Differential effects of phosphoramidon on contractile responses to angiotensin II in rat blood vessels

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1 Cumulative concentration-tension response (C-R) curves to angiotensin II (AII), big endothelin-1 (big ET-1), ET-1 and arginine vasopressin (AVP) were determined in endothelium intact-ring preparations of aorta, mesenteric artery and tail artery isolated from adult male Sprague-Dawley rats in the presence or absence of the neutral metalloprotease inhibitor, phosphoramidon.

2 The order of sensitivity of the three rat vascular smooth muscle preparations to AII, big ET-1 and ET-1 was aorta > mesenteric artery > tail artery whereas that for AVP was reversed, namely, tail artery > mesenteric artery > aorta.

3 Phosphoramidon blocked the responses to AII in a concentration-dependent manner, whereas even very high concentrations of phosphoramidon (100 µM) failed to affect the tension responses evoked by ET-1 and AVP in all three preparations. Low concentrations of phosphoramidon (10 µM) produced significant increases in EC₅₀ values for AII in tail artery ($P < 0.01$) and mesenteric artery ($P < 0.05$) but not in aorta. The rank order of sensitivity to the inhibition by phosphoramidon was tail artery > mesenteric artery > aorta. Phosphoramidon-evoked rightward shifts in the C-R curves to AII were much higher than those to big ET-1 in both mesenteric artery and tail artery.

4 In endothelium-denuded preparations, AII failed to evoke any increases in tension in tail artery while the responsiveness of the mesenteric artery to AII was reduced significantly relative to endothelium-intact tissues with a rightward shift in the C-R curve and a decrease in the maximal response. On the other hand, the C-R curve to AII was shifted to the left in aorta following removal of the endothelium. Importantly, ET-1 and AVP evoked vasoconstrictor responses were unaffected by the inclusion of a high concentration of phosphoramidon (100 µM) in endothelium-denuded aorta and mesenteric artery.

5 The results suggest that AII-evoked tension responses of blood vessels such as tail artery are completely endothelium-dependent; in relatively larger blood vessels such as mesenteric artery they are partially endothelium-dependent while in much bigger conduit type blood vessel such as aorta, they are endothelium-independent. It is concluded that the vasoconstrictor responses to AII in mesenteric artery and tail artery may be mediated by the release of endothelins from the endothelium by increased formation from big ET, an effect that is blocked by phosphoramidon.

Keywords: Angiotensin II; endothelins; vasopressin; phosphoramidon; endothelium; endothelin converting enzyme; vascular smooth muscle; aorta; mesenteric artery; tail artery

Introduction

Recent studies have shown that prolonged incubation with angiotensin II (AII) promotes the release of endothelin-1 (ET-1) from endothelial cells and rat mesangial cells in culture (Emori *et al.*, 1991; Kohno *et al.*, 1992). Perfusion of the mesenteric arterial bed with AII (100 nM) for 5 h potentiated the vascular responses to noradrenaline via release of ET-1 in the spontaneously hypertensive rat (SHR) but not in the normotensive Wistar-Kyoto (WKY) rat (Dohi *et al.*, 1992). Despite these observations with either cultured endothelial cells or perfusion model systems, there is no direct evidence implicating a role for ETs in AII-mediated changes in vascular smooth muscle tone of normotensive rats. Although heterogeneity in the vascular responsiveness to AII has been shown (Toda *et al.*, 1990), the possibility that altered production of ETs could contribute to the differences in sensitivity has not been investigated in the same species with vascular smooth muscle tissues from different vascular beds.

It is now well-established that the 21 amino acid vasoconstrictor peptide, ET-1, is not stored in endothelial cells but that it is generated by cleaving a 39-(porcine) or 38-(human) amino acid intermediate, termed big ET-1. This unusual proteolytic processing is achieved by a putative ET peptide converting enzyme, a neutral metalloproteinase present in both vascular smooth muscle cells and endothelial cells. This

enzyme is sensitive to blockade by phosphoramidon (Ikegawa *et al.*, 1991). Phosphoramidon has been shown to inhibit both the *in vivo* pressor effect and the *in vitro* contractile actions of big ET-1 without affecting the vasoconstrictor properties of exogenous ET-1 (Kashiwabara *et al.*, 1989; Fukuroda *et al.*, 1990; Auguet *et al.*, 1992; Oppenorth *et al.*, 1992). Therefore, our major aim was to investigate whether tension responses to AII are attenuated when ET converting enzyme is inhibited by phosphoramidon. We have used ring preparations of rat arteries, of varying sizes, namely aorta, mesenteric artery and tail artery obtained from normotensive adult Sprague-Dawley rats. In order to elucidate whether the observed effects are unique to AII, comparative studies with other vasoconstrictor peptides such as ET-1, arginine vasopressin (AVP) and big ET-1 were performed.

Methods

Tension measurement

Ring preparations of the thoracic portion of the aorta (length 5 mm aorta), the superior mesenteric artery (3 mm, mesenteric artery), and the ventral caudal artery (2 mm, tail artery) isolated from adult Sprague Dawley rats (14–16 weeks) were set up for isometric tension measurements in 20 ml organ baths containing modified Krebs physiological salt solution

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(PSS) oxygenated with 95% O₂/5% CO₂ at 37°C. The composition of the PSS (in mM) was: NaCl 118, KCl 4.7, KH₂PO₄ 1.2, MgCl₂·6 H₂O 1.2, CaCl₂·2H₂O 1.8, NaHCO₃ 25.0 and glucose 11.1 (Gopalakrishnan *et al.*, 1985). We confirmed that all three vascular smooth muscle preparations were optimally stretched for maximum force development. This was carried out by plotting maximum tension developed at a fixed concentration of ET-1 (100 nM) at various levels of preload tension. Aorta and mesenteric artery developed maximum force when resting tensions of 2 g and 1 g respectively were applied to these vessels. However, tail artery generated a maximal tension response at a preload level of 0.8 g. At a preload tension of 0.5 g, it developed 93% of its maximal tension. To avoid undue stretching of this minute blood vessel, we maintained 0.5 g of preload tension for this vessel. Several laboratories have used a similar preload tension for the tail artery (Kosaki *et al.*, 1993; Szocik *et al.*, 1993).

The increases in isometric tension under resting and agonist(s) stimulated conditions were recorded on a Grass 7E polygraph with FTO.3 force transducers. After an initial 1 h equilibration period with repeated washings every 20 min, the tissues were challenged with KCl (80 mM) with isotonicity being maintained by appropriate reduction in NaCl. The presence of a functional endothelium was confirmed by studying the relaxation responses to 1 µM acetylcholine (ACh) in all three vascular smooth muscle tissues pre-contracted with a standard 1 µM concentration of phenylephrine (PE). In these initial responses, peptide agonists were not used in order to avoid desensitization. In other experiments, endothelium was removed through chemical stripping by incubating the tissues for 3 s with Triton X-100 (0.5% in PSS) followed by several repeated washings with normal PSS. Previous studies have shown that brief exposure to this detergent selectively destroys the endothelial layer of both vascular and cardiac preparations (Brutsaert *et al.*, 1988). The absence of endothelium was confirmed by lack of relaxation responses to ACh in tissues pre-contracted with PE.

Aorta, mesenteric artery and tail artery rings obtained from the same animal were set up for measurements of tension with intact endothelium (on the same day) for comparison of differences in responsiveness to AII. Each tissue was exposed to only a single cumulative concentration-response (C-R) determination for AII (either in the presence or absence of endothelium) to avoid tachyphylaxis. Thus, all determinations were carried out only once and a single cumulative concentration-response curve was generated for each preparation. In order to determine if tachyphylaxis occurred during the cumulative addition of AII, preliminary experiments in parallel rings were conducted as follows. Each ring was exposed to one single maximal concentration of AII (10 µM) or a similar concentration achieved by a step wise addition in a cumulative fashion.

Whenever phosphoramidon (10–100 µM) was included, it was added 30 min before the addition of the agonist. Ring preparations that did not receive any phosphoramidon were set up in parallel and served as controls. At the end of the experiment, tissues were blotted dry, weighed, and the cross sectional areas were calculated as described earlier (Wyse, 1980). This was necessary in order to express the data for maximal tension ($E_{\max}$) developed as g mm⁻² after the addition of the agonist in each preparation taking into consideration the cross sectional area of the preparation.

Drugs used

Angiotensin II octapeptide (AII), endothelin-1 (ET-1), big ET-1 and arginine vasopressin (AVP) were obtained from either Peninsula Laboratories (Belmont, CA, U.S.A.) or Bachem Inc. (Torrance, CA, U.S.A.). Phenylephrine hydrochloride (PE) and acetylcholine chloride (ACh), Triton X-100 and phosphoramidon were purchased from Sigma (St. Louis, MO, U.S.A.). In order to ensure the reproducibility of

the effects of phosphoramidon, it was also obtained from another source (Peninsula Laboratories, Belmont, CA, U.S.A.). Drugs were added to the organ baths in volumes between 40–100 µl to provide the required final bath concentrations.

Data analyses

Each cumulative C-R curve was analysed individually to estimate the concentration required to produce 50% of the maximal response (EC_{50}) and the maximal increase in tension ($E_{\max}$) by using ALL FIT programme (De Lean *et al.*, 1978). The data were expressed as mean $\pm$ s.e.mean values. The comparison of mean values between various groups was performed by analysis of variance (Super ANOVA package Macintosh). Simultaneous multiple comparisons were examined by Scheffe's *F* test.

Results

Responses to AII in the presence of endothelium

AII evoked concentration-dependent increases in vascular smooth muscle tone. Similar levels of maximal tension were obtained either by stepwise cumulative addition of AII or by the addition of a single maximal concentration of AII (10 µM). These data suggest that there was no tachyphylaxis to AII responses during the first cumulative C-R curve in these tissues. Initial experiments performed with a fixed concentration of AII (1 µM) revealed that preincubation with increasing concentrations of phosphoramidon (10–50 µM) for a period of 30 min led to concentration-dependent diminution in the maximal tension responses to AII. However, the inhibitory effect of AII-evoked responses by phosphoramidon was relatively less in aorta whereas incubation with a high concentration of phosphoramidon (50 µM) led to partial and complete abolition of the tension responses to AII in the mesenteric artery and tail artery rings respectively. Increasing the duration of incubation with phosphoramidon to 1 h did not result in further inhibition of AII responses (data not shown). The rank order of sensitivity to AII amongst these three blood vessels was: aorta > mesenteric artery > tail artery (Table 1).

Pretreatment with phosphoramidon produced concentration-dependent, non-competitive blockade with rightward shifts in the C-R curves and reduction of maximal responses to AII (Figure 1a, b, c). While both maximal tension responses ($E_{\max}$ values) and potency (EC_{50} values) were significantly diminished in mesenteric artery ($P < 0.05$) and tail artery ($P < 0.01$) with the lowest concentration of phosphoramidon (10 µM), the responses of aorta to AII remained unaffected. Incubation with a higher concentration of phosphoramidon (50 µM) led to complete abolition of the AII responses in tail artery whereas the responses of aorta to AII was only slightly but significantly diminished by this concentration of phosphoramidon. The order of sensitivity for blockade of AII-evoked tension responses by phosphoramidon was: tail artery > mesenteric artery > aorta (Table 1).

Responses to other peptides in aorta, mesenteric artery and tail artery with intact endothelium

In order to elucidate whether the inhibitory effect of phosphoramidon was selective for AII-evoked responses, comparative studies with other peptides such as big ET-1, ET-1 and AVP were performed. The cumulative C-R curves to big ET-1, ET-1 and AVP obtained either in the presence or the absence of a very high concentration of phosphoramidon (100 µM) are shown in Figure 2a, b and c respectively. The pooled data of EC_{50} and $E_{\max}$ values obtained from these C-R curves ($n = 4$) is shown in Table 2. The rank order of sen-

sitivity of these blood vessels to ET-1 and big ET-1 were similar to AII (aorta > mesenteric artery > tail artery) whereas the responsiveness of these blood vessels to AVP showed a reverse rank order of tail artery > mesenteric artery > aorta. Aorta was the least sensitive of the three blood vessels to AVP.

The C-R curves to big ET-1 were shifted to the right by phosphoramidon (100 μ M) in all three blood vessels (Figure 2a and Table 2). However, as for AII, the non-competitive nature of blockade by phosphoramidon was most clearly evident for big ET-1-evoked responses only for tail artery. The evoked tension responses were almost completely

Table 1 Analyses of the cumulative concentration-tension response curves to angiotensin II (AII) in the ring preparations of rat aorta, mesenteric artery and tail artery with intact endothelium in the presence and absence of various concentrations of phosphoramidon (Phos) maintained in the bathing medium for 30 min

Treatment	Aorta		Mesenteric artery		Tail artery	
	EC_{50} (nM)	E_{max} (g mm $^{-2}$)	EC_{50} (nM)	E_{max} (g mm $^{-2}$)	EC_{50} (nM)	E_{max} (g mm $^{-2}$)
Control	13.2 $\pm$ 1.1	0.30 $\pm$ 0.01	92.5 $\pm$ 4.2†	0.72 $\pm$ 0.04†	167.7 $\pm$ 7.2†	0.70 $\pm$ 0.04†
Phos 10 μ M	13.4 $\pm$ 1.5	0.29 $\pm$ 0.01	138 $\pm$ 6.2*	0.64 $\pm$ 0.04*	2001 $\pm$ 40.8**	0.32 $\pm$ 0.05**
20 μ M	17.0 $\pm$ 1.1	0.29 $\pm$ 0.02	190.3 $\pm$ 4.1**	0.61 $\pm$ 0.03*	> 2 μ M**	0.12 $\pm$ 0.03**
50 μ M	21.7 $\pm$ 1.7*	0.24 $\pm$ 0.03*	386.7 $\pm$ 31.8**	0.57 $\pm$ 0.04**	NR**	NR**

NR denotes – no response. Each value is mean $\pm$ s.e.mean of 6 separate tissues from different animals.

* P < 0.05 and ** P < 0.01 compared to respective control group data; † P < 0.01 compared to respective control data for rat aorta.

Figure 1 Cumulative concentration-tension response curves (C-R) to angiotensin II (AII) in ring preparations of rat aorta (a), rat mesenteric artery (b) and rat tail artery (c) with intact endothelium either in the presence or the absence (control group, ○) of increasing concentrations of phosphoramidon (10 μ M ●, 20 μ M ■ and 50 μ M ▲) maintained in the medium for a period of 30 min prior to the addition of AII. It was ascertained that there was no tachyphylaxis with the first C-R curve and each tissue was exposed to only one C-R curve. Each data point is the mean $\pm$ s.e.mean of 6 separate experiments.

Figure 2 Cumulative concentration-response (C-R) curves to big endothelin-1 (big ET-1, a), endothelin-1 (ET-1, b) and arginine vasopressin (AVP, c) in the ring preparations of aorta (Control ○, phosphoramidon ●), mesenteric artery (Control □, phosphoramidon ■) and tail artery (Control △, phosphoramidon ▲) with intact endothelium either in the presence or the absence (control group) of a fixed concentration of phosphoramidon (100 μ M). Each data point shown is the mean $\pm$ s.e.mean of 4 separate experiments using tissues obtained from different animals.

Table 2 Analyses of the cumulative concentration-tension response curves to big endothelin-1 (big ET-1), ET-1 and arginine vasopressin (AVP) in the ring preparations of rat aorta, mesenteric artery and tail artery with intact endothelium either in the presence or the absence of phosphoramidon (Phos) 100 μ M for 30 min

Tissue	Treatment	Big ET-1		ET-1		AVP	
		EC_{50} (nM)	E_{max} (g mm ⁻²)	EC_{50} (nM)	E_{max} (g mm ⁻²)	EC_{50} (nM)	E_{max} (g mm ⁻²)
Aorta	Control	46.3 $\pm$ 3.0	0.31 $\pm$ 0.01	1.1 $\pm$ 0.1	0.47 $\pm$ 0.02	44.2 $\pm$ 2.1	0.42 $\pm$ 0.03
	Phos	60.3 $\pm$ 2.3*	0.32 $\pm$ 0.01	1.3 $\pm$ 0.2	0.45 $\pm$ 0.02	44.1 $\pm$ 2.3	0.38 $\pm$ 0.03
Mesenteric artery	Control	62.6 $\pm$ 1.8	0.64 $\pm$ 0.04	2.7 $\pm$ 0.2	0.99 $\pm$ 0.03	18.5 $\pm$ 0.5	0.76 $\pm$ 0.03
	Phos	87.1 $\pm$ 3.7*	0.64 $\pm$ 0.02	2.8 $\pm$ 0.2	0.91 $\pm$ 0.03	18.2 $\pm$ 0.6	0.76 $\pm$ 0.04
Tail artery	Control	74.7 $\pm$ 2.9	0.56 $\pm$ 0.03	4.5 $\pm$ 0.3	0.84 $\pm$ 0.02	5.5 $\pm$ 0.2	0.77 $\pm$ 0.03
	Phos	NR**	0.22 $\pm$ 0.01**	4.6 $\pm$ 0.3	0.74 $\pm$ 0.04	5.4 $\pm$ 0.2	0.81 $\pm$ 0.03

* $P < 0.05$; ** $P < 0.01$ compared to their respective control values in the same tissues. NR denotes – no response. Each value shown is mean $\pm$ s.e.mean of 4 separate experiments performed either in the presence or the absence (control group) of Phos using paired ring preparations set up on the same day.

Figure 3 Cumulative concentration-response (C-R) curves to angiotensin II (AII) in endothelium-denuded ring preparations of aorta (Control O, phosphoramidon, ●), mesenteric artery (Control, □, phosphoramidon, ■) and tail artery (Control Δ, phosphoramidon ▲) either in the presence or the absence (control group) of a fixed concentration of phosphoramidon (100 μ M) maintained in the incubation medium for 30 min prior to the addition of AII. Endothelium was removed by a 3 s exposure of the tissues to Triton X-100 (0.5%) followed by repeated washings in PSS. The absence of a functional endothelium was confirmed by the attenuation of the relaxation response to 1 μ M acetylcholine in phenylephrine precontracted tissues. Each data point shown is the mean $\pm$ s.e.mean of 4 separate experiments on tissues obtained from different animals. Note: Endothelium-denuded tail artery rings failed to elicit any tension responses to AII; however, the same tissues did respond to AVP and ET-1 with a closely similar response pattern to that in tail artery rings with intact endothelium.

abolished in tail artery rings exposed to phosphoramidon (100 μ M). In the case of aorta and mesenteric artery, pretreatment with phosphoramidon (100 μ M) led to a small but significant ($P < 0.05$) increase in EC_{50} without significant change in the maximal response (Figure 2a and Table 2).

In contrast to AII and big ET-1, preincubation with a very high concentration of phosphoramidon (100 μ M) failed to affect the C-R curves to ET-1 (Figure 2b) and AVP (Figure 2c) in all three arteries with intact endothelium. There were no significant differences in EC_{50} and E_{max} values between the control and phosphoramidon (100 μ M) pretreated group (Table 2). Moreover, increasing the preincubation period to 1 h for phosphoramidon did not affect the responses to ET-1 and AVP (data not illustrated). PE-evoked tension responses were also unaffected by the inclusion of phosphoramidon (100 μ M) in all three preparations (data not shown).

Responses to AII in endothelium-denuded aorta, mesenteric artery and tail artery

In endothelium-denuded tissues, incubation with very high concentrations of phosphoramidon (100 μ M) failed to evoke

significant alterations in the control C-R curves to AII in all the three preparations (Figure 3).

Discussion

This study demonstrates that there are regional variations in sensitivity to AII in rat arteries of varying sizes. To the best of our knowledge, this is the first paper which compares differences in sensitivity to AII in these three blood vessels. It provides a possible explanation that the release of ET evoked by AII could vary in different vascular beds and that this could be one amongst several factors that could contribute to the differences in sensitivity. The study also provides a comparison of differences in the rank order of sensitivity among these three blood vessels to AVP, AII and ET-1.

Previously, we have shown that AII evokes tension responses in conduit type blood vessels such as aorta by the direct stimulation of vascular AT_1 receptors, whereas in the case of blood vessels like the mesenteric artery and tail artery, the responses to AII are mediated by the activation of both vascular (direct) and endothelial (indirect) AT_1 receptors. Activation of endothelial AT_1 receptors are possibly linked to processes capable of endothelin release (Chen *et al.*, 1993; Gopalakrishnan *et al.*, 1994). In the present study, using the ET converting enzyme inhibitor, phosphoramidon, we provide further evidence for the probable participation of ET release from endothelium in AII-evoked tension responses in vascular smooth muscle preparations such as tail artery and mesenteric artery. The lack of responsiveness of these blood vessels to AII could not be due to tachyphylaxis or desensitization to AII because the inhibitory effects of phosphoramidon on AII responses were seen in tissues exposed to the first cumulative C-R curve to AII. Previously, desensitization/tachyphylaxis to AII has been characterized in aortic rings only for the second and third cumulative response determinations (Kuttan & Sim, 1993). The present data confirm that the level of dependence on indirect ET release may be much less in conduit type blood vessels such as aorta, whereas the responses of tail artery to AII are mediated mainly by the release of ETs. Increased expression of prepro ET mRNA by both AVP and AII has been demonstrated in cultured cells and isolated vascular beds subjected to perfusion for a prolonged time (Emori *et al.*, 1991; Kohno *et al.*, 1992; Dohi *et al.*, 1992; Tomobe *et al.*, 1993). However, these studies failed to examine whether acute addition of AII or AVP is linked to the immediate regulation of vascular smooth muscle tone via release of ETs. Earlier studies that attempted to examine the heterogeneity in vascular smooth muscle responsiveness to AII explained it on the basis of differences in the levels of eicosanoids (Toda *et al.*, 1990; Lin & Nasjletti, 1991) or the levels of sympathetic tone in different vascular beds (Malik & Nasjletti, 1976; Story & Ziogas, 1986). In the present study, we suggest that AII releases ET from endothelium, particularly in blood

vessels with smaller lumen such as tail artery and mesenteric artery and this could also contribute to the differences in sensitivity.

Since the discovery of ETs, the identification and characterization of endothelin converting enzyme has been the subject of intense investigation (Fukuroda *et al.*, 1990; Ikegawa *et al.*, 1991; Opgenorth *et al.*, 1992). Although earlier studies have demonstrated that AII and other peptides enhanced ET release via increased gene transcription, none of these studies has looked at the possibility that these effects could also take place at the posttranscription/posttranslational locus of increased conversion from big ET to ET. While it has been proposed that several different enzymes such as aspartic protease and cathepsin E are involved in the formation of ET-1 from big ET-1, it is now recognized that the neutral endopeptidase that is susceptible to blockade by phosphoramidon, plays a crucial and perhaps a physiologically important role in the formation of ET-1 (Opgenorth *et al.*, 1992). Our observation that AII-evoked tension responses were abolished selectively by phosphoramidon in a concentration-dependent and non-competitive manner in the mesenteric artery and the tail artery preparations with intact endothelium suggests that AII could promote the formation of ET from big ET.

Three key observations made in this study strongly support the suggestion that ET release from endothelial cells could play a crucial role in the tension responses to AII in blood vessels such as tail artery and mesenteric artery. Firstly, in endothelium-denuded ring preparations, AII failed to evoke any increase in tension in tail artery and in the case of mesenteric artery, the responses were significantly attenuated. These data parallel those in the same preparations with intact endothelium preincubated with phosphoramidon suggesting that the endothelin converting enzyme inhibition by phosphoramidon occurs at the level of the endothelium. Secondly, there were no significant differences in the C-R curves between the control and phosphoramidon pretreated groups in endothelium-denuded preparations, confirming that phosphoramidon failed to evoke further inhibition after the removal of endothelium. The observations that the responses to AVP and ET-1 could be demonstrated in tail artery with either intact endothelium incubated with high concentration of phosphoramidon (100 μ M) or endothelium-denuded tail artery preparations that failed to respond to AII confirmed that these tissues exhibited selective loss of responsiveness to AII. Thirdly, and most importantly, studies with big ET-1 revealed that the shifts in the C-R curves in phosphoramidon pretreated tissues were much higher in the case of mesenteric artery and that there was almost complete attenuation of responses to big ET-1 in the case of tail artery rings with intact endothelium. Thus, both AII and big ET-1 evoked responses were affected in a similar manner by phosphoramidon in mesenteric artery and tail artery. In support of our major observation, a recent report has also shown that AII potentiated the increased perfusion pressure induced by noradrenaline in tail artery *in vitro*, and that this effect

of AII was abolished by removal of the endothelium. This report attributed this response to the existence of an endothelium-derived contracting factor (EDCF) whose release was stimulated by AII (Thorin & Atkinson, 1994). Previously, it was proposed that ET-1 could be the EDCF involved in potentiating noradrenaline-induced vasoconstriction in tail artery (Reid *et al.*, 1991).

Comparison of AII responses with other agonists

It is well known that phosphoramidon inhibits both *in vitro* and *in vivo* vasoconstrictor responses to big ET-1 and not those evoked by ET-1 (Fukuroda *et al.*, 1990). In the present study, although it was observed that higher concentration of phosphoramidon (100 μ M) did produce rightward shifts in the C-R curves to big ET-1 in both aorta and mesenteric artery, the maximal responses could be restored. Moreover, the degrees of shift in the EC₅₀ values attained for big ET-1 were much lower than those evoked by a low concentration of phosphoramidon (50 μ M) for AII in both aorta and mesenteric artery. These data provide evidence that phosphoramidon produces more effective inhibition of AII-evoked vasoconstrictor responses than those caused by big ET-1 in these three blood vessels with intact endothelium. Although several studies have shown that phosphoramidon causes complete inhibition of the pressor response to big ET-1, it is known that *in vitro* vasoconstrictor effects of big ET-1 are not completely attenuated by phosphoramidon. For example, the C-R curve to big ET-1 was shifted to the right in rabbit saphenous artery but not in saphenous vein (Auguet *et al.*, 1992). These data suggest that big ET-1 can evoke vasoconstriction either by directly activating ET receptors on vascular smooth muscle cells or by being converted to ET-1 by processes independent of phosphoramidon-sensitive ET converting enzyme. Like AII, prolonged perfusion of the rat mesenteric artery bed with AVP was also shown to augment ET release (Tomobe *et al.*, 1993). However, in the present study, we failed to see any inhibition of AVP-evoked responses by the ET converting enzyme inhibitor. These data confirmed the selective inhibition of AII responses by phosphoramidon, a finding that has not been reported earlier.

In summary, the data obtained with phosphoramidon, suggest that AII can evoke ET release from endothelium and that this effect accounts for a major part of the evoked vasoconstrictor response to AII in small blood vessels such as tail artery and mesenteric artery than in the aorta. It is likely that locally generated AII could reach concentrations high enough to stimulate ET release in blood vessels with smaller lumens thereby contributing to the maintenance of enhanced peripheral vascular smooth muscle tone.

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