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Sensitivity of rabbit aorta and mesenteric artery to norepinephrine: role of tyrosine kinases

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Abstract

We tested the hypothesis that the differential sensitivity of rabbit aorta and mesenteric artery to norepinephrine is due to tyrosine kinase activity. The EC_{50} of aorta to norepinephrine was 6.5 times more sensitive than that in mesenteric artery. Basal myosin light chain phosphorylation was significantly greater in aorta as compared to mesenteric artery. Vanadate increased norepinephrine sensitivity significantly more in mesenteric artery than aorta, whereas genistein had the opposite effect. Basal phosphotyrosine levels were significantly higher in aorta than in mesenteric artery, the percentage increase in total tyrosine phosphorylated protein was significantly higher in mesenteric artery. These results suggest that the higher basal phosphotyrosine levels in the aorta may be responsible for the higher basal level of myosin light chain phosphorylation and this may be the basis for the higher sensitivity of the aorta to norepinephrine when compared with the mesenteric artery.

Keywords: Norepinephrine; Smooth muscle; Tyrosine kinase; Myosin light chain phosphorylation

1. Introduction

Regional differences in the response of mammalian blood vessels to α-adrenoceptor agonists in vitro have long been recognized (Altura and Altura, 1970). The sensitivity of rabbit and rat aorta to norepinephrine have been reported to be higher than those of its branches (Tayo and Bevan, 1987; Adegunloye and Sofola, 1997b) while the sensitivity of rat mesenteric artery to serotonin was shown to be much higher than that of the aorta (Adegunloye and Sofola, 1997a). Altura and Altura (1970) found that the maximum isometric active force produced in the rabbit aorta by epinephrine and several other drugs decreased with distance from the arch. In fetal sheep, the in vivo vasoconstrictor response to α-adrenoceptor agonist stimulation is less in the pulmonary circulation compared with the systemic circulation (Shaul et al., 1990).

This variability in smooth muscle response has been shown to depend on several factors which include age (Gurdal et al., 1995), sex (Kanashiro and Khalil, 2001), vessel diameter (Owen et al., 1983) and species (Forstermann et al., 1984). It has been suggested that the maximal active stress induced by α-adrenoceptor agonists in different parts of the aorta is directly correlated with the regional concentration of receptors in the smooth muscle cells; the greater the number of receptors the larger the maximal active stress (Griendling et al., 1984; Laher and Bevan, 1985; Shaul et al., 1990). There are also reports that the regional differences in sensitivity of α-adrenoceptors of vascular smooth muscle to norepinephrine are due to differences in receptor affinities (Oriowo et al., 1992; Satoh et al., 1998). Earlier reports indicate that differential sensitivity of the aorta and its branches to α-adrenoceptor agonists is due to differences in the source of Ca^{2+} mobilized for contraction (Cauvin et al., 1984; Tayo and Bevan, 1987). Because myosin light chain phosphorylation, the primary determinant of contraction, is dependent on intracellular calcium concentration ($[Ca^{2+}]$), it is possible that there may be differences in norepinephrine stimulated myosin light chain phosphorylation levels between the aorta and the mesenteric artery.

In addition to the myosin light chain kinase, tyrosine kinases are also important in contractile regulation. Receptor agonists such as angiotensin II, vasopressin, bombesin, and endothelins can mediate part of their action through stimulation of non-receptor tyrosine kinases (Hollenberg, 1994). Stimulation of vascular smooth muscle cells by the α-

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adrenoceptor agonist phenylephrine results in tyrosine phosphorylation of several proteins including Src, focal adhesion kinase, paxillin, Pyk2, Jak2, Tyk2, and STAT1 (Sasaguri et al., 2000). Protein tyrosine phosphorylation may participate in the regulation of $[Ca^{2+}]$ (Di Salvo et al., 1994) and mechanisms that couple receptor activation to modulation of myofilament Ca^{2+} sensitivity (Di Salvo et al., 1994). Inhibitors of tyrosine kinases block contractions of the porcine coronary artery preparation induced by either angiotensin II or arginine vasopressin but not contractions induced by acetylcholine (Laniyonu et al., 1995). Likewise, tyrosine kinase inhibitors decreased the potency of serotonin as well as the maximal contraction in response to serotonin in rat arterial strips denuded of endothelium (Watts et al., 1996), while they were incapable of attenuating the contractile responses to serotonin in sheep pulmonary arteries. Moreover, tyrosine kinase inhibitors were shown to inhibit contraction in response to carbachol and norepinephrine (Di Salvo et al., 1993b), whereas in another study they were without effect against phenylephrine- and phorbol ester-induced stimulation (Sauro and Thomas, 1993). These observations imply that there are differences in how tyrosine kinases modulate the contractile responses of different types of smooth muscle.

Thus, the goal of this study was to test the hypothesis that the increased sensitivity of aorta to norepinephrine as compared to mesenteric artery may be related to differences in norepinephrine-induced tyrosine kinase activity in these tissues. In addition, we also examined if the difference in response to norepinephrine is a reflection of a difference in basal and stimulated levels of myosin light chain phosphorylation.

2. Materials and methods

2.1. Animals

All experimental protocols were approved by the Institutional Animal Care and Use Committee of the Drexel University College of Medicine. Male white New Zealand rabbits weighing 2.5 to 3.2 kg were injected in the ear vein with 100 mg/kg sodium pentobarbital. The thoracic and abdominal cavities were opened and the thoracic aorta and branches of the superior mesenteric artery removed and placed in a beaker containing physiological salt solution at 4 °C. The physiological salt solution (PSS) contained (in mM): 140 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.6 $CaCl_2$, 1.2 Na_2HPO_4, 20 MOPS (pH 7.4), 5.5 D-glucose, and 0.02 Na_2EDTA.

2.2. Preparation of the aorta and mesenteric artery for isometric force measurement

For measurement of isometric force, the aorta and small branches of the superior mesenteric artery (O.D. ~ 800 μm) were cleaned of adhering fat and connective tissue and cut into 2.5–3-mm rings or near-circumferential strips. The endothelium was removed by gently rubbing the lumen with a 20-gauge needle (aorta) or fine stainless steel rod (mesenteric artery). The rings were suspended by a stainless steel hook and a rod in a 20-ml organ bath containing physiological salt solution and bubbled with O_2 at 37 °C with a pH of 7.4. The strips were mounted between two small clips in a similar chamber. The aortic rings were stretched to a passive force of 3 g; the aortic strips were stretched to a passive force of 1.5 g. The mesenteric arterial rings were stretched to a passive force of 1.5 g; the mesenteric arterial strips were stretched to a passive force of 750 mg. Isometric contractions were recorded by the use of a Grass FT.03 force transducer connected to a Model 7D Grass Polygraph (Astro-Med, West Warwick, RI). The arteries were equilibrated for 90 min during which they were stimulated three to four times with PSS containing 110 mM KCl (equimolar substitution for NaCl). Removal of the endothelial layer was confirmed by the complete absence of an acetylcholine (1 μM)-induced relaxation of an arterial preparation contracted by the addition of 0.1 μM norepinephrine.

After consistent contractions were elicited in response to 110 mM KCl, cumulative concentration–response curves to norepinephrine were performed (0.01 to 10 μM). A 45-min recovery period was allowed between cumulative concentration–response curves. The role of tyrosine kinases in the differential responses to norepinephrine-induced contractions was assessed by incubating the arterial preparations with either 50 μM genistein to inhibit tyrosine kinase activity or with 200 μM sodium orthovanadate (Na_3VO_4) to inhibit tyrosine phosphatase activity. The arterial preparations were incubated in either compound for 20 min prior to the generation of a second concentration–response relationship to norepinephrine. Time control concentration–response curves were performed to ensure stability in the response of both aorta and mesenteric artery to norepinephrine (data not shown).

2.3. Determination of myosin light chain phosphorylation levels

Arterial preparations used for quantitation of myosin light chain phosphorylation levels were treated identically as those used for force measurement. The preparations were stimulated with 10 μM norepinephrine for varying times and then rapidly frozen in a dry ice/acetone slurry containing 6% trichloroacetic acid and 10 mM 1,4-dithiothreitol. The frozen tissues were immediately transferred to −80 °C for storage. Within 5 days, the frozen tissues were removed from storage and allowed to slowly warm to room temperature. The thawed tissues were washed with acetone containing 10 mM 1,4-dithiothreitol and then air dried before homogenization using glass/glass homogenizers in a solution containing: 6 M urea, 50 mM Tris pH 6.8, 10 mM 1,4-dithiothreitol, 10 mM EGTA, 5 mM EDTA, and 5 mM NaF.

Homogenized samples were assayed for total protein using a kit based on the Bradford Technique (Bio-Rad Laboratories, Richmond, CA). The samples (5 μg) were then subjected to two-dimensional gel electrophoresis followed by transfer to nitrocellulose membranes as previously described (Moreland et al., 1992). Blotted proteins were visualized by gold staining (AuroDye Forte, Amersham Biosciences, Piscataway, NJ). Myosin light chain phosphorylation levels were quantified by densitometric analysis (Scion Image, Scion, Frederick, MD) of optically scanned images (Epson ES-1200U scanner, Long Beach, CA). Phosphorylation levels were calculated using the volume of the spot corresponding to the monophosphorylated myosin light chain as a percent of the total volume of the spots corresponding to the unphosphorylated and monophosphorylated myosin light chain.

2.4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblot

Equilibrated aorta and mesenteric arteries were frozen in an acetone/dry ice slurry containing 6% TCA and 10 mM 1,4-dithiothreitol at rest and after a 5-min stimulation with 10 μM norepinephrine. The frozen arterial preparations were slowly thawed to room temperature, washed with acetone containing 10 mM 1,4-dithiothreitol and allowed to air dry before homogenization in a solution containing: 2% SDS, 20 mM Tris (pH 7.0), 1 mM 1,4-dithiothreitol, 1 mM phenylmethylsulfonyl flouride, 1 mM benzamidine and 25 μg/ml leupeptin at 4 °C using glass–glass homogenizers. The homogenized tissue was clarified by centrifugation (6000 rpm) and the supernatant assayed for total protein. Ten-microgram protein was subjected to SDS-PAGE followed by transfer to nitrocellulose membrane. The efficiency of the transfer was confirmed by staining the nitrocellulose membrane with Ponceau S stain and the post-transfer gel with Coomassie blue. The nitrocellulose membranes were incubated for 45 min in a Tris-buffered saline solution (TBSS) containing 1% bovine serum albumin and 0.05% Tween 20. The membranes were washed in TBSS (3 × 15 min) and then incubated with mouse horseradish peroxidase-conjugated antiphosphotyrosine antibodies (1:2000 monoclonal mouse antibody, Zymed Laboratories, South San Francisco, CA) for 2 h in TBSS. Antibody binding was visualized using Enhanced Chemiluminescence (Amersham Bioscience).

Films exposed to Enhanced Chemiluminescence were subjected to quantitative scanning densitometry using a GS-800 Bio-Rad Scanning Densitometer (Bio-Rad Laboratories). Peak height of each band was determined and normalized to the first peak termed band 1 (lowest molecular weight band on blot). The density of band 1 was similar across any given phosphotyrosine immunoblot, resting and stimulated, for both arterial sources and as such was a reasonable band to use to ensure internal consistency between different blots.

2.5. Chemicals and statistics

All standard chemicals were obtained from Fisher Scientific (Pittsburgh, PA) and were analytical grade or better. Electrophoresis and blotting supplies were obtained from Bio-Rad Laboratories. Genistein and sodium orthovanadate were obtained from Sigma (St. Louis, MO). All results are expressed as the mean ± S.E.M. with n representing the number of samples. Data were compared for statistical significance using Student's t-test, $P < 0.05$ was taken as significant. The EC_{50} values were calculated using correlation regression analysis.

3. Results

3.1. Contraction to norepinephrine

The cumulative addition of norepinephrine to the aortic and mesenteric arterial preparations produced a concentration-dependent increase in the magnitude of contraction. Maximal force development in response to norepinephrine was achieved at 2.5 μM in the aorta and at 10 μM in the mesenteric artery (Fig. 1). Averaged data of several concentration–response curves are shown in Fig. 2. The EC_{50} for norepinephrine-induced contractions in the aorta was significantly lower than that for the mesenteric artery (0.09 ± 0.01 μM aorta vs. 0.61 ± 0.11 μM mesenteric artery; $P < 0.001$, $n = 8$). This represents a 6.5-fold greater sensitivity of the aorta to norepinephrine as compared to the mesenteric artery. The maximal force generated by the aortic rings to norepinephrine was slightly but not significantly

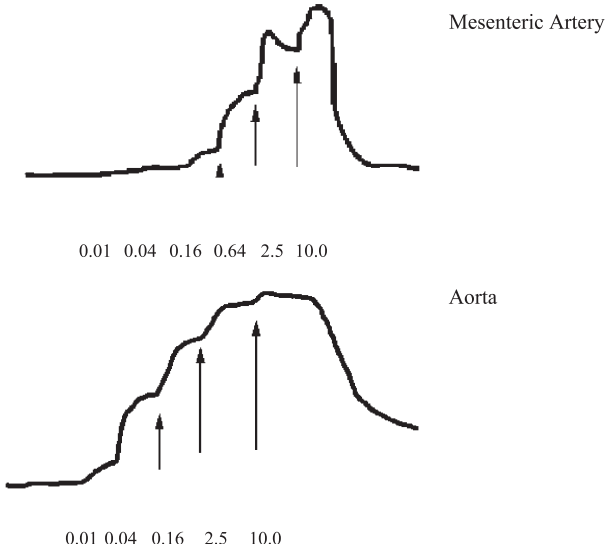

Fig. 1. Representative tracing of the concentration-dependent contraction of rabbit aorta and mesenteric artery to the cumulative addition of norepinephrine. The aorta shows higher sensitivity to norepinephrine than the mesenteric artery but there was no significant difference in the maximal level of force development. Numbers shown are [norepinephrine] in μM.

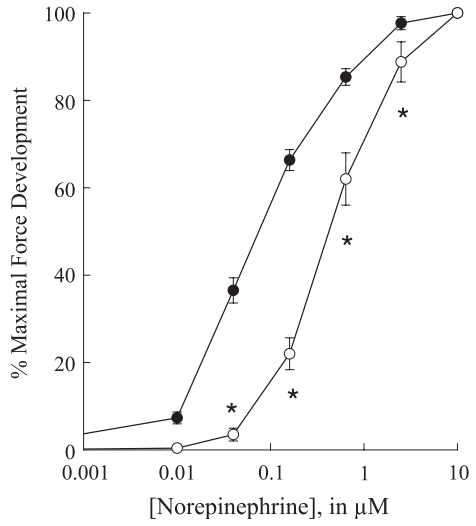

Fig. 2. Concentration–response curve of the rabbit aorta and mesenteric artery to norepinephrine. Rabbit aorta (●) and mesenteric artery (○) were subjected to the cumulative addition of norepinephrine (0.001 to 10 μM). Both vascular preparations responded robustly to the addition of norepinephrine, however the aorta exhibited a higher sensitivity to the agonist. Data are presented as a % of the maximal response in each preparation. Values shown are means ± S.E.M. of at least eight determinations. *Denotes significance at the $P < 0.05$ level.

greater (4.2 ± 0.3 g, $n = 8$) when compared to that developed by the mesenteric arterial rings (4.0 ± 0.2 g, $n = 8$). However, if the forces were normalized to tissue mass, the force generated per g tissue weight was significantly greater ($P < 0.001$) in the mesenteric arterial rings (14.1 ± 0.7 g/g dry tissue weight, $n = 8$) as compared to that generated by the aortic rings (2.8 ± 0.2 g/g dry tissue weight, $n = 8$).

3.2. Myosin light chain phosphorylation

Basal and norepinephrine-induced increases in myosin light chain phosphorylation are presented in Fig. 3. The basal value of myosin light chain phosphorylation was significantly higher in the rabbit aorta than in the mesenteric artery (0.16 mol Pi/mol MLC aorta vs. 0.09 mol Pi/mol MLC mesenteric artery). Upon stimulation with 10 μM norepinephrine, there was a time-dependent increase in myosin light chain phosphorylation values in both tissues. However, the peak increase in myosin light chain phosphorylation values was significantly higher ($P < 0.001$, $n = 4$) in the mesenteric artery as compared to the aorta. The typical transient increase in myosin light chain phosphorylation levels was faster in the mesenteric artery as compared to the aorta. Both the transient increase as well as the decline to suprabasal values was faster in the mesenteric artery.

3.3. Role of tyrosine kinases on norepinephrine responses: effect of Na_3VO_4

In order to determine if the differential response of the aortic and mesenteric preparations to norepinephrine involves tyrosine kinase activity, we performed two sets of experiments. We first utilized Na_3VO_4 to inhibit tyrosine phosphatase activity. If the higher norepinephrine sensitivity of the aorta is due, in part, to tyrosine kinase activity, then one would expect that inhibition of tyrosine phosphatase activity would increase the apparent tyrosine kinase activity in the mesenteric artery and increase norepinephrine sensitivity. Fig. 4 shows the results of these experiments. Incubation of the vascular preparations with 200 μM Na_3VO_4 significantly increased the sensitivity of both the aorta and mesenteric artery to norepinephrine. The EC_{50} value for norepinephrine in the aorta decreased from 0.09 ± 0.02 μM in the absence of Na_3VO_4 to 0.04 ± 0.01 μM in its presence which represents a ~ 2-fold increase in sensitivity. Similarly, the EC_{50} for norepinephrine in the mesenteric artery decreased from 0.62 ± 0.09 μM in the absence of Na_3VO_4 to 0.11 ± 0.01 μM in its presence which represents a 5.6-fold increase in sensitivity. Thus, the potentiating effect of Na_3VO_4 was greater in the mesenteric artery as compared to that in the aorta ($P < 0.005$).

3.4. Role of tyrosine kinases on norepinephrine responses: effect of genistein

The second set of experiments tested the converse hypothesis. If tyrosine kinase activity is important in the greater norepinephrine sensitivity of the aorta as compared to the mesenteric artery, then inhibition of tyrosine kinases should depress norepinephrine sensitivity in the aorta. We

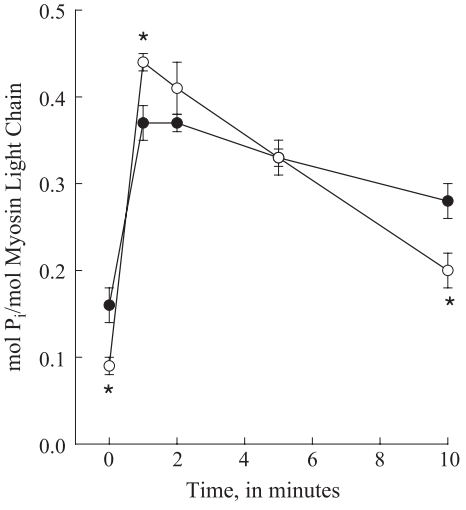

Fig. 3. Myosin light chain phosphorylation levels in rabbit aorta and mesenteric arterial preparations. Myosin light chain phosphorylation levels were quantified at rest and following stimulation with 10 μM norepinephrine in aortic (●) and mesenteric arterial (○) preparations. Basal levels of myosin light chain phosphorylation were significantly higher in the aorta as compared to those measured in the mesenteric artery. The transient peak in myosin light chain phosphorylation was higher and the decline faster in mesenteric arterial as compared to aortic preparations. Values shown are means ± S.E.M. and are presented in mol Pi/mol myosin light chain. Data were obtained from four different sets of experiments, each experiment representing samples pooled from two to three rabbits.

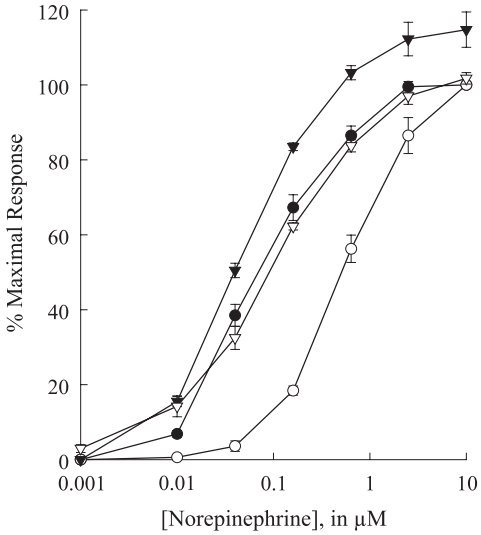

Fig. 4. Cumulative concentration–response curve of the rabbit aorta and mesenteric artery to norepinephrine in the absence and presence of 200 μM sodium orthovanadate. Rabbit aorta (●) and mesenteric arteries (○) were subjected to the cumulative addition of norepinephrine (0.001–10 μM). The tissues were rinsed, allowed to return to baseline force and then 200 μM sodium orthovanadate was added (20-min incubation). The aorta (▼) and the mesenteric arteries (▽) were then subjected to a second cumulative addition of norepinephrine. Maximal response to norepinephrine in the absence of sodium orthovanadate was taken as 100%. Values shown are the means ± S.E.M. of at least four observations.

used 50 μM genistein to inhibit tyrosine kinase activity in the aortic and mesenteric arterial preparations. Fig. 5 depicts the results of cumulative concentration–response curves to norepinephrine in the presence and absence of genistein. Consistent with our hypothesis, genistein significantly decreased norepinephrine sensitivity in the aorta (0.11 ± 0.01 μM control; 0.75 ± 0.03 μM in the presence of genistein, $n = 7$). In addition, genistein also significantly decreased norepinephrine sensitivity in the mesenteric artery (0.69 ± 0.06 control; 2.92 ± 0.08 in the presence of genistein, $n = 6$). Also consistent with the differential change in norepinephrine sensitivity caused by Na_3VO_4, the fold change in EC_{50} induced by genistein was greater in the aorta than in the mesenteric artery.

3.5. Phosphotyrosine levels

Western blot analysis (Fig. 6A and B) showed that both the aorta and mesenteric arteries contain several proteins with phosphorylated tyrosine residues. Proteins containing phosphorylated tyrosine residues were present over the broad range of molecular weights separable on a 10% SDS-PAGE. However, the majority of phosphotyrosine containing proteins appeared to be in the range of 38–155 kDa. Phosphotyrosine antibody binding was demonstrated in unstimulated aortic and mesenteric arterial preparations. The magnitude of tyrosine phosphorylation was greater in the unstimulated aorta (Fig. 6A, Lane 2) as compared to the mesenteric artery (Fig. 6A, Lane 1). The unstimulated aorta showed a distinctly different pattern of phosphotyrosine containing proteins as compared to the unstimulated mesenteric artery. Most notably a protein in the range of 60 kDa showed high levels of antiphosphotyrosine antibody binding in the aorta which was relatively absent in the mesenteric artery. Stimulation of the two vascular tissues (Fig. 6A, Lanes 3 and 4) with 10 μM norepinephrine resulted in a significant increase in phosphotyrosine levels. Densitometric analysis of the blots showed that total phosphotyrosine protein levels were 1.9 times higher in the unstimulated aorta as compared to that in the unstimulated mesenteric artery. Stimulation with 10 μM norepinephrine resulted in a significantly greater percent increase in phosphotyrosine levels in the mesenteric artery as compared to in the aorta (70% increase in mesenteric artery vs. 22% increase in the aorta).

To further investigate the differences in phosphotyrosine levels in resting and stimulated aorta and mesenteric artery, the immunoblots were scanned and the density of each peak normalized to the lowest molecular weight band termed band 1 (Fig. 6B). The left panel shows the results for unstimulated aorta and mesenteric artery and the right panel shows the results for the stimulated tissues. In addition to more clearly demonstrating the generally higher levels of phosphotyrosine binding in unstimulated aorta as compared to the unstimulated mesenteric artery, several specific points are exposed. Most notable are the densities at band #2 which is visible only in stimulated aorta and band #8 which is present in the unstimulated and in greater amounts in the norepinephrine stimulated aorta. Neither band is visible in

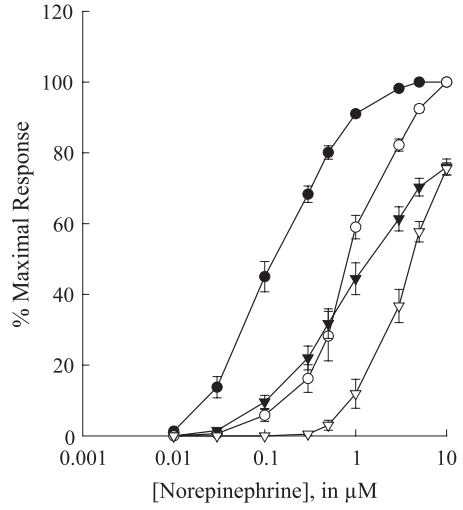

Fig. 5. Cumulative concentration–response curve of the rabbit aorta and mesenteric artery to norepinephrine in the absence and presence of 50 μM genistein. Rabbit aorta (●) and mesenteric arteries (○) were subjected to the cumulative addition of norepinephrine (0.01–10 μM). The tissues were rinsed, allowed to return to baseline force and then 50 μM genistein was added (20-minute incubation). The aorta (▼) and the mesenteric arteries (▽) were then subjected to a second cumulative addition of norepinephrine. Maximal response to norepinephrine in the absence of genistein was taken as 100%. Values shown are the means ± S.E.M. of at least four observations.

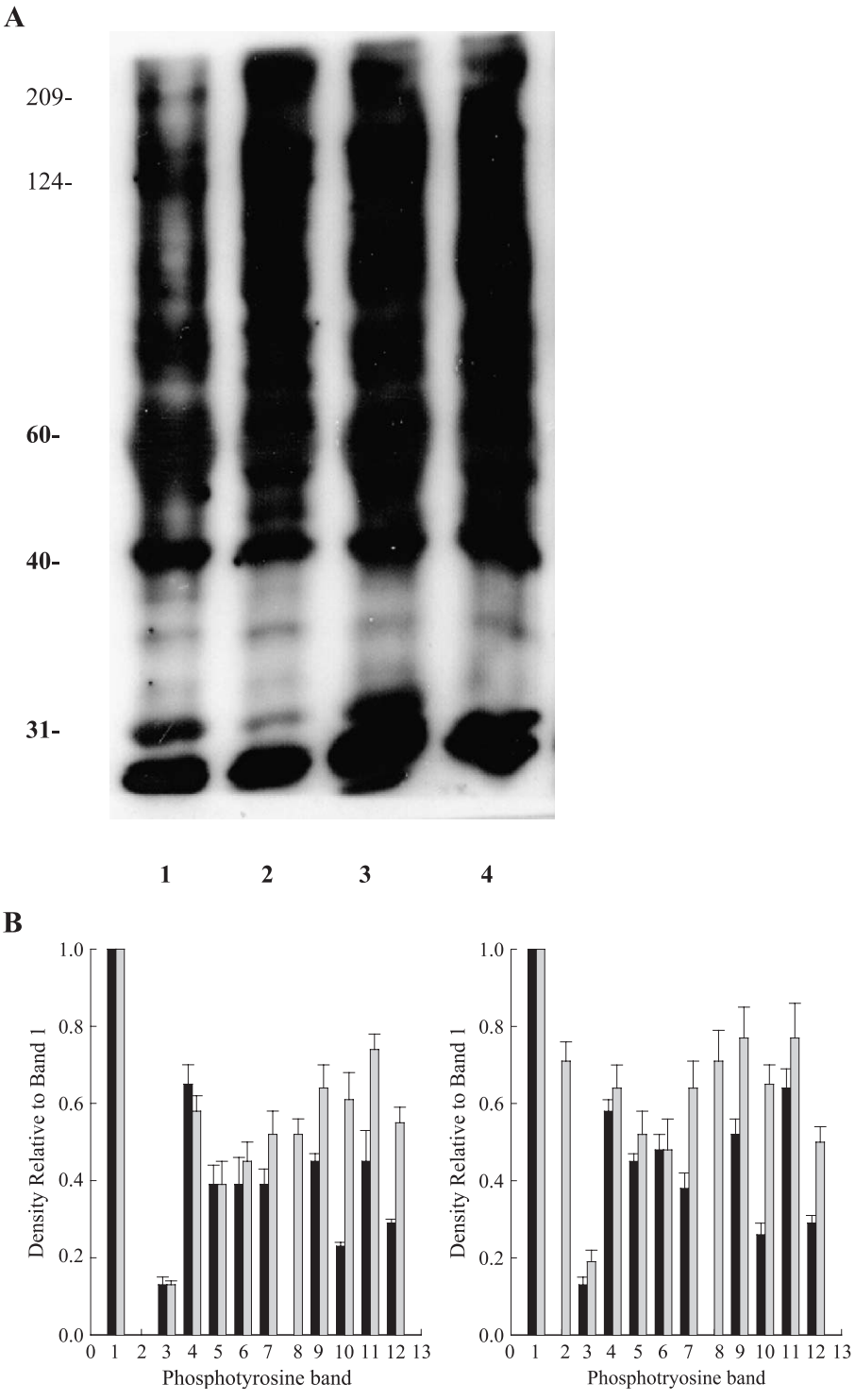

Fig. 6. (A) Phosphotyrosine immunoblot of unstimulated and norepinephrine stimulated aorta and mesenteric artery. Resting, unstimulated mesenteric artery (Lane 1) and mesenteric artery stimulated by 10 μM norepinephrine (Lane 3) were subjected to 10% SDS-PAGE followed by transfer to nitrocellulose membrane and exposure to antiphosphotyrosine antibodies. Similar procedures were applied to resting, unstimulated aorta (Lane 2) and aorta stimulated by 10 μM norepinephrine (Lane 4). Immunoblot shown is representative of four different experiments. (B) Phosphotyrosine immunoblots were subjected to scanning densitometry and band density were normalized to band 1. Left panel shows resting unstimulated values; right panel shows stimulation-induced values. Black bars = mesenteric artery; grey bars = aorta.

the mesenteric artery. In addition, band #7 increases to a greater degree with norepinephrine stimulation of the aorta as compared to the mesenteric artery and band #11 increases to a greater degree with norepinephrine stimulation of the mesenteric artery as compared to the aorta. Therefore, the histograms shown in Fig. 6B provide further evidence that

phosphotyrosine levels are differentially regulated in the two arterial preparations.

4. Discussion

In this study, we have demonstrated that the rabbit aorta is more sensitive to norepinephrine than the mesenteric artery. This finding is consistent with previous work in the same species (Tayo and Bevan, 1987) as well as in rat aorta and mesenteric artery (Adegunloye and Sofola, 1997b). We also demonstrated that the absolute amount of force produced by the rabbit aorta and mesenteric artery were similar but that the amount of force per unit weight of vessel was greater in the mesenteric artery as compared to the aorta. These findings are again consistent with previous studies showing that smaller vessels have a greater capacity to develop tension (Bevan et al., 1999). What we believe to be important in this present study is that we have extended these previous findings to provide evidence as to the mechanism responsible for the enhanced sensitivity to norepinephrine in the aorta as compared to the mesenteric artery.

The contractile response of smooth muscle is initiated by a rise in cytosolic free calcium concentration ($[Ca^{2+}]$). Ca^{2+} can then bind calmodulin to form the Ca^{2+}/calmodulin complex which activates myosin light chain kinase and in turn phosphorylates the 20-kDa myosin light chain of myosin. Myosin light chain phosphorylation activates myosin allowing it to interact with actin resulting in an increase in cross-bridge cycling and in turn, force generation (Kamm and Stull, 1985). Based on this model of contraction, anything that alters either the concentration of activator Ca^{2+} or myosin light chain phosphorylation levels would alter vascular responsiveness to norepinephrine. Our results demonstrated that basal levels of myosin light chain phosphorylation are significantly elevated in the aorta as compared to the mesenteric artery (Fig. 3). This would suggest that the aorta has a higher resting $[Ca^{2+}]$ and can be considered ''primed'' prior to stimulation with norepinephrine. We would suggest that this ''Ca^{2+}-priming'' is not associated with a greater basal value of tone, but that for any given amount of calcium released following norepinephrine stimulation, the final achieved value of activator $[Ca^{2+}]$ is higher in the aorta as compared to the mesenteric artery.

It is well documented however that $[Ca^{2+}]$ does not always correlate with the level of myosin light chain phosphorylation and contraction in vascular smooth muscle. Contractile responses induced by G-protein coupled receptor activation can produce greater force as compared to membrane depolarization, at any given concentration of Ca^{2+} (Somlyo and Somlyo, 2000). This pathway has been shown to be functional in all smooth muscles examined and is therefore an important modulatory event in contractile regulation. In addition, another modulatory pathway has been identified in smooth muscle which may have a direct bearing on our current results. Several laboratories have presented evidence suggesting that tyrosine kinases are important in vascular contractile regulation (Hollenberg, 1994). For instance, inhibitors of tyrosine kinase such as genistein have been reported to inhibit smooth muscle contraction (Di Salvo et al., 1993b; Gould et al., 1995). These studies are consistent with our results demonstrating that genistein significantly decreases contractile sensitivity. Conversely, sodium vanadate has been used to inhibit tyrosine phosphatase activity and produce an increase in cellular tyrosine phosphorylation levels (Di Salvo et al., 1993a; Candura et al., 1994; Laniyonu et al., 1994). Vanadate, presumably by enhancement of tyrosine phosphorylation levels, increases contraction of several smooth muscles such as the human bronchus (Zhou et al., 1997), rat gastric smooth muscle (Shimada et al., 1986), rat aorta (Laniyonu et al., 1994), and guinea pig taenia coli (Christensen and Mulvany, 1993). The results shown in Fig. 6 suggest that tyrosine kinase phosphorylation may play a significant role in the differential response of rabbit aorta and mesenteric artery to norepinephrine. The significant difference in phosphotyrosine levels in the basal state as well as the differential response in phosphotyrosine levels to norepinephrine stimulation in the two vascular preparations supports this hypothesis. Of particular interest is that the results shown in Fig. 6B suggest that some phosphotyrosine peaks appear to be specific for the unstimulated and norepinephrine-stimulated aorta as compared to the mesenteric artery.

In addition to the biochemical changes noted in phosphotyrosine levels, our more direct pharmacological results also support a role for tyrosine kinases in setting the level of vascular sensitivity to norepinephrine. The greater inhibition of norepinephrine-stimulated aortic contractions by genistein as compared to that of mesenteric artery (Fig. 5) could be explained by a greater role of tyrosine kinases in the aorta as compared to the mesenteric artery. Conversely, the smaller increase of the norepinephrine-stimulated aortic contractions by vanadate as compared to mesenteric artery (Fig. 4) could be explained again by an already greater role and activity of cellular tyrosine kinases in the aorta.

The mechanism(s) by which tyrosine kinase activity modulates smooth muscle contraction remains uncertain, however a number of possibilities exist. An increase in tyrosine kinase phosphorylation correlates with an elevation in vascular $[Ca^{2+}]$ and vasoconstriction (Christensen and Mulvany, 1993; Shimada et al., 1986; Zhou et al., 1997). It has also been suggested that tyrosine kinases are involved in the mechanisms that mediate Ca^{2+} release from the sarcoplasmic reticulum (Liu and Sturek, 1996). This possibility is supported by the observation that genistein decreases the rate of serotonin induced inositol trisphosphate production (Liu and Sturek, 1996). Tyrosine kinase activity may also be involved in the regulation of Ca^{2+} entry as inhibitors of tyrosine kinase attenuate Ca^{2+} entry following agonist-mediated Ca^{2+} store depletion (Low, 1996). This observation is consistent with the hypothesis that the release of

intracellular Ca^{2+} activates a tyrosine kinase that in turn, stimulates Ca^{2+} entry (Huckle et al., 1992). There have also been reports to suggest that inhibitors of tyrosine kinase activity modulate K^+ currents in vascular smooth muscle cells (Hughes and Wijetunge, 1998). These observations are consistent with a study showing that the genistein-dependent decrease in $[Ca^{2+}]$ quantitatively accounted for the decrease in myosin light chain phosphorylation in swine carotid arteries (Gould et al., 1995). However, it has also been shown that sodium orthovanadate significantly increased myoplasmic Ca^{2+}-sensitivity in vascular smooth muscle (Masui and Wakabayashi, 2000).

In summary, we have shown that the rabbit aorta is significantly more sensitive to norepinephrine than the mesenteric artery. We have also demonstrated that the enhanced sensitivity of the aorta can be reversed by inhibitors of tyrosine kinase while the lower sensitivity of the mesenteric artery can be enhanced by inhibitors of tyrosine phosphatase. The rabbit aorta has significantly higher basal values of myosin light chain phosphorylation which correlate with higher basal levels of tyrosine phosphorylation. We suggest that the results of our study are consistent with a hypothesis that the higher basal tyrosine kinase activity in the aorta may result in higher basal $[Ca^{2+}]$ and myosin light chain phosphorylation levels. This may produce a muscle state that could be considered "primed" for stimulation.

Acknowledgements

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