Endogenous Angiotensin II Enhances Phenylephrine-Induced Tone in Hypertensive Rats

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Abstract  Subthreshold concentrations of angiotensin II (Ang II) potentiate agonist-induced tone in a variety of blood vessels. We measured in vivo the mesenteric artery diameter and blood flow in 12-week-old normotensive Wistar-Kyoto (WKY) rats (n=20) and spontaneously hypertensive rats (SHR, n=20); systemic blood pressure was monitored continuously. Phenylephrine (10 μmol/L) superfused on the exteriorized mesentery reduced arterial diameter from 480±40 to 256±18 μm (P<.05) in WKY rats and from 562±26 to 273±7 μm (P<.05) in SHR, whereas blood flow was lowered by 77% in WKY rats and 76% in SHR (P<.05 in both strains). Topical superfusion of an angiotensin-converting enzyme inhibitor (perindoprilat, 10 and 100 μmol/L) attenuated the phenylephrine-induced decrease in diameter and blood flow in both strains (P<.05). The Ang II type 1 receptor blocker losartan (10 μmol/L) attenuated the phenylephrine-induced decrease in diameter and blood flow in both strains (P<.05). The relaxing effect of losartan was significantly accentuated by the addition of perindoprilat (10 μmol/L) to the superfusate (P<.05 in both strains). Systemic blood pressure was unaffected by the topical application of phenylephrine (10 μmol/L), perindoprilat (10 and 100 μmol/L), or losartan (10 μmol/L). We conclude that phenylephrine-induced tone impairment by angiotensin-converting enzyme inhibition and Ang II type 1 receptor blockade in vivo probably reflects the role of endogenous Ang II in the potentiation of the adrenergic response during the control of vascular tone. This role is identical in both normotensive and hypertensive rats. (Hypertension. 1994;24:317-321.)

Key Words • drug synergism • phenylephrine • muscle, smooth, vascular • angiotensin II • angiotensin converting enzyme inhibitors • renin-angiotensin system

Angiotensin II (Ang II) is one of the most potent vasoconstrictors and is produced by transformation of Ang I by a vascular Ang I-converting enzyme. Converting enzyme inhibitors (CEIs) as well as Ang II receptor blockers such as losartan lower blood pressure in hypertensive patients and animal models, suggesting a role for Ang II in hypertension even in hypertensive animals with normal or low Ang II levels. In vitro, the effect of both CEIs and losartan is more controversial,1 even though it is clear that subthreshold Ang II concentrations increase the contractile response of arteries in vitro to sympathetic nerve activation,2, 4 norepinephrine,5-7 clonidine,8 thrombin,9 histamine,8 potassium-induced depolarization, and caffeine in the absence of extracellular Ca2+.6 The amplification of vascular responses by Ang II requires receptor occupation9 and does not involve the release of vasoactive factors from the endothelium.4,6,7,10 Previous studies have shown that Ang II administered chronically in supressor animals by an intraperitoneal osmotic pump in the rabbit could potentiate the pressure effects of subsequently infused norepinephrine.11 In vivo experiments in humans have also given conflicting results, with evidence for12-14 and against15 the existence of Ang II potentiation of vascular tone. However, it remains possible that such a potentiation could occur in the control of vascular tone and could be involved in hypertension in addition to the direct pressor effect of Ang II. No in vivo study has yet determined directly the occurrence of such a phenomenon under physiological conditions, especially in hypertension. We have tested in vivo the hypothesis that phenylephrine-induced changes in diameter and flow could be potentiated by endogenous Ang II. We measured in anesthetized normotensive Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) the external diameter and blood flow rate of mesenteric arteries. To test a possible potentiation of phenylephrine-induced tone by endogenous Ang II, we investigated the effect of the CEI perindoprilat and of the Ang II type 1 receptor blocker losartan.

Methods

Twelve-week-old normotensive WKY rats and age-matched SHR were anesthetized with sodium pentobarbital (50 mg/kg IP). All rats were obtained from C.E.R.J. (Janvier). Fig 1 shows the experimental model used to measure mesenteric artery diameter and mesenteric blood flow. A medial laparotomy was performed, and the last loop of the small intestine was exposed on a plastic container and irrigated with a buffered Tyrode's solution (pH 7.4) maintained at 37°C and aerated with 95% O2/5% CO2. The mesenteric loop was being without stretch. A 1- to 2-mm-long segment of a second-generation branch (external diameter, 400 to 600 μm) was dissected free of fat tissue under a binocular lens (Microcontrol), and the mesenteric artery was separated from the vein to allow a clear observation of the vessel. A video image of the artery was obtained from a video camera mounted on the binocular lens, allowing a total enlargement of ×120 (Microcontrol) and determination of the artery diameter. For continuous recording of mesenteric flow rate, another 3-mm-long
segment of the main branch (external diameter, 800 μm) was dissected as described above, and a flow probe (Transonic 1R) was positioned around the artery and connected to a flowmeter (Transonic). A polyethylene catheter (internal diameter, 0.9 mm) was introduced via the right femoral artery up to the location of the superior mesenteric artery. The catheter was connected to a pressure transducer (Gould P23ID), and systemic arterial pressure was recorded continuously.

After measurement of systemic pressure, mesenteric artery diameter, and mesenteric blood flow under control conditions, phenylephrine (10 μmol/L) was added to the superfusate for 5 minutes before addition of the CEI perindoprilat (10 or 100 μmol/L) for 20 minutes. Finally, the direct nitric oxide donor SIN-1 (linsidomine hydrochloride, 10 mmol/L) was added to the superfusate for 20 minutes to fully dilate the vessel.

In another group of experiments, after measurement of systemic pressure, mesenteric artery diameter, and mesenteric blood flow under control conditions, phenylephrine (10 μmol/L) was added to the superfusate for 5 minutes before addition of the Ang II receptor blocker losartan (10 μmol/L) for 20 minutes. This was followed by the further addition of perindoprilat (10 μmol/L) to the superfusate for 20 minutes. Finally, SIN-1 (10 mmol/L) was added to the superfusate for 20 minutes to fully dilate the vessel.

Each of these two protocols involved 10 SHR and 10 WKY rats.

Statistical Analysis

Results are expressed as mean±SEM; n represents the number of observations. The significance of differences between means of the different groups was determined by two-way ANOVA followed by a Newman-Keuls test when significant. Probability values less than .05 were considered significant.

Drugs

Phenylolephrine was purchased from Sigma Chemical Co. Perindoprilat was supplied by IRIS (Servier Research Institut, Courbevoie, France), losartan by MSD, and SIN-1 by Hoechst. All other reagents were of analytical grade.
superfusate (Figs 2 and 4). In all experiments, the addition of SIN-1 (10 mmol/L) further relaxed the artery to a diameter not significantly different from the control diameter (Figs 2, 3, and 4).

In SHR, topical application of phenylephrine (10 μmol/L) lowered artery diameter from 562±26 to 273±7 μm (n=10, P<.05). This represented a significantly higher fall in diameter than in WKY rats (289±8 versus 224±18 μm, n=10 per group, P<.05). Perindoprilat (10 and 100 μmol/L) and losartan (10 μmol/L) both significantly attenuated the phenylephrine-induced decrease in diameter (Figs 3 and 4). The effect of losartan was maintained over a period of 30 minutes (data not shown) and was significantly accentuated by the addition of perindoprilat (10 μmol/L) to the losartan-containing superfusate (Fig 4). The addition of SIN-1 (10 mmol/L) relaxed the artery to its control level (Figs 3 and 4). There was no significant difference in mesenteric artery diameter between WKY rats and SHR when either perindoprilat or losartan was used alone or in combination.

In both strains, neither perindoprilat (10 and 100 μmol/L) nor losartan (10 μmol/L) modified significantly the mesenteric artery diameter in the absence of phenylephrine (10 μmol/L) (data not shown).

**Mesenteric Artery Blood Flow**

In WKY rats, topical application of phenylephrine (10 μmol/L) significantly reduced the main branch mesenteric artery blood flow (3.2±0.25 mL/min, n=10) to 23% of its control level (n=10, P<.05, Fig 5). The CEI perindoprilat (10 and 100 μmol/L) significantly attenuated the phenylephrine-induced decrease in blood flow, and the further addition of SIN-1 (10 mmol/L) increased mesenteric blood flow to a level not significantly different from the control level (Figs 2, 5, and 6). Losartan (10 μmol/L) significantly attenuated the phenylephrine-induced decrease in blood flow (Fig 5). This effect was persistent over a period of 30 minutes (data not shown). The effect of losartan was significantly accentuated by the further addition of perindoprilat (10 μmol/L) to the losartan-containing superfusate (Fig 6).

In SHR, phenylephrine (10 μmol/L) significantly decreased mesenteric blood flow (3.3±0.13 mL/min) to 24% of its control level (n=10, P<.05, Fig 5). Perindoprilat (10 and 100 μmol/L) and losartan (10 μmol/L) attenuated significantly phenylephrine-induced decrease in blood flow (Figs 5 and 6). The effect of losartan was persistent over a period of 30 minutes (data not shown) and was significantly accentuated by the addition of perindoprilat (10 μmol/L) to the losartan-containing superfusate (Fig 6).

In all cases there was no significant difference between mesenteric blood flow in WKY rats and SHR regarding the effects of perindoprilat (10 and 100 μmol/L), losartan (10 μmol/L), or a combination of perindoprilat (100 μmol/L) and losartan (10 μmol/L).

In both SHR and WKY rats, neither perindoprilat (10 and 100 μmol/L) nor losartan (10 μmol/L) significantly...
affected mesenteric blood flow in the absence of phenylephrine (10 μmol/L) (data not shown).

Discussion

This study provides the first direct evidence that endogenous Ang II might amplify vascular tone caused by phenylephrine in vivo in both normotensive and hypertensive rats. The main experimental findings are that a phenylephrine-induced decrease in mesenteric artery diameter and blood flow was similarly attenuated by perindoprilat and losartan and that there was no difference between mesenteric responses of normotensive and hypertensive rats to perindoprilat, losartan, or both.

The method used in the present study allows the simultaneous measurement of mesenteric artery diameter, mesenteric blood flow, and systemic blood pressure in vivo in anesthetized animals and allows the superfusion of drugs acting locally on a mesenteric artery. The absence of effect of the superfused drugs on the systemic circulation avoids interferences caused by changes in arterial pressure that would trigger baroreflexes and other regulatory mechanisms. Indeed, nonspecific changes in baseline blood pressure in vivo have been shown to influence vascular reactivity, and this makes the interpretation of results more difficult.

Both the diameter of a conductance mesenteric artery and the mesenteric blood flow under phenylephrine stimulation were increased by losartan and perindoprilat without modification of systemic blood pressure. In addition, as the control values of blood flow and artery diameter were not significantly altered by perindoprilat or losartan, it is unlikely that Ang II exerts a direct pressor effect in the large-diameter mesenteric artery studied under our experimental conditions. Large-diameter arteries have much less spontaneous tone than small resistance arteries as they respond to dilator stimuli to a much smaller extent. Nevertheless, we cannot exclude the possibility that the lack of effect of perindoprilat and losartan on diameter and flow in control conditions might reflect the disruption of the sympathetic innervation during dissection of the arterial segment.

Previous studies have shown a positive interaction between Ang II and different agonists. The potentiation phenomenon depends on protein kinase C activation that increases the sensitivity of intracellular contractile mechanisms associated with Ca²⁺-dependent vasoconstriction and is probably not mediated by depolarization of vascular smooth muscle cells as previously demonstrated.

No study has yet clearly demonstrated in vivo the possibility of such a mechanism involved in the regulation of vascular tone. Ang II potentiation has been demonstrated in hypertensive patients with renin-angiotensin system activation caused by sodium depletion. Under normal pressure conditions, the situation is less clear. In pithed rats, several studies have shown that interruption of the renin-angiotensin system with CEI or nephrectomy decreases the pressor response to sympathetic stimulation. The pressor response can be restored by infusion of Ang II as well as vasopressin. Nevertheless, in these experiments systemic blood pressure was altered by the different treatments, and changes in baseline blood pressure have been shown to modify per se the pressor response to sympathetic stimulation in both two-kidney and nephrectomized pithed rats. Chronic antihypertensive treatment with CEIs has been reported to produce a regression of structural and functional abnormalities of resistance arteries in essential hypertension. In the SHR, losartan has been reported to decrease blood pressure to normal values and to suppress vascular hyperreactivity. Moreover, in the SHR isolated perfused mesenteric vascular bed, Ang II potentiation of the contraction elicited by electrical stimulation is antagonized by losartan; and in isolated rings of aorta or tail artery from SHR, losartan decreases serotonin- and norepinephrine-induced tone, whereas hydralazine does not.

In the present experimental conditions, no change in systemic blood pressure was detected in the presence of local phenylephrine, perindoprilat, or losartan, excluding a possible nonspecific pressure effect. Thus, direct measurements of mesenteric arterial diameter and blood flow were performed in vivo without changes in systemic pressure. This allows us to attribute the effects of perindoprilat and losartan to their local inhibition of the potentiating effect of endogenous Ang II on phenylephrine-induced vasospasm.

In addition to inhibiting the conversion of Ang I to Ang II, CEIs also prevent the breakdown of bradykinin. We observed that the reduction by the Ang II receptor blocker losartan of the phenylephrine-induced tone was enhanced by perindoprilat, suggesting that the CEI attenuated phenylephrine-induced tone not only by inhibition of Ang II production but probably also by its activity on bradykinin metabolism. Bradykinin breakdown would be decreased by perindoprilat, and its concentration would rise, leading to a further dilation as observed when perindoprilat was added after losartan. This is supported by the observation that CEIs such as trandolapril and perindoprilat increase endothelium-dependent relaxation caused by bradykinin in vitro. Furthermore, we have previously reported that under control conditions, i.e., nonstimulated vasomotor tone, CEIs significantly relax the carotid arterial wall in both normotensive and hypertensive rats. Thus, the relaxant effect of perindoprilat observed in the present experiments might be the combination of the suppression of Ang II potentiation and an increase in bradykinin-induced dilation.

An alternative explanation to the effect of perindoprilat could be a facilitation of the endothelium-dependent relaxation. Indeed, perindoprilat increases acetylcholine-induced relaxation, and shear stress-induced release of endothelium-derived relaxing factor has been proposed to be enhanced by CEIs. In the present study such a possibility may not apply, as no relaxing effect was detected on baseline mesenteric arterial diameter and blood flow after application of perindoprilat or losartan.

In SHR, vascular hyperreactivity has been extensively reported. In the present work, although the phenylephrine-induced decrease in diameter in SHR (289 μm) was greater (P<.05) than that (224 μm) in WKY rats, the percent changes (53% versus 49%) were very similar. In the same way, both losartan and perindoprilat attenuated phenylephrine-induced decrease in diameter and blood flow similarly in WKY rats and SHR. CEIs are commonly used as antihypertensive drugs with the different possible mechanisms described above. They can also decrease blood pressure in patients or animals with normal blood pressure or in hypertensive patients with normal or even low plasma renin levels.
could reflect the existence of a local vascular renin-angiotensin system. Our present experimental results contrast with other studies suggesting a role for Ang II in the occurrence of hypertension in SHR. There are at least two possible explanations for this discrepancy. First, Ang II potentiation might not be exaggerated in large (conductance) vessels and therefore not involved in vascular resistance. Another possibility is that we studied Ang II potentiation in arteries submitted to their normal operating pressures in both SHR and WKY rats, whereas other studies have used CEIs or losartan to decrease SHR systemic blood pressure. Nevertheless, this issue remains to be clarified.

In conclusion, our study suggests for the first time directly and in vivo that endogenous Ang II potentiation of the contractile responses of mesenteric arteries to phenylephrine could represent a mechanism of regulation of vascular tone in hypertensive and normotensive rats. The relaxant activity of CEIs larger than that of Ang II receptor antagonists might be related to the combined action of CEIs on Ang II potentiation and on bradykinin breakdown.

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