

RESEARCH ARTICLE

Phenylephrine induces relaxation of longitudinal strips from small arteries of goat legs

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Abstract

Alpha adrenergic stimulation is known to produce vasoconstriction. We have earlier shown that, in spiral strips of small arteries Phenylephrine (PE) caused vasorelaxation under high nitric oxide (NO) environment. However, on further experimentation it was realized that the PE-induced vasorelaxant response occurred only with longitudinal strips of small arteries even under normal NO environment while circular strips showed contraction with PE even under high NO environment. Such PE-induced vasorelaxation of longitudinal strips was blocked by Phentolamine, an alpha-adrenergic receptor blocker. On delineation of specific receptor subtype, PE-induced relaxation was found to be mediated through alpha 1D receptor. However, this phenomenon is specific to small artery, as longitudinal smooth muscle of aorta showed only contractile response to adrenergic stimulation. There is no prior report of longitudinal smooth muscle in small artery up to our knowledge. The results of this study and histological examination of vessel sections suggest the presence of longitudinal smooth muscle in small artery and their relaxant response to alpha adrenergic stimulation is a novel phenomenon.

Introduction

Phenylephrine (PE) is an alpha-adrenergic agonist which is known to cause contraction of vascular smooth muscle. We had recently reported that Phenylephrine caused relaxation of spiral strips made from small arteries from goat legs in a high nitric oxide environment, while causing contraction in normal circumstances. However, with further experimentation we have realized that longitudinal strips made from small arteries always relaxed with Phenylephrine, even in normal nitric oxide environment. On the other hand, PE caused contraction of circular strips of small arteries even in a high nitric oxide environment. Spiral strips used in earlier

experiments have led to erroneous inferences, as the method of making the strip is prone to experimenter bias.

In this paper, we compare the responses of transverse (or circular) and longitudinal preparations made from aorta and small arteries from goats. While PE increases vascular tension in both transverse and longitudinal strips made from aorta, as do circular strips of small arteries, longitudinal strips from small arteries show reduction in vascular tension in the presence of PE.

An important determinant of arterial pressure is peripheral vascular resistance, which in turn is primarily determined by the diameter of small arteries and arterioles which are referred to as resistance vessels [1]. The walls of the resistance vessels have smooth muscle cells arranged in a concentric fashion in the tunica media which is referred to as circular smooth muscle [2]. Contraction of circular smooth muscle reduces lumen diameter and therefore increases vascular resistance [1, 3]. The balance between vasoconstrictor and vasodilator signals acting on the circular smooth muscle determines the diameter and therefore the tone of resistance vessels.

While muscular arteries (medium-sized) are documented to have concentrically arranged circular smooth muscle in the tunica media, veins have longitudinal smooth muscle just next to the intima [4]. There is evidence for the presence of longitudinal smooth muscle in the walls of large arteries and aorta too [5]. Function of longitudinal smooth muscle in the large arteries has not been clearly understood.

There is, however, no prior report of longitudinal smooth muscle in small arteries, up to our knowledge. The results of this study suggest the presence of longitudinal smooth muscle in small arteries, and their relaxant response to alpha adrenergic stimulation is a novel phenomenon.

Materials & methods

This study was approved by Institutional Review Board of Christian medical College, Vellore, (IRB number 10959 dated 7th November 2017).

Solutions used

The composition of the mammalian ECF solution used in the experiments was as follows (in mmol/L): NaCl 100; KCl 3; CaCl₂ 1.3; NaH₂PO₄ 0.5; Na₂HPO₄ 2; NaHCO₃ 25; MgCl₂ 2; HEPES 10; Glucose 5; pH was corrected to 7.4 with 1mol/L sodium hydroxide. All salts for mammalian ECF solution were purchased from SIGMA. Phenylephrine hydrochloride, L-Arginine, Sodium Nitroprusside (SNP) and L-NNA were also purchased from SIGMA.

10 mmol/L stock solution was prepared for phenylephrine and L-Arginine in distilled water. Appropriate amount of the drug was added both to the organ bath and to the drug reservoir to obtain the final concentration.

Isolation of small sized artery

Fresh goat leg (*Capra hircus*) from a registered slaughterhouse, Rafi's slaughterhouse, accreditation number: 309/13 (Co-ordinates 12.93°N 79.13°E) was procured on the day of experiment, washed, and the skin removed. A vascular bundle close to the muscle below the knee was identified. The artery in the bundle was identified by patency of its lumen. Considerable length of the artery was dissected and transferred to a petri dish containing cold ECF solution. Adventitious tissue attached to the artery was removed and the artery was cut into segments of 1.5–2 cm length. It was ensured that the arterial segments were devoid of side branches. Two different arterial preparations were made:

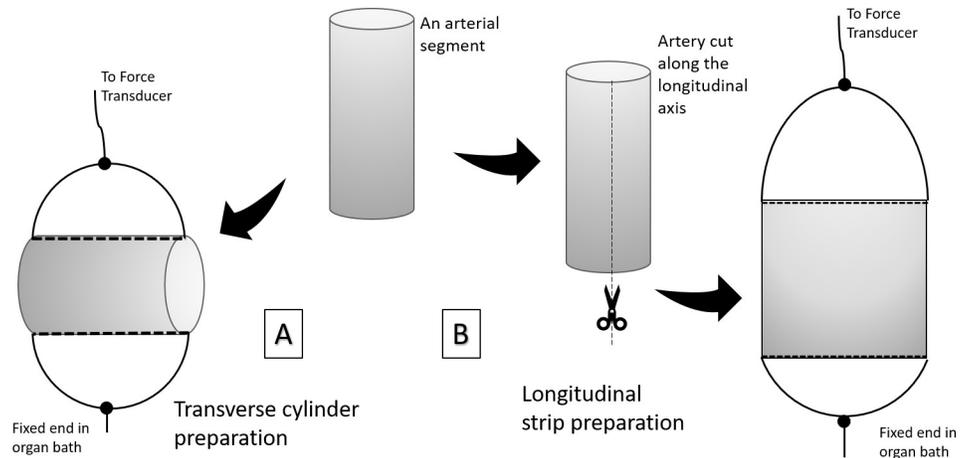


Fig 1. Diagrammatic representation of transverse cylinder preparation and longitudinal strip preparation of small artery.

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- i. Transverse cylinder
- ii. Longitudinal strip

Transverse cylinder preparation: Two fine threads were inserted through the lumen of the arterial segment and loops were made on opposite sides. One loop was connected to a force transducer and the other loop was attached to a hook in the organ bath such that the tissue was suspended transversely. (Fig 1)

Longitudinal strip preparation: An arterial segment of length 1.5–2 cm was selected and cut open longitudinally using an iris scissors thereby exposing the endothelium. One end of the longitudinal axis was connected to the force transducer while the other end was connected to the base of the organ bath. (Fig 1)

Isolation of aorta

Fresh goat heart procured from a registered slaughterhouse (details as before) was transported to lab in ice cold mammalian ECF solution. The heart was washed, pericardial sac and fat removed, and ascending aorta was identified by the patency of its lumen. The aorta was then dissected from its point of origin to the point before it branches. The dissected aorta was transferred to a petri dish containing cold ECF solution and the adventitious tissue removed. Two different preparations were made.

- i. Aortic circular strip
- ii. Aortic longitudinal strip

Aortic circular strip preparation: A ring of aorta was cut, with thickness of 5 mm. One end of the ring was attached to the force transducer and the other end to the base of the organ bath.

Aortic Longitudinal strip Preparation: A length of aortic cylinder was cut open in longitudinal orientation with surgical scissors exposing the endothelium. Opposite ends of the longitudinal strip were connected to a force transducer and the base of the organ bath.

Data acquisition and analysis. The force transducer was connected to Powerlab data acquisition system for recording vessel tension. The thread was kept taut with an optimum preload and the resting tension was maintained between 0.2 to 0.6g. Change in tension was

recorded when drugs were added into the organ bath. The data were acquired at 1 KHz and was processed using MATLAB R2018a software. Change in vascular tension with interventions is expressed as scatter plots. The percentage change in tension is also shown as bar diagrams showing mean and SD.

Statistical analysis

Statistical analysis was done using SPSS software ver.16.0. Comparison of vascular tension before and after intervention in same group was done using Wilcoxon signed rank (WSR) test. Changes in vascular tension between different groups were done using Mann-Whitney U (MWU) test. P value ≤ 0.05 was considered statistically significant.

Histological examination of vessel preparations

Transverse and longitudinal preparations of small artery and aorta were immersed in 10% buffered formalin for 2–3 days for fixation. Ascending grades of alcohol were used to dehydrate the tissues which were finally cleared with xylene. Then paraffin blocks of the tissues were made by impregnating with liquid paraffin. 5 μ m thick sections were made using microtome. Tissue sections were subjected to dewaxination and were hydrated with descending grades of alcohol and were washed in distilled water. Staining was done using hematoxylin and eosin and sections were observed under light microscope.

Results

A summary of the major results obtained is presented in Table 1 and the details are provided:

PE induces contraction of circular smooth muscle of aorta under normal and high NO environments

In circular strips of aorta, PE 100 μ mol/L increased tension from 0.37 to 0.51g. (Median, n = 5, P value = 0.043 with WSR test comparing vascular tension before and after PE), Fig 2, (i). The concentration of PE for the experiments on aortic as well as small artery preparations was chosen as 100 μ mol/L because the small artery preparation did not show a response to 10 μ mol/L PE.

L-Arginine 100 μ mol/L was added to the bath to increase NO levels in the circular strip of aorta. No change in tension was observed with L-Arginine. Subsequent addition of PE 100 μ mol/L increased tension from 0.31g to 0.65g. (Median, n = 6, P value = 0.028 with WSR test comparing tension before and after PE), Fig 2, (ii).

Addition of sodium nitroprusside (SNP) 400 μ mol/L to the bath to create high NO environment, did not change vascular tension in the circular aortic strip. Subsequent addition of PE increased tension from 0.35g to 0.49g. (Median, n = 5, P value = 0.043 with WSR when tension before and after PE in presence of SNP were compared), Fig 2, (iii).

Table 1. Summary of major results of the study.

	Aorta circular strip	Aorta longitudinal strip	Small artery transverse Strip	Small artery longitudinal strip
PE 100 μ mol/L	Contraction	Contraction	Contraction	Relaxation
L-Arginine + PE	Contraction	Contraction	Contraction	Relaxation
SNP+ PE	Contraction	Contraction	-	-
L-NNA + PE	-	-	No Contraction	No Relaxation
Phentolamine + PE	-	-	No Contraction	No Relaxation

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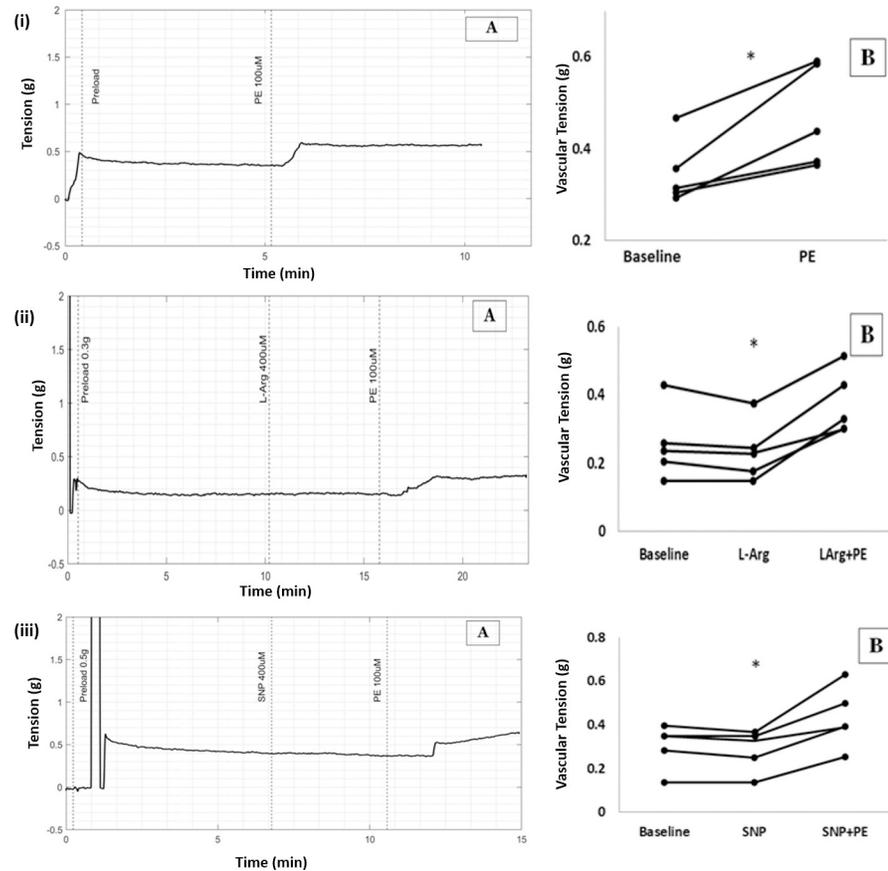


Fig 2. (A) Raw tracings showing changes in vascular tension in circular strips of aorta (B) Scatter plots of results from all five experiments demonstrating increase in vascular tension with PE from baseline (* $p = 0.043$, WSR). (i) PE 100µmol/L (ii) PE 100µmol/L in high NO environment caused by L-Arginine (iii) PE 100µmol/L in presence of SNP.

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There was no significant difference when percentage change in aortic vascular tension due to PE with and without L-Arginine (P value = 0.273 with MWU) were compared and also when PE with and without SNP (P value = 0.754 with MWU) were compared, showing that PE-induced vasoconstriction in aortic circular muscle was not affected by high levels of NO.

PE induces contraction of longitudinal smooth muscle of aorta under normal and high NO environment

PE 100µmol/L increased the tension of longitudinal smooth muscle in aorta from 0.31g to 0.43g. The increase in tension by PE was statistically significant. (Median, $n = 5$, P value = 0.043, WSR), Fig 3, (i).

High NO environment created by addition of L-Arginine 100µmol/L did not cause change in tension of the longitudinal strip of aorta. However subsequent addition of PE 100µmol/L increased tension from 0.22g to 0.32g. (Median, $n = 5$, P value = 0.043, WSR), Fig 3, (ii).

In another set of experiments, in the presence of SNP, a NO donor, PE 100µmol/L increased tension of longitudinal smooth muscle from 0.32g to 0.39g. (Median, $n = 5$, P value = 0.043 with WSR test when tension before and after PE in presence of SNP were compared), Fig 3, (iii).

There was no significant difference when the percentage change in vascular tension due to PE with and without L-Arginine were compared (P value = 0.076 with Mann-Whitney U test,

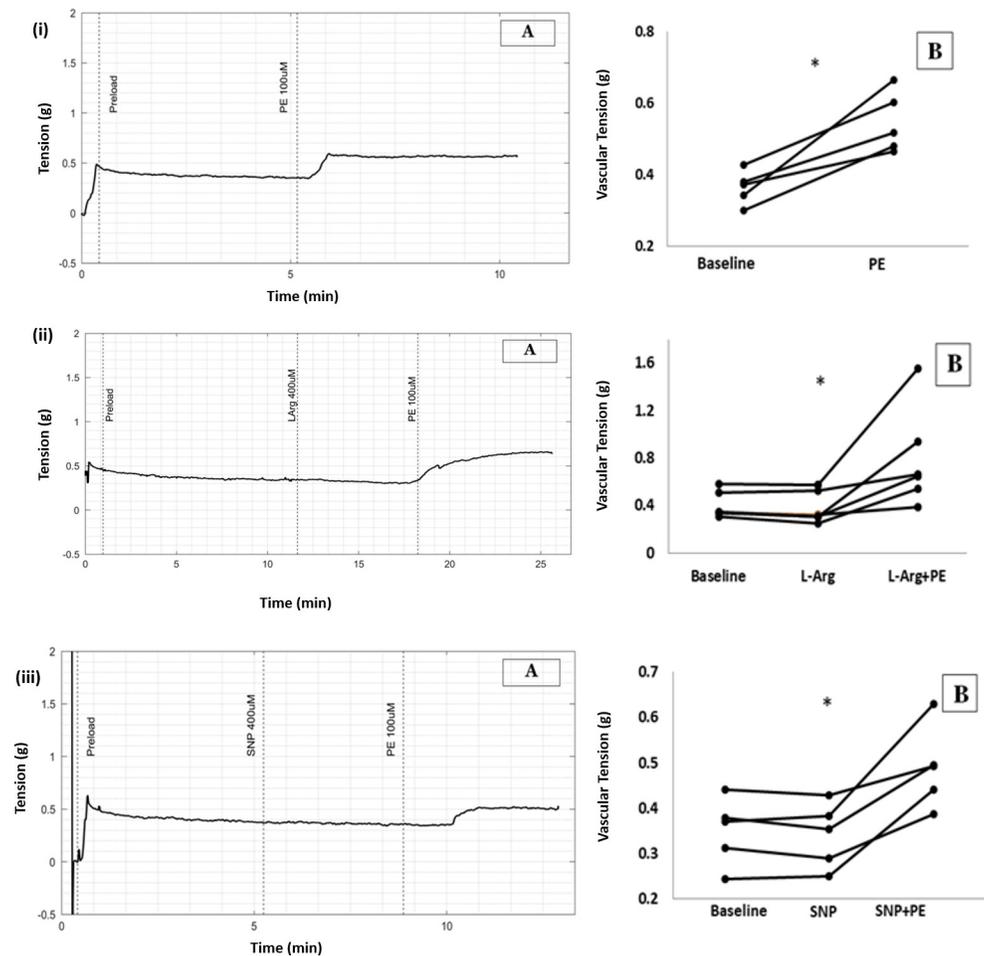


Fig 3. (A) Raw tracings showing changes in vascular tension in longitudinal strips of aorta (B) Scatter plots of results from all five experiments demonstrating increase in vascular tension with PE from baseline (* $p = 0.043$, WSR). (i) PE 100µmol/L (ii) PE 100µmol/L in high NO environment caused by L-Arginine (iii) PE 100µmol/L in presence of SNP.

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MWU) and also when percentage changes in vascular tension due to PE with and without SNP were compared (P value = 0.251 with MWU) showing that PE-induced vasoconstriction in aortic longitudinal muscle was not affected by high NO conditions.

PE induces contraction of circular smooth muscle of small artery under normal and high NO environments

10µmol/L concentration of PE did not alter the tension in small-artery preparation, but at 100 µmol/L concentration, PE increased the tension of circular smooth muscle of small artery from 0.09g to 0.54g. (Median, $n = 5$, P value = 0.028 with Wilcoxon signed rank test when tension before and after PE was compared, Fig 4, (i).

No change in basal tension was observed when high NO environment was created by adding L-Arginine 100µmol/L. Subsequent addition of PE 100µmol/L increased the tension of circular strip of small artery from 0.07g to 0.79g (Median, $n = 5$, P value = 0.043 with WSR test, Fig 4, (ii).

There was no significant difference in percentage change in vascular tension due to PE with or without L-Arginine (P value = 0.273 with MWU test).

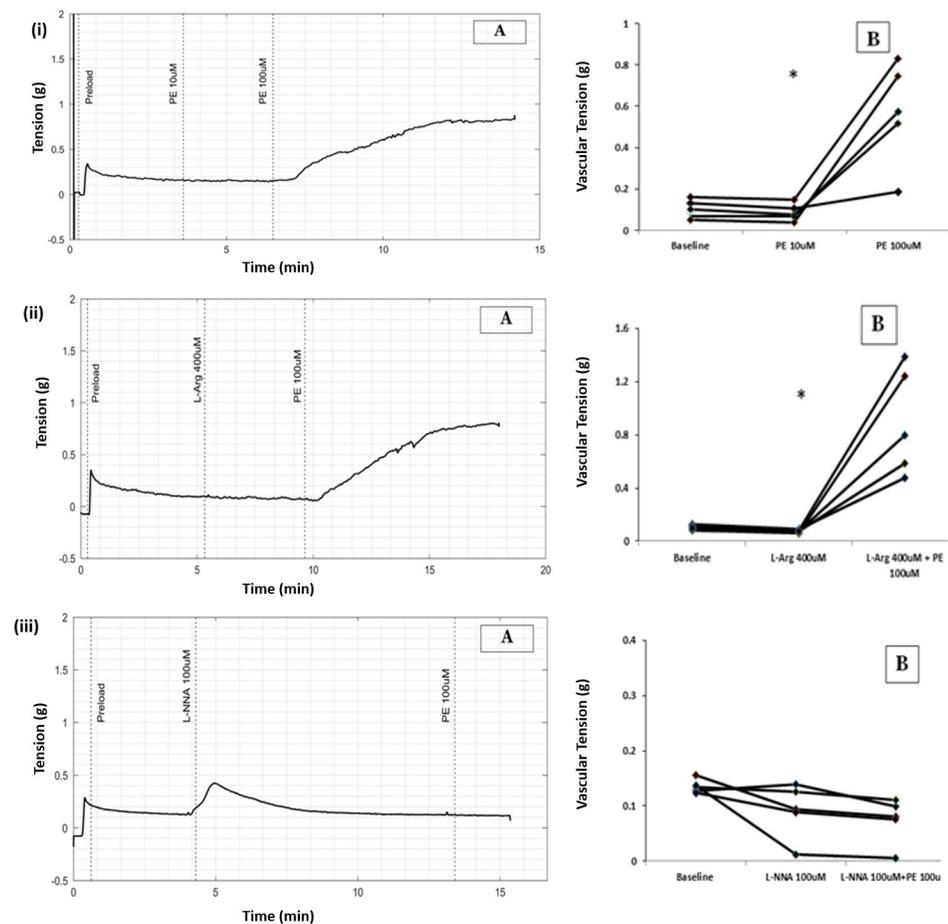


Fig 4. (A) Raw tracing showing changes in vascular tension with PE 100µmol/L in transverse cylinder preparation of small artery. (B) Scatter plots of results from all five experiments demonstrating change in vascular tension with PE from baseline. (* $p = 0.043$, WSR), (i) PE 100µmol/L (ii) PE in the presence of L-Arginine (iii) PE in the presence of NO synthase blocker L-NNA.

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L-NNA inhibited PE-induced contraction of circular smooth muscle in small artery

Addition of L-NNA, a competitive inhibitor of Nitric oxide synthase, caused a transient increase in tension which returned to baseline. Subsequent addition of PE 100µmol/L failed to produce contraction of circular smooth muscle. The vessel tension before and after addition of PE in the presence of L-NNA were 0.09 and 0.08g (median, $n = 5$, P value = 0.08 with WSR test), Fig 4, (iii).

The lack of response to PE in the presence of L-NNA was statistically significant as compared to the contractile response to PE alone (Median, $n = 5$, P value = 0.008, with MWU test).

PE induces relaxation of longitudinal smooth muscle of small artery both under normal and high NO environments

PE 100µmol/L reduced the tension of longitudinal smooth muscle from 0.25g to 0.09g in small arteries. (Median, $n = 5$, P value = 0.043, WSR), Fig 5, (i).

While L-Arginine 100µmol/L did not reduce the basal tension by itself, subsequent addition of PE 100µmol/L reduced the tension from 0.28 to 0.07g (Median, $n = 5$, P value = 0.043, WSR), Fig 5, (ii).

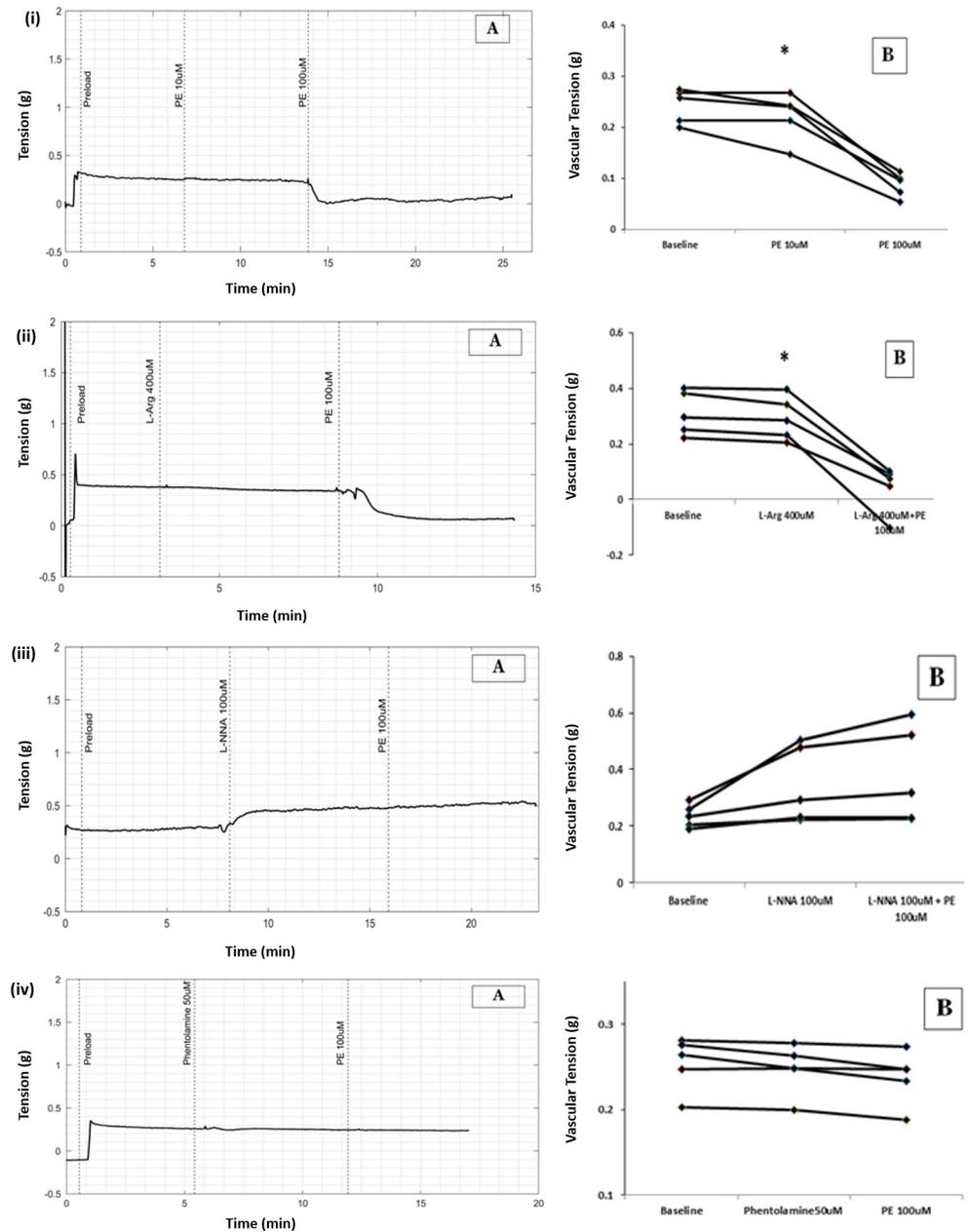


Fig 5. (A) Representative raw tracing showing changes in vascular tension with PE in longitudinal strip of small artery. (B) Scatter plots of results from all five experiments demonstrating changes in vascular tension with PE from baseline (* $p = 0.043$, WSR), (i) PE 100µmol/L (ii) PE in the presence of L-Arginine (iii) PE in the presence of NO synthase blocker L-NNA (iv) PE in presence of an alpha adrenoceptor blocker, Phentolamine.

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There was no significant difference in percentage change in tension due to PE under normal NO environment and in presence of L-Arginine (P value = 0.08, MWU test).

PE-induced relaxation of longitudinal smooth muscle in small artery was prevented by L-NNA

L-NNA (100µmol/L) itself increased the basal tension of the vessel. PE 100µmol/L failed to cause relaxation of longitudinal smooth muscle in the presence of L-NNA (100µmol/L). The

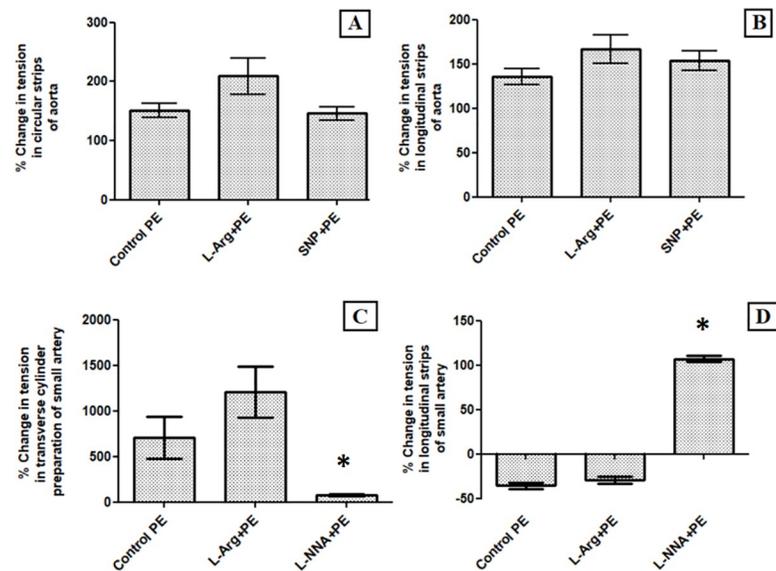


Fig 6. Bar diagrams representing percentage change in tension from baseline in, (A) Circular Strip preparation of aorta, (B) Longitudinal strip preparation of aorta, (C) Transverse cylinder preparation of small artery and (D) Longitudinal strip preparation of small artery. There was no significant difference between the contractile responses to PE in the presence or absence of L-Arginine (P value = 0.273 with MWU) as well as SNP (P value = 0.754 with MWU) in circular and longitudinal strips of aorta. There was no significant difference in percentage change in tension due to PE in the presence or absence of L-Arginine in transverse cylinder preparation (P value = 0.273, MWU test) and longitudinal strip preparation of small artery (P value = 0.08, MWU test). In panel C and D lack of response to PE in presence of L-NNA was statistically significant (*).

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vessel tension before and after addition of PE in the presence of L-NNA were 0.29g and 0.31g. (Median, n = 5, P value = 0.13 with WSR test), Fig 5, (iii).

The lack of response to PE in the presence of L-NNA was statistically significant as compared to the relaxant response to PE alone (Median, n = 5, P value = 0.008, with MWU test).

PE-induced relaxation of longitudinal smooth muscle is alpha adrenoceptor-mediated

PE failed to produce relaxation of longitudinal smooth muscle in the presence of phentolamine 50 μ mol/L, an alpha adrenoceptor blocker. The vascular tension before and after the addition of PE (in the presence of phentolamine 50 μ mol/L) were 0.24g and 0.24g respectively (median, n = 5, P value = 0.08, WSR test). Fig 5, (iv).

The lack of relaxant response to PE in the presence of phentolamine was statistically significant as compared to the relaxant response to PE alone (Median, n = 5, P value = 0.008, with MWU test).

Fig 6 shows the summary of percentage change in tension after PE under normal and high NO environment from baseline tension in circular strip and longitudinal strip preparation of aorta and small artery.

PE-induced relaxation of longitudinal smooth muscle is mediated through alpha-1D receptor subtype

Delineation of the specific subtype of alpha-1 adrenergic receptor responsible for PE-induced vasorelaxation was done by testing the effect of PE in the presence of different blockers of alpha-adrenergic receptor subtypes. Alpha-1A receptor blocker, RS17053 (10 μ mol/L) and

alpha-1B receptor blocker, CEC (10µmol/L) were not able to prevent the PE-induced reduction in tension in longitudinal strip. However, an alpha-1D receptor blocker, BMY 7378 (10µmol/L) prevented PE-induced reduction in tension in longitudinal strip.

The vascular tension before addition of PE (in the presence of 10µmol/L RS 17053) was 0.32g and after addition of PE was 0.24g (median, n = 5, p value = 0.043 with WSR test), Fig 7, (i).

A statistically significant difference was not observed (P value = 0.347, MWU test) when percentage changes of vascular tension due to PE in the presence and absence of presence of RS 17053 10µmol/L were compared.

Tension recordings in the longitudinal strip before and after addition of PE (in the presence of CEC 10µmol/L) were 0.30g and 0.18g respectively (median, n = 5, P value = 0.043 with WSR test), Fig 7, (ii).

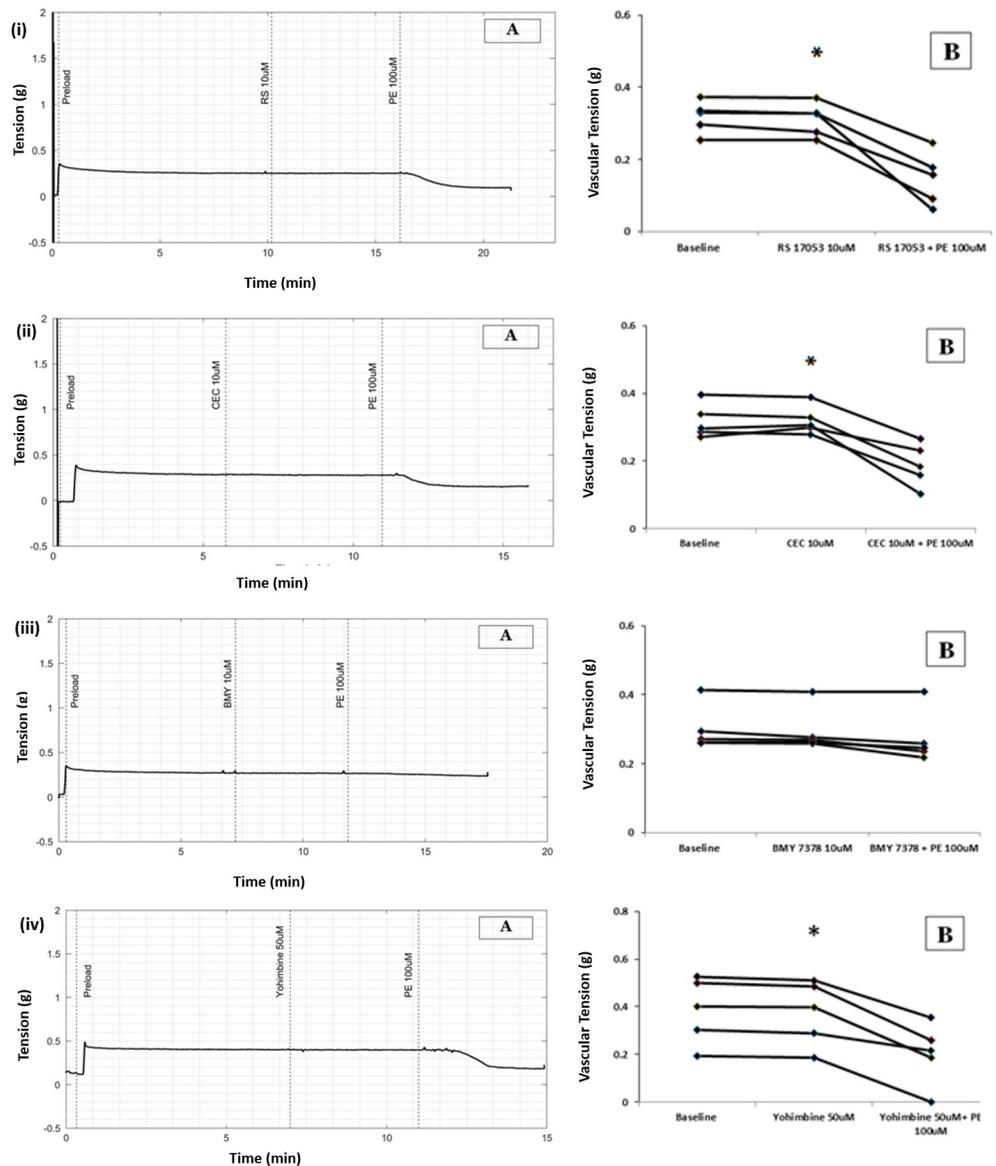


Fig 7. (A) Raw tracing showing changes in tension with PE in the presence of specific blockers in longitudinal strip preparation of small artery. (B) Scatter plots of results from all five experiments demonstrating PE-induced changes in vascular tension in presence of specific blockers, (i) alpha-1A blocker (ii) alpha-1B blocker (iii) alpha-1D blocker (iv) alpha-2 blocker.

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There was a 37% reduction of baseline tension by PE in the absence of CEC 10 μ mol/L, (Median, n = 5) whereas the reduction of baseline tension by PE in the presence of CEC 10 μ mol/L was about 56% (Median, n = 5). However, for this sample size there was no significant difference when percentage changes in tension due to PE with and without CEC were compared (P value = 0.056 with MWU test).

The tension in longitudinal strip before addition of PE (in the presence of BMY 7378 10 μ mol/L) was 0.26g and after addition of PE was 0.24g (median, n = 5, P value = 0.08 with WSR test), Fig 7, (iii).

There was a significant difference between the control group (PE alone) and BMY 7378/PE group when the percentage changes in tension were compared (P value = 0.008 with MWU test).

PE-induced relaxation of longitudinal smooth muscle is not mediated through alpha-2 receptor

To identify if PE-induced vasorelaxation in longitudinal strip of small artery is mediated through alpha-2 adrenergic receptor, yohimbine 50 μ mol/L, an alpha-2 antagonist was used. Tension in the longitudinal strip prior to the addition of PE (in the presence of Yohimbine 50 μ mol/L) was 0.39g and after addition of PE was 0.21g (median, n = 5, P value = 0.043 with WSR test), Fig 7, (iv).

There was no statistically significant difference in percent change of vascular tension between control group (PE alone) and PE in the presence of yohimbine 50 μ mol/L (P value = 0.117, MWU test)

PE-induced relaxation of longitudinal smooth muscle is not mediated through cAMP

To delineate the role of cAMP in PE-induced vasorelaxation in longitudinal strips of small artery, MDL 12330A, an adenylyl cyclase inhibitor was used. Tension in the longitudinal strip prior to the addition of PE (in the presence of MDL 50 μ mol/L) was 0.22g and after addition of PE was 0.05g (median, n = 5, P value = 0.043 with WSR test), Fig 8, (i).

There was no significant difference when the percent changes in tension were compared between the control group (PE alone) and MDL 12330A/PE group P value = 1.00, with MWU test).

Forskolin (FSK), an adenylyl cyclase stimulator failed to bring about reduction in tension in longitudinal strips of small artery by itself. Tension in the longitudinal strip prior to the addition of FSK 100 μ mol/L was 0.24g and after addition of FSK was 0.23g (median, n = 5, P value = 0.08, with WSR test), Fig 8, (ii).

Fig 9 shows the summary of percent change in tension from baseline tension due to PE in the presence various blockers from in longitudinal strip preparation of small artery.

Histological examination

To look for anatomical evidence for presence of longitudinal smooth muscle in small artery, histological examination was done. Light microscopic images of transverse cylinder preparation showed elliptical nucleus of circular smooth muscle predominantly while a few nuclei were seen circular representing a different arrangement of muscle fibres (Fig 10A and 10B). In light microscopic images of longitudinal strip, nuclei of circular smooth muscle were seen circular and a few were seen elliptical representing the longitudinal smooth muscle fibres (Fig 10C and 10D).

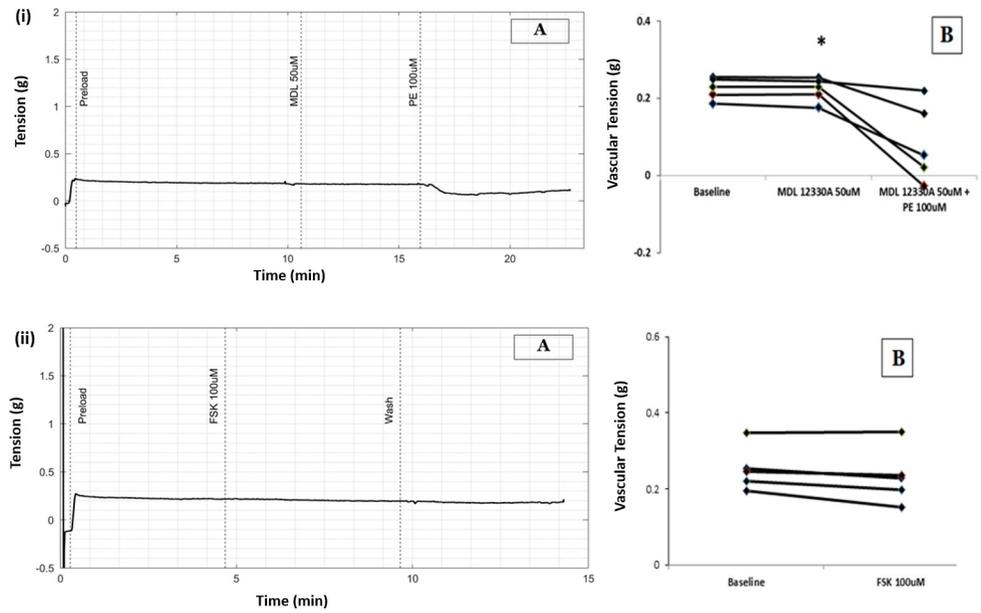


Fig 8. (A) Raw tracing showing changes in tension in longitudinal strip preparation of small artery. (B) Scatter plots showing reduction in vascular tension. (i) Effect of PE in presence of Adenylyl cyclase blocker, MDL 12330A (ii) Effect of Forskolin.

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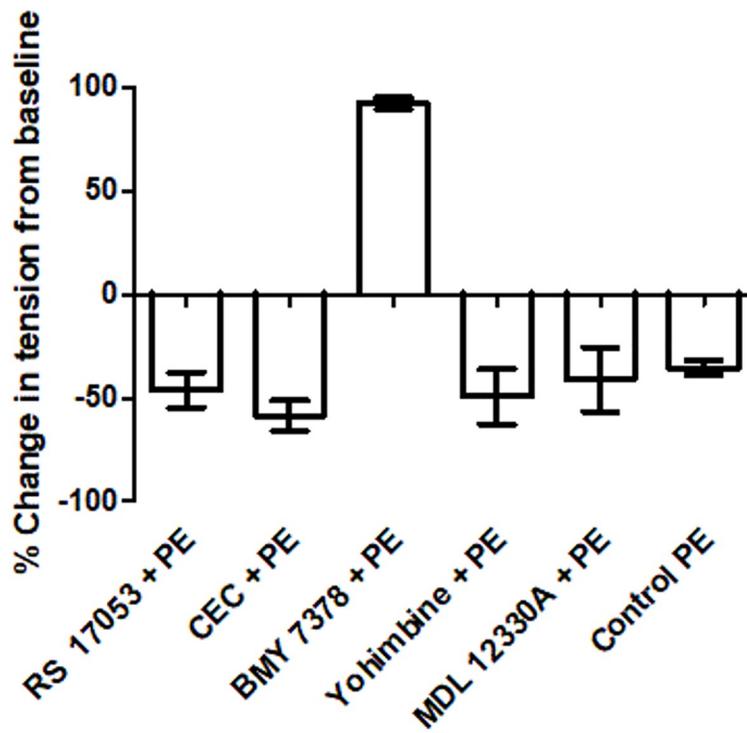


Fig 9. Bar diagram representing percent change in tension from baseline due to PE in the presence of blockers in longitudinal strip preparation of small artery. BMY 7378 an alpha 1D blocker, blocked the relaxant response to PE in a statistically significant manner (P value < 0.05 with MWU test).

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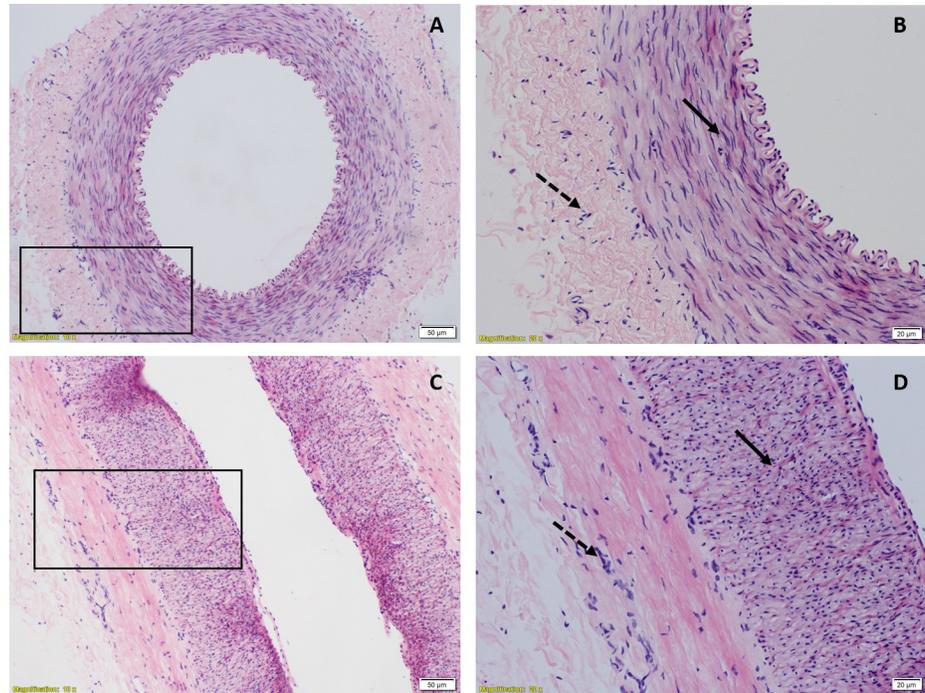


Fig 10. Hematoxylin & eosin stained light microscopic view of small artery. (A) & (B) Transverse cylinder preparation showing elliptical nuclei of circular smooth muscle fibres (solid arrow) and circular nuclei of longitudinal muscle fibres (dashed arrow) under 10X & 20X magnification respectively, (C) & (D) Longitudinal strip preparation showing elliptical nuclei of longitudinal smooth muscle fibres and circular nuclei of circular smooth muscle fibres under 10X & 20X magnification. The boxed areas in A & C have been magnified in B & D respectively.

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Similar picture is seen in aorta in which the existence of longitudinal smooth muscle is well known (Fig 11A, 11B, 11C & 11D).

Discussion

Sympathetic stimulation is known to cause vasoconstriction through alpha adrenergic receptors [6, 7]. Adrenergic receptors are broadly classified as alpha adrenoceptors and beta adrenoceptors [8, 9]. Alpha adrenoceptors are further classified as alpha-1 and alpha-2 adrenergic receptors [6]. Alpha-1 adrenoceptors in turn fall into 3 subtypes namely alpha-1A, alpha-1B and alpha-1D. Subtypes of alpha-2 adrenoceptors include alpha 2A, alpha 2B and alpha 2C [6]. Beta adrenoceptor subtypes are beta-1, beta-2 and beta-3 [10]. All adrenergic receptors are G-protein coupled receptors [9]. Alpha and beta-2 adrenoceptors are located on vascular smooth muscle. [11].

Vasoconstriction by alpha adrenergic stimulation occurs in two ways—either stimulation of myosin light chain kinase (MLCK) by calcium/Calmodulin complex [12] or inhibition of myosin light chain phosphatase by (MLCP) by phosphorylated CPI-17 [13, 14]

It has been shown earlier that PE could cause alpha-adrenoceptor mediated vasorelaxation in spiral strips of small arteries in a high nitric oxide (NO) environment [15]. However, it was realized that whether a spiral strip will relax or constrict depended not on NO, but in the manner in which it was prepared. There was PE-induced relaxation with strips prepared with fewer spirals (in hindsight this would include more longitudinal component) and PE-induced contraction even in a high NO environment with strips prepared in a manner to include more concentric muscle. To clarify this paradox this study focuses on the effect of PE on two

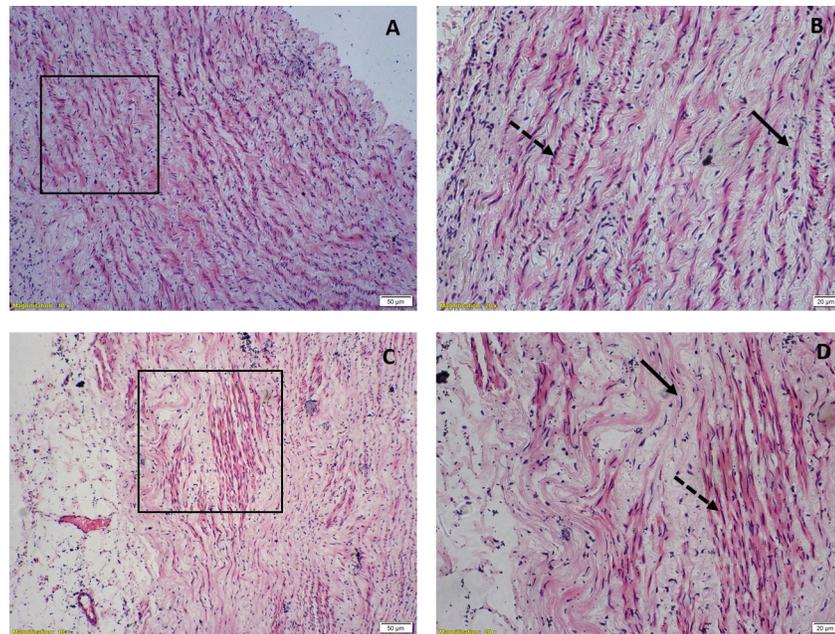


Fig 11. Hematoxylin & eosin stained light microscopic view of aorta, (A) & (B) Circular strip preparation showing elliptical nuclei of circular smooth muscle fibres (solid arrow) and round nuclei of longitudinal muscle fibres (dashed arrow) under 10X & 20X magnification respectively, (C) & (D) Longitudinal strip preparation showing elliptical nuclei of longitudinal smooth muscle fibres (dashed arrow) and round nuclei of circular smooth muscle fibres (solid arrow) under 10X & 20X magnification respectively. The boxed areas in A & C have been magnified in B & D respectively.

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different preparations—transverse cylindrical arterial strip (circular smooth muscle) and longitudinal strip (longitudinal muscle) respectively. Both these types of preparations were made with small arteries and aorta. It is shown here that there is a component of longitudinal muscle in small arteries which relaxes on alpha adrenergic stimulation. However, circular strips of small arteries demonstrated the well-known vasoconstrictor response with alpha adrenergic stimulation (with PE). The relaxant response of longitudinal smooth muscle to PE that was observed in small arteries did not occur in the aorta. Both circular and longitudinal preparations of aorta showed a vasoconstrictor response with PE.

Phenylephrine, a selective alpha agonist [16] is a known vasoconstrictor, the effects of which are mediated via alpha-1 and alpha-2 adrenergic receptors. The action of Phenylephrine on alpha 1 receptor is mediated through phospholipase-C on the cell membrane [17]. Phospholipase-C causes breakdown of phosphatidylinositol-4,5 bisphosphate (PIP₂) on cell membrane to inositol 1,4,5-triphosphate (IP₃) and 1,2—diacylglycerol (DAG). IP₃ then enters the cytoplasm, acts on IP₃ receptors on sarcoplasmic reticulum and causes release of calcium. The released calcium then binds to calmodulin, a regulatory protein forming calcium/calmodulin complex [18]. Calcium/Calmodulin complex causes stimulation of MLCK which causes phosphorylation of myosin light chain thereby causing contraction [12]. Calcium released from sarcoplasmic reticulum combines with DAG and stimulates protein kinase C (PKC). PKC causes phosphorylation of a smooth muscle phosphoprotein, CPI-17. CPI-17 inhibits MLCP, which prevents dephosphorylation of myosin light chain thereby supporting contraction [13, 14]. Activation of alpha-2 adrenoceptor stimulates G_i type of G-protein, which in turn inhibits adenyl cyclase and decreases cAMP, again supporting contraction [18].

Raj et al., [15] have demonstrated in spiral strip preparation of goat arteries, scenarios in which PE could cause vasorelaxation from basal tone, one being high NO environment. It was

shown that PE/NO induced vasorelaxation is cGMP independent and requires alpha adrenergic activation. However, further experiments showed that contractile or relaxant response depends on the manner of spiral strip preparation rather than on the level of NO.

The results show that PE induces only contraction of circular smooth muscle both under normal NO and high NO environment as known earlier. However, the effect of PE on longitudinal component of smooth muscle is only relaxation both under normal NO and high NO environment. L-Arginine is reported to produce relaxant response by increasing NO levels [19]. However, we observed that L-Arginine by itself did not induce relaxation in both small artery and aorta. It is not as if the preparations were not capable of relaxation because in the case of longitudinal strip of small artery at least, PE could induce vasorelaxation. Similarly, SNP, a NO donor also failed to produce relaxation in both circular and longitudinal strips of aorta. These effects of both L-Arginine and SNP need further evaluation. The earlier observations of L-Arginine-induced relaxation were all observed in pre-constricted strips and not in unconstructed strips [20]. In the current study, we show that L-Arginine and SNP have no relaxant effect from baseline tension in unconstructed strips. We have also demonstrated structural integrity of the endothelium histologically. However, it is a limitation of the study that functional integrity of the endothelium was not assessed.

It was also found that such PE-induced vasorelaxation in longitudinal smooth muscle is alpha-adrenergic dependent, as phentolamine, an alpha adrenoceptor antagonist prevented such response. In order to delineate the receptor subtype involved in PE-induced vasorelaxation, four alpha adrenoceptor subtype blockers (alpha-1A, alpha-1B, alpha-1D and alpha-2) were used. Among them only alpha-1D blocker, BMY 7378 was successful in preventing PE induced vasorelaxation. BMY can block alpha-2C adrenoceptors when used at a concentration above 0.3 μmol/L [21]. Though the concentration that we have used is 10 μmol/L, its effect of blocking vasorelaxation in longitudinal strips is unlikely to be due to alpha 2C blockade, as Yohimbine, which is a non-selective antagonist of alpha-2 subtypes did not prevent the vasorelaxation induced by PE. Hence, prevention of PE-induced vasorelaxation by BMY 10 μmol/L is through blockade of alpha-1D receptor.

The role of the second messenger cAMP, in PE-induced vasorelaxation was also studied. To decipher this, forskolin an adenylyl cyclase activator, and MDL 12330A, an adenylyl cyclase inhibitor were used. Forskolin by itself failed to produce vasorelaxation in longitudinal strips of small artery and MDL 12330A failed to prevent PE-induced vasorelaxation showing that PE-induced vasorelaxation in longitudinal strip is not mediated through cAMP.

It is also shown that L-NNA, a competitive inhibitor of NO synthase inhibited both PE-induced contraction and relaxation in small artery. The probability of L-NNA being a blocker of alpha-adrenergic receptors too, rather than being just a NO synthase blocker has to be evaluated further. Also, L-NNA caused a transient increase in tension in transverse cylinder preparation whereas it produced a sustained increase in tension in longitudinal preparation of small artery. This differential behaviour of L-NNA also needs further evaluation.

Further, anatomical evidence for the existence of longitudinal smooth muscle in small arteries has been demonstrated through histology.

Presence of longitudinal smooth muscle in aorta is well-known. However, the effect of PE on both circular and longitudinal smooth muscle of aorta is only vasoconstriction, unlike in small arteries.

This is the first time within our knowledge, the existence of longitudinal smooth muscle in small arteries is being reported. It is also shown here that the longitudinal smooth muscle of small artery relaxes in response to α_{1D} adrenergic stimulation and such relaxant response is independent of cAMP. However, the physiological significance for the existence of such

longitudinal component of smooth muscle in small arteries and their relaxant response to alpha adrenergic stimulation needs to be ascertained.

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References

1. Tykocki NR, Boerman EM, Jackson WF. Smooth muscle ion channels and regulation of vascular tone in resistance arteries and arterioles. *Compr Physiol*. 2017; 7(2):485–581. <https://doi.org/10.1002/cphy.c160011> PMID: 28333380
2. Liu Y, Buerk DG, Barbee KA, Jaron D. A dynamic computational network model for the role of nitric oxide and the myogenic response in microvascular flow regulation. *Microcirculation*. 2018; e12465. <https://doi.org/10.1111/micc.12465> PMID: 29885064
3. Davis MJ, Hill MA, Kuo L. Local regulation of microvascular perfusion. *American Cancer Society*; 2011; p. 161–284.
4. Cohen ML, Wiley KS. Comparison of arteries with longitudinal and circular venous muscle from the rat. *Am J Physiol*. 1977; 232(2):H131–139. <https://doi.org/10.1152/ajpheart.1977.232.2.H131> PMID: 842644
5. Tennant M, McGeachie JK. Blood vessel structure and function: a brief update on recent advances. *Aust N Z J Surg*. 1990; 60(10):747–53. <https://doi.org/10.1111/j.1445-2197.1990.tb07468.x> PMID: 2206118
6. van Brummelen P, Jie K, van Zwieten PA. Alpha-adrenergic receptors in human blood vessels. *Br J Clin Pharmacol*. 1986;21 Suppl 1:33S–39S.
7. Kiowski W, Bühler FR, van Brummelen P, Amann FW. Plasma noradrenaline concentration and alpha-adrenoceptor-mediated vasoconstriction in normotensive and hypertensive man. *Clin Sci (Lond)*. 1981; 60(5):483–9.

8. Insel PA. Structure and function of alpha-adrenergic receptors. *Am J Med*. 1989; 87(2A):12S–18S. [https://doi.org/10.1016/0002-9343\(89\)90108-3](https://doi.org/10.1016/0002-9343(89)90108-3) PMID: 2548380
9. Strosberg AD. Structure, function, and regulation of adrenergic receptors. *Adv Pharmacol*. 1998; 42:511–3 [https://doi.org/10.1016/s1054-3589\(08\)60801-7](https://doi.org/10.1016/s1054-3589(08)60801-7) PMID: 9327952
10. Perez DM. The Adrenergic receptors in the 21st century (The Receptors). Humana Press; 2005. pp. 416.
11. Molinoff PB. Alpha- and beta-adrenergic receptor subtypes properties, distribution and regulation. *Drugs*. 1984;28 Suppl 2:1–15.
12. Basu S, Proweller A. Autoregulatory control of smooth muscle myosin light chain kinase promoter by notch signaling. *J Biol Chem*. 2016; 291(6):2988–99. <https://doi.org/10.1074/jbc.M115.679803> PMID: 26703474
13. Kim JI, Urban M, Young GD, Eto M. Reciprocal regulation controlling the expression of CPI-17, a specific inhibitor protein for the myosin light chain phosphatase in vascular smooth muscle cells. *Am J Physiol—Cell Physiol*. 2012; 303(1):C58–68. <https://doi.org/10.1152/ajpcell.00118.2012> PMID: 22538237
14. Grassie ME, Moffat LD, Walsh MP, MacDonald JA. The myosin phosphatase targeting protein (MYPT) family: A regulated mechanism for achieving substrate specificity of the catalytic subunit of protein phosphatase type 1δ. *Arch Biochem Biophys*. 2011; 510(2):147–59. <https://doi.org/10.1016/j.abb.2011.01.018> PMID: 21291858
15. Raj RR, Subramani S. Phenylephrine Decreases Vascular Tension in Goat Arteries in Specific Circumstances. *PLOS ONE*. 2016; 11(6):e0158551. <https://doi.org/10.1371/journal.pone.0158551> PMID: 27362703
16. Guo TZ, Tinklenberg J, Olikier R, Maze M. Central alpha 1-adrenoceptor stimulation functionally antagonizes the hypnotic response to dexmedetomidine, an alpha 2-adrenoceptor agonist. *Anesthesiology*. 1991; 75(2):252–6. <https://doi.org/10.1097/00000542-199108000-00013> PMID: 1677547
17. Adolfo García-Sáinz J. α1-adrenergic action: Receptor subtypes, signal transduction and regulation. *Cell Signal*. 1993; 5(5):539–47. [https://doi.org/10.1016/0898-6568\(93\)90049-r](https://doi.org/10.1016/0898-6568(93)90049-r) PMID: 8312131
18. Exton JH. Mechanisms involved in alpha-adrenergic phenomena. *Am J Physiol-Endocrinol Metab*. 1985;248(6):E633–47.
19. Tarry WC, Makhoul RG. L-arginine improves endothelium-dependent vasorelaxation and reduces intimal hyperplasia after balloon angioplasty. *Arterioscler Thromb*. 1994; 14(6):938–43. <https://doi.org/10.1161/01.atv.14.6.938> PMID: 8199185
20. Sakuma I, Stuehr DJ, Gross SS, Nathan C, Levi R. Identification of arginine as a precursor of endothelium-derived relaxing factor. *Proc Natl Acad Sci U S A*. 1988; 85(22):8664–7 <https://doi.org/10.1073/pnas.85.22.8664> PMID: 3263652
21. Cleary L, Murad K, Bexis S, Docherty JR. The alpha (1D)-adrenoceptor antagonist BMY 7378 is also an alpha (2C)-adrenoceptor antagonist. *Auton Autacoid Pharmacol*. 2005; 25(4):135–41. <https://doi.org/10.1111/j.1474-8673.2005.00342.x> PMID: 16176444