in carotid arteries with endothelium from control animals. Furthermore, L-NAME increased the potency and Emax for Phe in carotid arteries from trandolapril-treated animals (Figs. 6A and B; Tables 1 and 2).
Fig. 6. Concentration–dependent contraction curves for Phe (A) and Ang II (B) in isolated carotid arteries with intact endothelium and following incubation with L-NAME (10^{-5} M) from either control or trandolapril-treated animals. Values are mean ± SEM and represent 5–11 animals.

Fig. 7. Concentration–dependent contraction curves for Phe (A) and Ang II (B) in isolated carotid arteries with intact endothelium and HOE140 (10^{-7} M) from either control or trandolapril-treated animals. Values are expressed as the mean ± SEM and represent 5–9 animals.

Effect of HOE140 on the arterial responsiveness to Phe or Ang II following trandolapril treatment

The B2-receptor antagonist HOE140 was used to test the possibility that BK participated in the trandolapril effect on the potencies of Ang II and Phe. The presence of the B2-antagonist *in vitro* did not affect the contraction of arteries from control animals to either Phe or Ang II. The presence of HOE140 restored the potencies of both Phe and Ang II in carotid artery
preparations from trandolapril-treated rats to similar values as those found in the control group (Figs. 7A and B; Tables 1 and 2).

Discussion

The present study provided evidence that treatment with the ACEI, trandolapril, decreases the potencies of both Phe and Ang II (pD2 values from concentration-effect curves) in isolated carotid arteries from normotensive animals. Several factors can influence the pD2 value including, receptor affinity, endothelium-derived molecules, second messengers, drug access to receptors and agonist degradation systems. To evaluate whether trandolapril treatment may have affected the desensitization-induced contraction of smooth muscle, we studied the effect of trandolapril treatment on KCl-induced contraction of carotid arteries without endothelium. Our data showed that trandolapril treatment did not influence the KCl response, thus suggesting that the ACEI effects on the potencies of both Phe and Ang II could be endothelium-dependent.

We then investigated the possible involvement of endothelium-derived relaxing factors in the trandolapril effect. First, we removed the endothelium from the arteries and the results showed that the arterial reactivity to both Phe and Ang II was increased in the absence of endothelium. The pD2 and Emax values were increased for each of these agonists in preparations from both control and treated rats. Indeed, the endothelial-related inhibitory component of the Phe response was greater in trandolapril-treated arteries than in control arteries, thus suggesting that trandolapril increased the participation of endothelium-derived factors in the final vascular response to Phe.

To analyse which endothelium-derived relaxing factor was responsible for the decrease in the potencies of both Phe and Ang II, we investigated the possible role of prostanoids and NO in the responses to both agonists. Inhibition of prostanoid synthesis decreased the responses to Phe and Ang II in arteries from control animals, but it did not change either the Phe or Ang II effects in carotid arteries from trandolapril-treated animals, suggesting that the trandolapril treatment has blocked prostaglandin modulation of vascular reactivity to both Phe and Ang II. These results are in accordance with a previous report by Vidal et al. (1994).

To examine the possible role of NO in the trandolapril effect, we inhibited NO synthesis in vitro using L-NAME, an inhibitor of NOS. L-NAME increased the potencies of both agonists in carotids from trandolapril-treated rats, thus suggesting that NO could be the endothelium-derived relaxing factor related to the trandolapril effect.

The clinical benefits of ACEI administration, apart from blocking Ang II release and prolonging the half-life of BK, can also involve the direct effects on BK receptors due to cross-talk between ACE and B2-receptors (Tschope et al., 2002). Several investigators have found that ACEI increase the effects of BK and also those of ACE-resistant analogues on B2-receptors. It was concluded that ACEI augmented the response to BK via B2-receptors, independently of blocking its enzymatic degradation (Auch-Schwelk et al., 1993; Hecker et al., 1997; Hecker et al., 1994). It has been suggested that ACEI can also abolish receptor desensitisation (Marcic et al., 1999), or delay their sequestration (Minshall et al., 1997; Erdos et al., 1999) and thereby potentiate the actions of BK. It has recently been proposed that ACEI directly activates B2-
receptors even in the absence of ACE (Ignjatovic et al., 2002).

Based on the above evidence, we studied the possible involvement of BK in the trandolapril treatment effect on both Phe and Ang II reactivity in the carotid artery of normotensive rats. The addition of the selective B2-receptor antagonist, HOE140, increased the potencies of both agonists only in arteries from trandolapril-treated rats suggesting that trandolapril decreased both Phe and Ang II potencies through a BK-dependent mechanism.

In conclusion, we have demonstrated that trandolapril treatment (0.3 mg Kg\(^{-1}\) day\(^{-1}\)) for a period of 6 days decreased the potencies of both Phe and Ang II in the carotid artery. This effect was not related to a change in depolarization-induced contraction of the smooth muscle but was endothelium-dependent. Additionally, it may be postulated that these trandolapril effects are BK-related and also involve stimulation of NO production.

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