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Differential responses to endothelial–dependent relaxation of the thoracic and abdominal aorta from male Sprague-Dawley rats

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Summary: Regional heterogeneity exists in reactivity of different vascular beds to vasoactive substances. Experiments were designed to determine if there are differences between thoracic and abdominal aorta response to acetylcholine-induced relaxation. Ten male Sprague-Dawley rats with a weighing between 200g–250g were used. The aorta was isolated and 3mm aortic rings were cut and suspended in organ baths containing physiological salt saline (PSS). Contractile and relaxation responses to noradrenaline (NA) and ACh, in the presence or absence of L-NNA and high K^+ concentration were studied. Contractile response to NA was similar along the aorta. At the higher doses, ACh elicited a greater ($p < 0.05$) relaxation in the abdominal aorta when compared with the thoracic aorta. However, inhibition of eNOS was more effective ($p<0.05$) in preventing ACh-induced relaxation in the thoracic aorta when compared with the abdominal aorta. Conversely, inhibition of endothelial hyperpolarizing factor (EDHF) by high K^+ concentration blocked ACh-induced relaxation to a greater extent in the abdominal aorta ($p<0.05$) when compared with the thoracic aorta. ACh-induced relaxation differs in the thoracic and abdominal aorta. Differences in the EDHF activity along the aorta underlie the differential response of the thoracic and abdominal aorta to ACh-induced relaxation.

Keywords*:* Nitric oxide, Potassium ions, Endothelium, Acetylcholine, abdominal aorta, Thoracic aorta.

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INTRODUCTION

Vascular smooth muscle is a key player contributing to the regulation of blood pressure by controlling the diameter of blood vessels and thereby regulating peripheral resistance. Many vascular reactivity studies have involved whole vessels, ring or helical strip preparation of vessels such as the aorta (Dominiczak *et al.,* 1991; Sofola *et al.,* 2003; Wong *et al.,* 2010), mesenteric (Misurski *et al.,* 2001; Tep – areenan *et al.,* 2002) or cerebral arteries (Gonzales *et al.,* 2004; Tsang *et al.,* 2004) of rats. Differences exist in the responses of vascular cells from different parts of the vascular tree and from different species to vasoactive substances. For example, rabbit aorta is more responsive to norepinephrine than its branches, a fact that has been ascribed to variations in response to extracellular calcium ions along the aorta (Yang and Bevan, 1987; Tayo and Bevan, 1987). Rat thoracic aorta and mesenteric artery have also been reported to exhibit differential responses to norepinephrine and serotonin (Adegunloye and Sofola, 1997), just as observed in the differential responses of mouse aorta and rat aorta to isoproterenol, angiotensin II, endothelin-1, histamine, and adenosine (Russel and Watts, 2000).

The rat aorta is often used for vascular studies because it produces stable and reproducible contractions which are essential in relaxation response studies (Matsumoto *et al.,* 1996). The vascular endothelium exerts a significant arterial vasodilator influence mediated by the production of NO, prostacyclin and a third mechanism that is resistant to the combined inhibition of NO synthase and cyclooxygenase. This mechanism is accompanied by smooth muscle hyperpolarization and is sensitive to high potassium ions concentration (Savage *et al.,* 2003). It is termed endothelial-derived hyperpolarizing factor (EDHF). Acetylcholine is an endothelial-dependent arterial vasodilator. A regional difference in relaxation response to ACh has been reported in different vascular beds (Hilgers *et al.,* 2006). We therefore, chose to investigate, if there are differences in the response to acetylcholine-induced

relaxation along regions of the aorta and to address the mechanisms that may underlie these differences.

MATERIALS AND METHODS

Ten adult male Sprague Dawley rats obtained from the Laboratory Animal Facility of the College of Medicine of the University of Lagos weighing 200g – 250g were used for this study. The protocol for this study was approved by the College of Medicine Research and Ethic Committee.

Isolation and Preparation of Aortic Rings

The rats were sacrificed by cervical dislocation. Thereafter the thoracic cage was opened and heparin was injected into the ventricle to prevent blood clotting. The aorta was cut along its length, from its arch and far down, close to the origin of the renal artery and was quickly placed in a Petri dish containing cold Physiological Salt Solution (PSS). The aorta was then freed of connective tissue and cut into 2-3mm ring segments. Special care was taken to avoid contact with the endothelial surface during the removal and mounting of the rings. The ring was then mounted horizontally between two fine stainless steel rods. The lower rod was connected to the base of the organ bath, while the upper rod was attached to the isometric force transducer (Model FT03, Grass Instruments, Mass, USA) which was coupled to the Grass polygraph (Model 7D, Grass Instruments) via a preamplifier and a driver amplifier to an ink writing stylus. This was used in recording the force displacement by the tissue. The rings were superfused in 20ml double jacketed organ bath with PSS at 37^{o}C and gassed with 95% O_2: 5% CO_2 mixture. The pH of the PSS was usually between 7.35-7.40, and all baths used simultaneously had a parallel connection to the source of PSS. The PSS consisted of (mM): NaCl, 119.0; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1-2; $NaHCO_3$, 24.9; $CaCl_2$, 1.6 and glucose, 11.5. The PSS contained 10^{-5}M indomethacin, an inhibitor of cyclooxegenase. This was to eliminate the effect / activity of prostaglandins in the response.

Experimental Protocol

After mounting, a passive tension of 2g was applied to each ring. The mounted ring was then allowed to equilibrate in the PSS for 90min, during which period each ring was subjected to a sub-maximal dose (10^{-7}M) of noradrenaline at 30 min interval. Each of the stabilizing stimulations lasted for 5 minutes after which the ring was rinsed with PSS. The PSS in the bath was replaced after every 15 minutes during the experiment. The stabilization was required to ensure consistent responses of the aortic rings throughout the experiment. At the end of the 90min stabilization period, the integrity of the endothelium was assessed by relaxation induced by 10^{-6} M acetylcholine in the noradrenaline (10^{-7} M) precontracted rings. Rings with less than 60% contraction to 10^{-7}M noradrenaline and / or less than 50% relaxation response to ACh were discarded. Fresh rings were used for each of the experimental protocols.

Cumulative Contractile Response to Noradrenaline

In the experiment to assess the differential contractile response of the thoracic and abdominal aorta to noradrenalin, cumulative doses of noradrenalin (10^{-8} – 10^{-5}M) were added to the organ bath sequentially.

Relaxation Response to Acetylcholine

For relaxation response to acetylcholine, aortic rings were precontracted with 10^{-7}M noradrenaline and after the contraction had reached a plateau, cumulative doses of ACh (10^{-8} – 10^{-5}M) were added to the organ bath while the pen deflection recorded continuously.

Relaxation Response to Acetylcholine in the Presence of L-NNA

Involvement of endogenous NO production on the relaxation response of the aorta to ACh in both thoracic and abdominal aorta was studied by using the eNOS inhibitor, L–NNA. The aortic rings were incubated with L-NNA (10^{-4}M), for 30 minutes. After the 30 minutes incubation period the rings were precontracted with 0.1µM noradrenaline, after which cumulative doses of Ach (10^{-8} – 10^{-5}M) were added to the organ bath.

Relaxation Response to Acetylcholine in the Presence of Increased Extracellular Concentration of Potassium ions (K^+)

To assess the role of EDHF in the differential response of thoracic and abdominal aorta to acetylcholine, the extracellular concentration of K^+ was elevated to 30mM (K_{30}) (Savage *et al.,* 2001), after which the rings were then precontracted with 10^{-7} M noradrenaline and cumulative doses of ACh (10^{-8} – 10^{-5}M) were added to the organ bath.

Relaxation Response to Acetylcholine in the Presence of LNNA and Increased Extracellular Concentration of Potassium ions (K^+)

To assess the role of EDRFs and EDHF in the differential response of thoracic and abdominal aorta to acetylcholine, the aortic rings were incubated with L-NNA (10^{-4}M), for 30 minutes. After the 30 minutes incubation period, the extracellular concentration of K^+ was elevated to 30mM (K_{30}), after which the rings were then precontracted with 10^{-7}M noradrenalin and cumulative doses of ACh (10^{-8} – 10^{-5}M) were added to the organ bath.

Chemicals and Drugs

Noradrenaline, acetylcholine and L- Nitroarginine (L-NNA were dissolved and diluted in distilled water.

Noradrenalin, acetylcholine, L-NNA indomethacin and KCl were products of Sigma-Aldrich Chemicals USA.

Statistical Analysis

The collected data were expressed as mean ± S.E.M. The data were analyzed using Student's t-test and one way analysis of variance (ANOVA) where applicable. Student-Newman-Keuls post Hoc test was used to identify differences between individual means after ANOVA. Confidence interval was placed at 95%, so that in all cases a value of $p < 0.05$ was considered significant. (Graphpad Prism 5 USA).

RESULTS

Cumulative Contractile Response to Noradrenalin

As shown in Figure 1, there was no significant difference in the contractile response of both thoracic and abdominal aorta to noradrenaline.

Relaxation Response to Acetylcholine

At lower concentrations of ACh (10^{-8}–10^{-6}), relaxation response appears to be similar in both thoracic and abdominal aorta. However, at higher concentrations, ACh (10^{-6}–10^{-5}) elicit greater ($p<0.05$) relaxation response in the abdominal aorta compared with the thoracic aorta (Figure 2).

Relaxation Response to Acetylcholine in the Presence of L-NNA

Experiment on the involvement of NO in the acetylcholine-induced relaxation along the aorta showed a significant decrease ($p < 0.05$) in relaxation response to ACh of thoracic aorta when compared with abdominal aorta (Figure3). Likewise the magnitude of reduction in relaxation response of

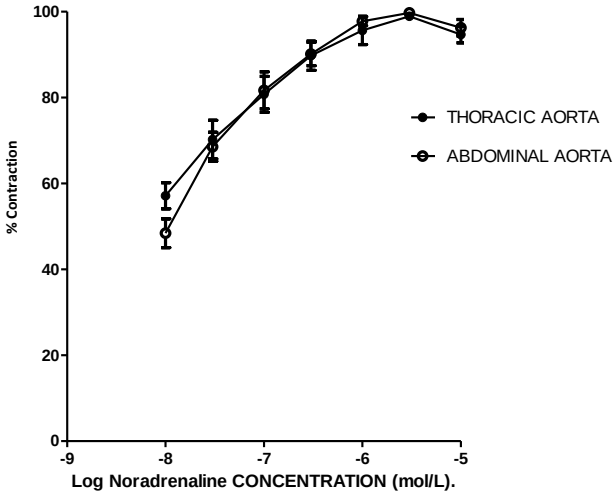

Fig.1. Cumulative contractile response curves to noradrenaline of thoracic and abdominal aorta. Data were expressed as mean ± S.E.M. n = 6

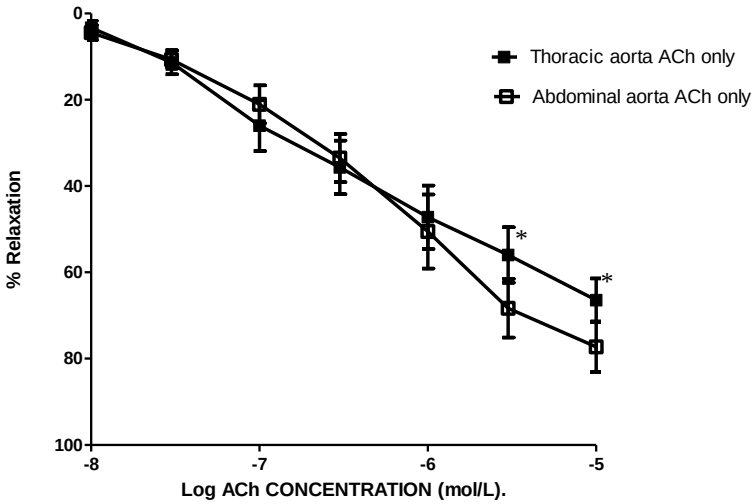

Fig.2. Cumulative relaxation response curves to acetylcholine of thoracic and abdominal aorta. Data were expressed as mean ± S.E.M. n = 6. *Significantly less ($p<0.05$) when compared with abdominal aorta.

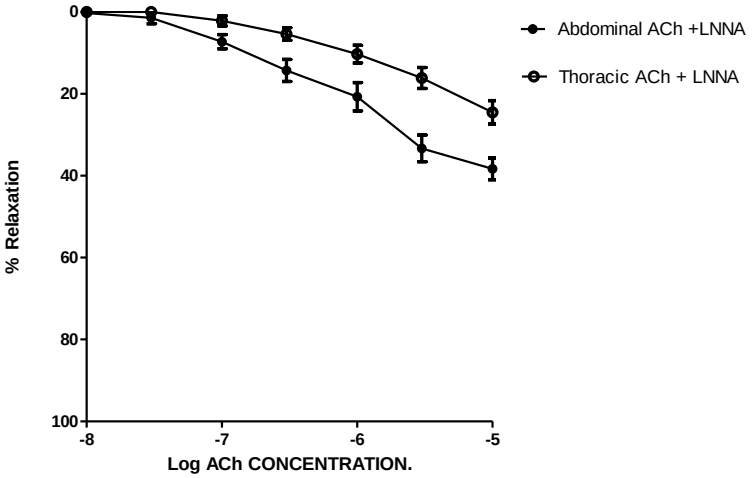

Fig. 3. Cumulative relaxation response curves of thoracic and abdominal aorta to acetylcholine in the presence of L-NNA. Data were expressed as mean ± S.E.M. n = 6. *Significantly less ($p<0.05$) when compared with abdominal aorta.

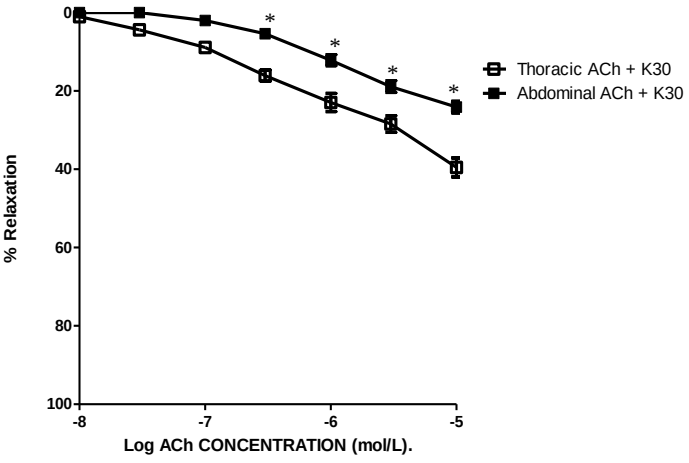

Fig. 4. Cumulative relaxation response curves thoracic and abdominal aorta to acetylcholine in high potassium ions extracellular concentration (K_{30}). Data were expressed as mean ± S.E.M. n = 6. *Significantly less ($p < 0.05$) when compared with thoracic aorta.

thoracic aorta to ACh in the presence of L-NNA was greater ($p<0.05$) when compared with that in the presence of high concentration of K^+. (Figure 6).

Relaxation Response to Acetylcholine in High Extracellular Concentration of K^+

Blocking of EDHF activity by elevated extracellular K^+ concentration elicited a significant ($p<0.05$) reduction of abdominal aorta relaxation response to ACh when compared with thoracic aorta (Figure 4). The magnitude of reduced relaxation response to ACh of abdominal aorta in the presence of blocked EDHF activity was greater than that of eNOS blockade (Figure 7).

It was observed that, elevation of the K^+ concentration to 30mM (K_{30}) contracted the thoracic and abdominal aorta to (56.72 ± 4.23%) and (61.34 ± 5.72%) respectively. Further addition of 10^{-7}M noradrenaline to the organ bath raised the contraction of the thoracic and abdominal aorta to (69.62 ± 3.54%) and (71.91 ± 5.01%) respectively. Addition of noradrenaline abolished the differences that existed in the responses of thoracic and abdominal aorta to K_{30}.

Relaxation response to Acetylcholine in High Extracellular Concentration of K^+ (K_{30}) in the Presence of L-NNA

As shown in Figure 5, blockade of both EDRFs and EDHF activities almost completely abolished acetylcholine–induced relaxation response in both thoracic and abdominal aorta. There was no significant difference in the response of thoracic and abdominal aorta to ACh in the combined blockade of eNOS and EDHF by L-NNA and K_{30} respectively.

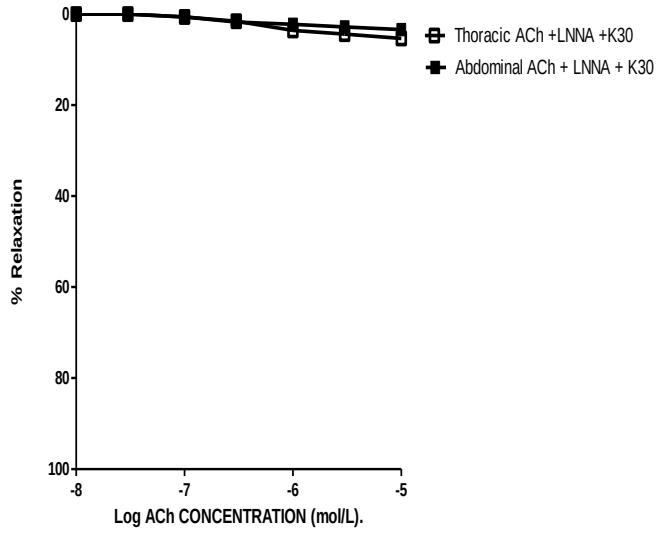

Fig.5. Cumulative relaxation response curves of thoracic and abdominal aorta to acetylcholine in the presence of LNNA and high potassium ions extracellular concentration (K_{30}). Data were expressed as mean ± S.E.M. n = 6.

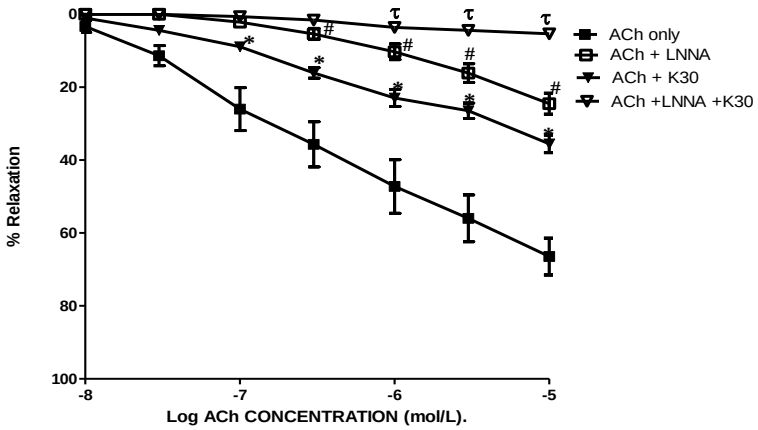

Fig.6. Cumulative relaxation response curves of thoracic aorta to acetylcholine in the presence or absence of LNNA and high potassium ions extracellular concentration (K_{30}). Data were expressed as mean ± S.E.M. n = 6. ᵗSignificant reduction ($p<0.05$) when compared with relaxation response to ACh in the presence or absence of L-NNA or K_{30}. #Significantly less ($p<0.05$) when compared with relaxation response to ACh in the presence or absence of K_{30}. *Significantly less ($p<0.05$) when compared with relaxation response to ACh.

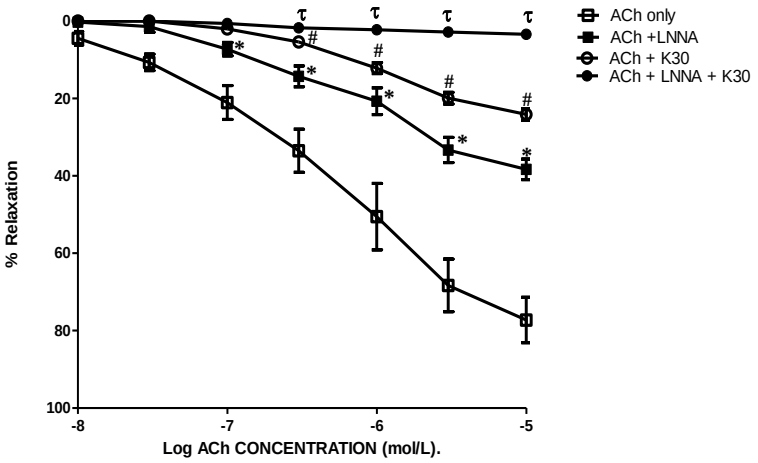

Fig.7. Cumulative relaxation response curves of abdominal aorta to acetylcholine in the presence or absence of LNNA and high potassium ions extracellular concentration (K_{30}). Data were expressed as mean ± S.E.M. n = 6.). Data were expressed as mean ± S.E.M. n = 6. ᵗSignificant reduction ($p<0.05$) when compared with relaxation response to ACh in the presence or absence of L-NNA or K_{30}. #Significantly less ($p<0.05$) when compared with relaxation response to ACh in the presence or absence ofL-NNA. *Significantly less ($p<0.05$) when compared with relaxation response to ACh.

DISCUSSION

Findings from this study indicate that relaxation of thoracic and abdominal aorta to acetylcholine appears similar and is dependent of endothelial vasoactive substances. However, differences exist in the mechanism that underlies acetylcholine-induced relaxation in thoracic and abdominal aorta. In the design of the experiments in this study, 10^{-6}M of

indomethacin (a cyclooxygenase inhibitor) was present in the PSS throughout the experimental period, thereby suppressing cyclooxegenase activity.

The greater relaxation response of abdominal aorta when compared with thoracic aorta to ACh even in the presence of eNOS blockade suggests that ACh-induced relaxation in thoracic aorta is more NO-dependent compared to abdominal aorta, and also that there is another more important mechanism that is involved in ACh-induced relaxation in the abdominal aorta.

The fact that the combined blockade of endogenous production of NO and prostacyclin by L-NNA and indomethacin respectively, do not completely abolish the acetylcholine-induced relaxation in the thoracic and abdominal aorta suggests there is another mechanism involved in relaxation response of the aorta to acetylcholine. Involvement of a third autacoid that contributes to endothelium-dependent vasodilatation mechanisms in many vascular beds had been earlier suggested (Chen *et al.,* 1988). This autacoid has been called endothelium dependent hyperpolarizing factor (EDHF) (Taylor and Weston, 1999).

Raising the extracellular concentration of potassium ions (K^+) has been demonstrated to inhibit the activity of the EDHF (Adeagbo and Triggle, 1993; Savage *et al.,* 2001). In the present study, the reduced relaxation response to acetylcholine of abdominal aorta in the presence of high K^+ concentration when compared with thoracic aorta suggests a higher and / or more important activity of EDHF in the abdominal aorta. The abolishment by noradrenaline of the differences that exist in the magnitude of contractile response to high K^+ between thoracic and abdominal aorta suggest that there is no difference in reactivity to noradrenaline along the aorta. This is consistent with absence of significant difference between thoracic and abdominal aorta in the cumulative contractile response to noradrenaline.

This finding is interesting and is consistent with the earlier submission of other workers that the EDHF activity increases with decreasing diameter of blood vessels (Ozkor and Quyyumi, 2011). Probably the aorta decreases gradually in diameter as it descends from the thorax into the abdomen.

We conclude that there are differences in the mechanisms underlying the response to ACh-induced relaxation, which is mediated by the differential EDHF activity along the aorta. In physiological experiments, the use of abdominal aorta will give more evidence of involvement of EDHF in relaxation responses when compared with the thoracic aorta.

Acknowledgement

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