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# The influence of constitutive Cox-2 in smooth muscle tissue on the contractile effect of phenylephrine in the rat abdominal aorta

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## 1. ABSTRACT

Prostanoids are involved in the phenylephrine-induced contraction of the aorta. Here, we examined whether or not constitutive cyclooxygenase-2 (phosholipases C and $A_2$) is the source of prostanoids in the smooth muscle of the arterial wall of the thoracic and abdominal aorta. Both cyclooxygenase isoforms (COX-1 and COX-2) were expressed in the two aortic segments, but their expression was not altered by phenylephrine, the protein synthesis inhibitor cycloheximide, or the phospholipase $A_2$ inhibitors arachidonyl trifluoromethyl ketone and methyl arachidonyl fluorophosponate. Indomethacin and NS398, which are a non-selective and selective COX-2 inhibitor, respectively, but not SC-560, which is a COX-1-selective inhibitor, inhibited the effect of phenylephrine on the abdominal, but not the thoracic, aorta. Similarly, U73122, which is a phospholipase C inhibitor, and RHC80267, which is a diacylglycerol lipase inhibitor, inhibited the effect of phenylephrine. These findings suggest that prostanoids, which are produced by constitutively active COX-2, influence the contractile response of the abdominal aorta and that the production of arachidonic acid relies on phospholipase C and diacylglycerol lipase.

## 2. INTRODUCTION

The effect of alpha$_1$–adrenergic agonists on the vascular tone is the result of a complex network of direct and indirect actions, which are influenced by many factors, including species (1), gender (2) and local or regional characteristics of the vascular bed. These characteristics may be modified by aging (3,4) or the development of diseases such as hypertension, diabetes or arteriosclerosis (5,6,7). Accordingly, studies of the contractile effects of norepinephrine in arteries from rabbits (8) as well as in both arteries and veins from canines (9,10) demonstrated that alpha$_1$-adrenoceptor-mediated arterial and vein sensitivity varied by species and region. Quantitative and qualitative regional variations in the alpha$_1$-adrenergic vasocontractile effects may be due to differences in the number, affinity or subtype of alpha$_1$-adrenergic receptors, as well as differences in the predominant intracellular pathways of distinct vascular regions (11,12,13,14). In addition to phosphoinositide turnover and calcium signaling (14), prostaglandins may also be involved in the vasocontractile effect that is mediated by alpha$_1$-adrenoceptors. Studies suggest that cyclooxygenase-2 (COX-2) is the COX isoform that is responsible for the contractile effects of prostanoids. According to some

authors, this enzyme is virtually absent in the healthy vasculature and only expressed in pathological conditions, such as atherosclerosis or diabetes (15,16,17). However, other researchers suggest that COX-2 is present in the healthy vasculature, mainly in the vascular endothelium, and upregulated in pathological conditions such as hypertension (18). Given the current controversy over the role of COX-2 in the vasculature, we decided to evaluate the capacity of rat aortic smooth muscle cells to produce contractile prostaglandins in response to phenylephrine (Phen), as well as the role of constitutively active COX-2 and the influence of the vascular region in this process. We also investigated the role of phospolipases C and $A_2$ in the production of arachidonic acid, which is necessary to produce the COX-2-derived prostanoids.

## 3. MATERIALS AND METHODS

The experiments were conducted using protocols approved by the Animal Care Committee of our institution, which are in agreement with the UK Animals (Scientific Procedures) Act of 1986.

### 3.1. Preparation of the aortic rings and measurement of tension

Male Wistar rats (250 to 300g) were kept in the animal colony until time of sacrifice. The animals were maintained on a 12/12 hrs light-dark cycle at a constant temperature (22 ± 2°C), with food and water freely available in their home cages.

The animals were euthanized by decapitation, and the aortic tissues were immediately excised, placed in cold solution Krebs, cleaned and freed from the surrounding connective tissue. Taking the diaphragm as a reference, the abdominal and thoracic segments of the aorta were split, and each segment was cut into rings (4-5 mm long) that were then placed in 10 ml chambers filled with Krebs-bicarbonate solution of the following composition (mM): NaCl, 118; KCl, 4.7; $KH_2PO_4$, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2; $CaCl_2 \cdot 2H_2O$, 2.5; $NaHCO_3$, 25; dextrose, 11.7; and calcium disodium EDTA, 0.026.

Tissue baths were maintained at 37°C, pH 7.4 and bubbled with a mixture of 95% $O_2$ and 5% $CO_2$. Rings were mounted on two stainless steel hooks in order to fix them to the bottom of the chamber and to a Grass FTO3 force displacement transducer connected to a 7D Grass Polygraph (Grass Instrument Co., Quincy MA, U.S.A), which was used to record the isometric tension developed by the aortic rings. The rings were given 2 g of initial tension and allowed to equilibrate for 2 h. Thirty minutes after setting up the organ bath, tissues were first exposed to Phen ($10^{-6}$ M) to test their contractile responses and then rinsed three times with Krebs solution to restore tension to the precontracted level. Optimal ring tension was selected from preliminary experiments in which the rings were stretched to elicit the greatest response to Phen ($10^{-6}$ M). We employed denuded aortic rings that were prepared by gently turning the rings several times on the distal portion of a pair of small forceps. The lack of endothelial integrity was pharmacologically assessed by acetylcholine-induced vasodilatation ($10^{-6}$ M). Segments showing no relaxation were considered to be endothelium-denuded.

### 3.2. Phenylephrine concentration-response curves in the presence or absence of different antagonists.

#### 3.2.1. Phenylephrine concentration-response curves

After the equilibration period, we obtained Phen concentration-response curves ($10^{-9}$ to $10^{-5}$ M) for aortic rings without endothelium from the thoracic and abdominal segments, in order to determine if there were regional differences in the contractile response to this drug.

#### 3.2.2. Phenylephrine concentration-response curves in the presence of indomethacin

In order to evaluate if prostanoids are involved in the phenylephrine-induced contraction and if this contractile effect is specific to the vascular region, the contractile response of the thoracic and abdominal aortic rings to Phen was analyzed in the absence and presence of indomethacin ($10^{-5}$ M). This drug was added 30 minutes before obtaining the Phen concentration-response curve.

#### 3.2.3. Phenylephrine concentration-response curves in the presence of NS-398 and SC-560

To determine the enzymatic source of the prostanoids involved in the phenylephrine-induced contraction, we tested the response of the aortic rings to phenylephrine in the presence of selective COX-1 (SC-560, $10^{-6}$ M) and COX-2 (NS-398 $10^{-7}$ M) inhibitors. We also used immunoblot analysis to detect the presence of COX-1 and COX-2 in the endothelium–denuded thoracic and abdominal aortic segments.

#### 3.2.4. Phenylephrine concentration-response curves in the presence of cycloheximide

To evaluate if the de novo synthesis of COX is involved in the contractile effect of Phen, we pretreated aortic rings with cycloheximide ($10^{-5}$ M), which is an inhibitor of protein synthesis, 30 minutes before obtaining a Phen concentration-response curve.

#### 3.2.5. Phenylephrine concentration-response curves in the presence of AATFMK and MAPF

To determine whether cytosolic and intracellular phospholipase $A_2$ ($cPLA_2$ and $iPLA_2$) were sources of the arachidonic acid needed to produce the prostanoids involved in the contractile effect of Phen, aortic rings were pretreated with arachidonyl trifluoromethyl ketone (AATFMK; 1.6 $10^{-5}$ M) and methyl arachidonyl fluorophosponate (MAPF; $10^{-6}$ M) 30 minutes before determining the Phen concentration-response curve of these rings.

#### 3.2.6. Phenylephrine concentration-response curves in the presence of U73122 and RHC80267

We also evaluated phosoholipase C (PLC) and diacylglycerol lipase as possible sources of arachidonic acid in the production of the contractile prostanoids. To do this, we incubated abdominal aortic rings for 30 min with either U73122, which is an inhibitor of PLC, or RHC80267, which is an inhibitor of diacylglycerol lipase, before examining these rings Phen concentration-response curves.

### 3.3. COX-1 and COX-2 immunoblot analysis

Briefly, samples were homogenized in Tris-HCl, pH 7.4 with a protease cocktail (MiniComplete-EDTA free, Roche, Mannheim, Germany), and the total protein content of the sample was analyzed by the Lowry method (Lowry *et al.*, 1951). Immunoblots were performed in duplicate using 50 µg of protein per lane on a 10% SDS-polyacrylamide gel. The samples were then transferred onto a polyvinylidene fluoride (PVDF) membrane (Hybond-P, Amersham Biosciences, UK). The PVDF membrane was blocked with TBS containing 5% skim milk and 0.05% Tween for 2 hours at room temperature, followed by an overnight incubation at 4°C with a 1:400 dilution of a polyclonal antibody against COX-1 or COX-2 (Santa Cruz Biotechnology, CA, USA). After the primary incubation, the membrane was washed and incubated with the corresponding secondary (anti-goat or anti-rabbit) HRP-labeled antibody (Zymed, USA), diluted 1:10,000 in the blocking solution, for two hours at room temperature. The blots were washed and developed using an ECL detection system (Luminol, Santa Cruz Biotechnology). The blots were then stripped and, as a control, beta-actin levels were determined using a polyclonal antibody. Images from films were digitally acquired, and a densitometry analysis was performed using the Quantity One Image Acquisition and Analysis Software (BioRad, Hercules, CA. USA). Data are expressed as normalized optical density (OD) arbitrary units.

### 3.4. Drugs

Phenylephrine HCl, acetylcholine HCl, NS-398, SC-560, indomethacin, arachidonyl trifluoromethyl ketone (AATFMK), methyl arachidonyl fluorophosponate (MAFP), U72122, RHC 80267 and cycloheximide were purchased from Sigma Chemical Co. (St. Louis, MO, USA.). NS 398 was purchased from ICN Biomedicals Inc. (Irvine, CA. USA). The majority of the drugs were prepared daily in deionized water and kept on ice until use. NS-398 aliquots ($10^{-4}$ M) were prepared in dimethyl sulfoxide and kept frozen at $-20^{0}$C until use. RHC 80267 and SC-560 were also diluted in dimethyl sulfoxide. Indomethacine was dissolved in 3% $Na_2CO_3$. Cycloheximide was diluted in ethanol and kept frozen. Control rings that were treated with corresponding concentrations of the solvent were studied simultaneously.

### 3.5. Data and statistical analysis

Data are presented as the mean +/- SEM throughout the paper. In all experiments, n equals the number of rats from which vessel segments were obtained. Non-linear regression curve fits were calculated for the concentration-response curves, and the -log $EC_{50}$ and $E_{max}$ were obtained. Statistical comparisons were performed by ANOVA in order to determine the differences in the data, followed by a post hoc test. In all cases, a p-value less than 0.05 was considered to be statistically significant.

## 4.-RESULTS

### 4.1. Phenylephrine concentration-response curves of thoracic and abdominal aortic rings without endothelium

Phen ($10^{-9}$ -$10^{-5}$ M) elicited a concentration-dependent contractile effect in aortic rings; the contractions were of similar magnitudes for both the thoracic and abdominal segments. The maximal responses to Phen were 1.67 +/- 0.12 g and 1.42 +/- 0.23 g, while the –log $CE_{50}$ was 7.03 +/- 0.08 and 6.85 +/- 0.11 M in the thoracic and abdominal segments, respectively. Similar results were obtained using aortic rings with endothelium or using another contractile agent, such as serotonin or angiotensin II, instead of Phen (data not shown).

### 4.2. Effect of indomethacin on the contractile response to phenylephrine

The role of prostanoids in the contractile effect of Phen was determined by using indomethacin, which is a nonspecific inhibitor of COX. Pretreatment with indomethacin (10-5 M) significantly depressed the contractile response to Phen in the abdominal, but not thoracic, rings (Figure 1). For the abdominal rings, the maximal effect changed from 1.42 +/- 0.23 g to 0.70 +/- 0.03 g in the presence of indomethacin, while the –log EC50 decreased from 6.85 +/- 0.11 to 6.52 +/- 0.07 M when pretreated with indomethacin.

### 4.3. Effect of SC-560 on the contractile response to phenylephrine

Pretreatment with the selective COX-1 inhibitor SC-560 ($10^{-6}$ M) did not modify the contractile response of the abdominal aortic rings to Phen (Figure 2a).

### 4.4. Effect of NS 398 on the contractile response to phenylephrine

Pretreatment with the selective COX-2 inhibitor NS 398 (10-6 and 10-5 M) depressed the contractile response of the abdominal aortic rings to Phen in a concentration-dependent manner (Figure 2b). The presence of NS 398 decreased the maximal response to Phen from 1.06 g +/- 0.087g to 0.63 +/- 0.050 at 10-5 M and 0.39 +/- 0.041 g at 10-6 M. The –log EC50 response to Phen was 6.6 +/- 0.22 M, 6.6 +/- 0.21 M and 6.2 +/- 0.10 M for no NS 398 pretreatment, pretreatment with 10-5 M NS 398 and pretreatment with 10-6 M NS 398, respectively (Figure 2b).

### 4.5. Immunoblot analysis of COX-1 and COX-2

The protein expression of the COX-1 and COX-2 receptors was assayed using specific antibodies against each receptor. These antibodies had been previously tested to ensure that they did not cross-react with a different enzyme subtype. COX-1 and COX-2 were expressed in both aortic segments, with higher relative expression in the thoracic aorta. When Phen was added to both tissues, the relative expression of both COX-1 and COX-2 did not change significantly (Figure 3).

### 4.6. Evaluation of the effect of cycloheximide on contractile response to phenylephrine

Pretreatment with cycloheximide did not influence the contractile effect of Phen in the abdominal rings (Figure 4). Similar results were seen for the thoracic rings (data not shown).

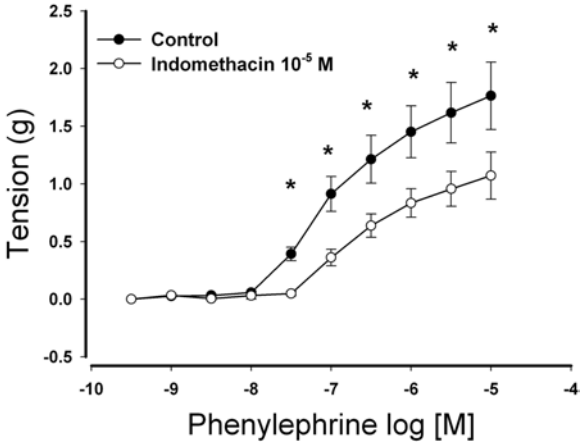


**Figure 1.** The contractile effect of phenylephrine on abdominal aortic rings without endothelium in the absence (control) and presence of indomethacin ($10^{-5}$ M). Data shown correspond to the mean ± S.E.M. of at least six experiments and are expressed in grams of developed tension. * $p < 0.05$.

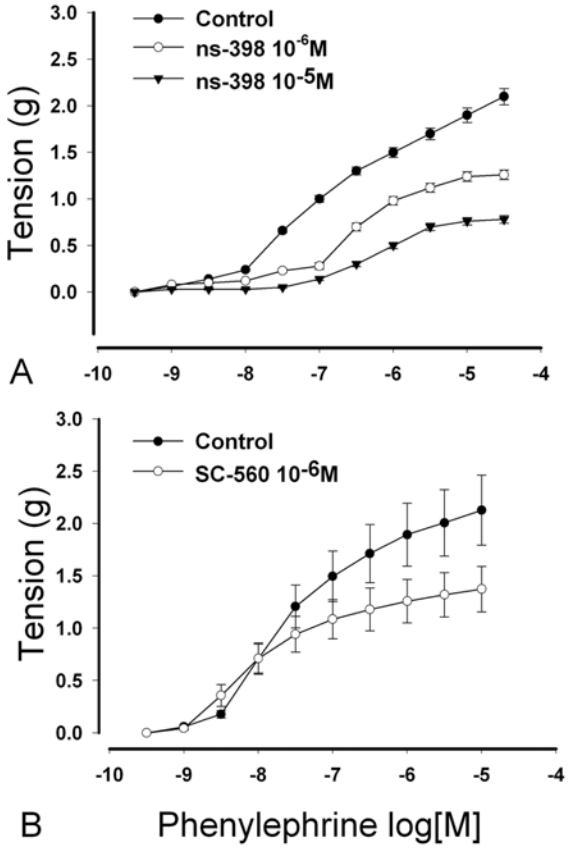


**Figure 2.** The contractile effect of phenylephrine on abdominal aortic rings without endothelium in (a) the absence (control) and presence of SC560 ($10^{-6}$ M) or (b) NS 398 ($10^{-6}$ and $10^{-5}$ M). Data shown correspond to the mean ± S.E.M. of at least six experiments and are expressed in grams of developed tension. * $p < 0.05$.

**4.7. Effect of AATFMK and MAFP on the contractile response to phenylephrin**

Pretreatment with AATFMK (1.6 x $10^{-5}$ M) or MAFP ($10^{-6}$ M), which are both potent inhibitors of the non-secretory forms of $PLA_2$ ($cPLA_2$ and $iPL_2$), did not affect the contractile response of the abdominal aortic rings to Phen (Figure 5). The maximal responses to Phen were 1.47 +/- 0.055 g and 1.54 +/- 0.062 g in the absence or presence of AATFMK, respectively, and 1.33 +/- 0.024 g and 1.31 +/- 0.03 g in the absence or presence of MAFP, respectively.

**4.8. Effects of U73122 and RHC80267 on the contractile response to phenylephrine.**

Pretreatment with U73122, which is an inhibitor of PLC, completely eliminated the contractile effect of Phen on the abdominal aortic rings (Figure 6a). In contrast, pretreatment with RHC80267, which is an inhibitor of diacylglycerol lipase, significantly shifted the concentration-response curve of Phen to the right (Figure 6b).

**5.-DISCUSSION**

The results of the present study did not show quantitative differences in the contractile effect of Phen in the different regions of the aorta of male Wistar rats. The magnitude of the contraction was similar in the thoracic and abdominal segments of the aorta, either in the absence or presence of endothelium. Similar results were observed using serotonin or angiotensin II as the contractile agents instead of Phen, which indicated, the region of the aorta was not a significant factor in the magnitude of the contractile effect and that the magnitude was independent of the drug employed. There are qualitative differences, however, since the mechanism of contraction in the rat abdominal aorta is different from that of the thoracic aorta. Prostanoids other than thromboxane $A_2$ are involved in the contraction elicited by the adrenergic amines in the abdominal, but not the thoracic, aorta[12]. Our results confirm those of Lamb *et al* (1994), since the non-selective COX inhibitor indomethacin inhibited the contractile effect of the $\alpha_1$-adrenergic agonist Phen only in the abdominal segment of the rat aorta.

Phen is a non-selective agonist of all three subtypes of $\alpha_1$-adrenergic receptors ($alpha_{1A}$, $alpha_{1B}$ and $alpha_{1D}$), which are coupled through $G_q/11$ proteins and phospholipase C to phosphoinositide turnover and calcium signaling (14). Activation of this pathway explains the vasocontractile effect of α1-adrenergic receptor agonists. However, this signaling pathway does not completely explain all of the $alpha_1$-adrenergic actions. Multiple signaling pathways are clearly involved in the activity of alpha1-adrenergic receptors, which seem to utilize different G proteins and depend on the specific signaling pathways expressed by different cell types (14,19,20,21). In addition to phospholipase C (PLC), both phospholipase A2 (PL A2 ) (22,23) and phospholipase D (PLD) (24) appear to be involved in alpha1-adrenergic activation. These three enzymes cause the release of arachidonic acid, which is the source of several vasoactive compounds. Differences in the distribution of the $alpha_1$–adrenoceptors subtypes between

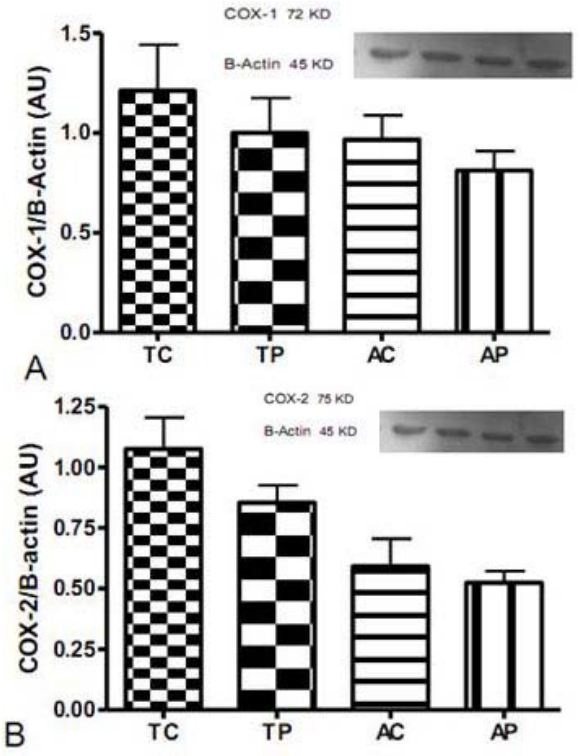


**Figure 3.** COX-1 and COX-2 expression in thoracic and abdominal aorta. Data are the mean ± SEM of three or four rats. AU: Arbitrary units. TC, Control thoracic aorta; TP, Phenylephrine-treated thoracic aorta; AC, Control abdominal aorta; AP, Phenylephrine-treated abdominal aorta. The insert shows the results from a typical experiment.

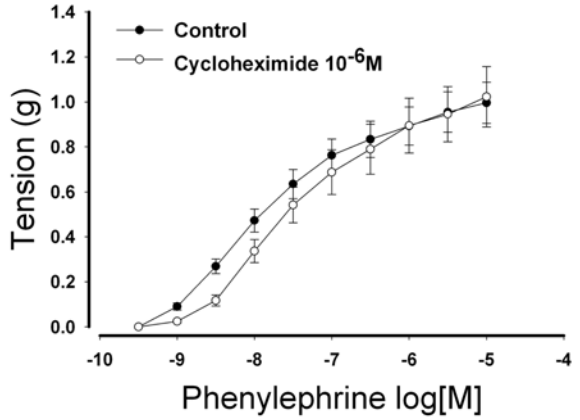


**Figure 4.** The contractile effect of phenylephrine on abdominal aortic rings without endothelium in the absence (control) and presence of cycloheximide ($10^{-6}$ M). Data shown correspond to the mean ± S.E.M. of at least six experiments and are expressed in grams of developed tension.

vessels, or even along the same vessel, could explain the variation in the mechanisms underlying the contractile response to alpha$_1$-adrenergic agonists (25). In the case of the rat aorta, however, this hypothesis does not explain our results, because alpha$_{1D}$ is the predominant subtype of alpha$_1$–adrenergic receptor in both segments of the aorta (14,26).

The regional differences in the mechanism of Phen in the aorta could also be related to the isoform of COX that is present in the different segments. Of the two well-known COX isoforms, COX-1 is considered to be constitutively expressed and involved in a variety of physiological processes. COX-2 is thought to be an inducible isoform that is upregulated by inflammatory citokines (27). However, recent reports have shown that both COX isoforms are expressed in many organs, including the lung, kidney and heart (28). COX-2 mediates the response to the prostanoid precursor, arachidonic acid, in the canine coronary vascular bed and in the rat lung, whereas COX-1 is the dominant isoform in the cerebral vascular bed of the mouse (29,30,31). Given the variability in the distribution of these COX isoforms, we analyzed the relative participation of the COX isoenzymes in the response to Phen by quantifying the levels of COX-1 and COX-2 expression. Although pretreatment of endothelium-denuded abdominal segments with SC-560 did not modify the contractile response to Phen, the selective COX-2 inhibitor NS 398 inhibited this effect in a concentration-dependent manner in the abdominal segment only. Given the similar potencies of NS 398 and indomethacin in the rat abdominal aorta, it seems likely that these agents act to prevent the formation of vasoconstrictor prostaglandins by COX-2 in vascular smooth muscle. Since we used endothelial-denuded aortic rings, the COX-2 that is involved in the contractile effect of Phen is likely located in the smooth muscle layer and perhaps the outer layer of the aorta as well. Our results differ from those of Alvarez *et al* (2005), who concluded that the COX-2 that is involved in the contractile effect of Phen is located in the endothelial layer of the aorta. These differences could be explained by the different ages and strains of rats used in the two studies (6-month old SHR and WKY rats vs. 3-month old WKY rats in the current study). In addition, the immunoblot results further support our conclusion, confirming the presence of COX-2 in the endothelial-denuded abdominal segments. The fact that this isoform is also found in the thoracic segments suggests there is an undiscovered mechanism within the abdominal, but not thoracic, segment of the aorta that couples the $\alpha_1$-adrenergic signal transduction pathway to the COX-2 system and that the presence of COX-2 is not enough to induce this effect.

Cycloheximide is an inhibitor of protein synthesis that helps determine whether these processes are necessary for various physiological or cellular adaptations. This drug prevented the production of prostaglandins in some tissues within 10 minutes of exposure, suggesting that prostaglandin production requires the production of a new polypeptide and that other metabolic processes are not affected (32). This result does not fully address the question of whether the COX-2 that is involved in the production of the Phen-induced contractile prostanoids in the abdominal aortic segment was inducible or constitutively active. Given

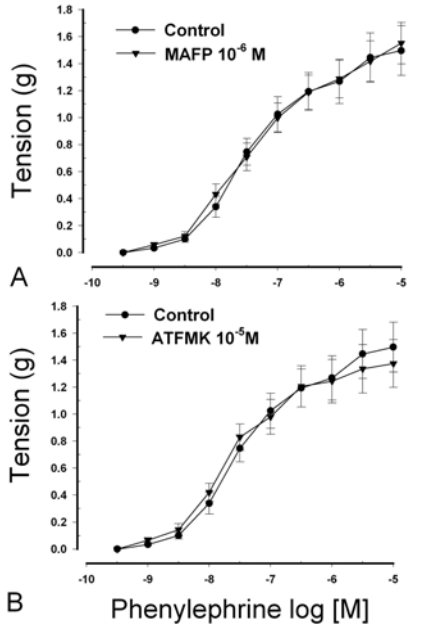


**Figure 5.** The contractile effect of phenylephrine on abdominal aortic rings without endothelium, in (left side) the absence (control) and presence of AATFMK (1.6 x $10^{-5}$ M) or (right side) MAFP ($10^{-6}$ M). Data shown correspond to the mean ± S.E.M. of at least six experiments and are expressed in grams of developed tension.

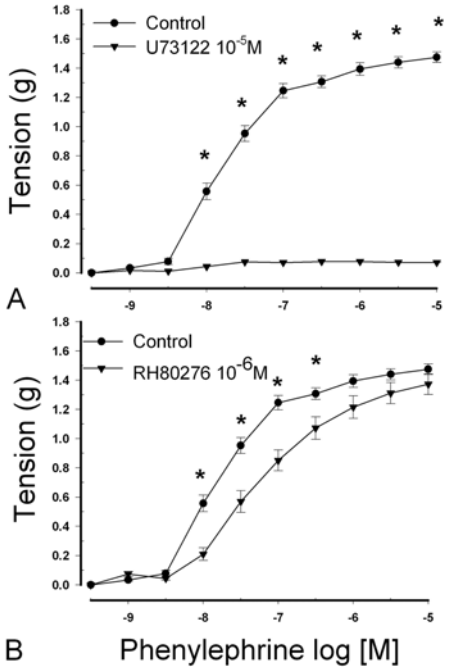


**Figure 6.** The contractile effect of phenylephrine on abdominal aortic rings without endothelium in (a, left side) the absence (control) and presence of U73122 ($10^{-5}$ M) or (b, right side) RHC 80267 ($10^{-5}$ M). Data shown correspond to the mean ± S.E.M. of at least six experiments and are expressed in grams of developed tension. * $p < 0.05$.

that the presence of Phen did not modify the expression of COX-2 and that cycloheximide did not affect the contractile response, we believe that the COX-2 involved in this response is constitutively active. Since pretreating the two aortic segments with cycloheximide did not modify the contractile effect of Phen, we conclude that the trauma of aortic dissection or endothelium removal likely did not induce COX-2 expression.

The generation of free arachidonic acid following receptor stimulation can result from the activity of a variety of different phospholipases, including $PLA_2$, PLC and PLD (33). $PLA_2$ has many subtypes with diverse functions, structures and regulatory mechanisms (34). Types I-III and V $PLA_2$ enzymes are secreted, do not show specificity for the fatty acid moiety at the sn-2-position of phosphatidylcholine and require millimolar concentrations of Ca for activity (35). Types IV and VI $PLA_2$ enzymes are cytosolic and show a preference for the hydrolysis of phosphatidylcholine, which possesses an arachidonyl moiety at the sn-2-position that leads to the release of arachidonic acid (35). Both type IV $PLA_2$ ($cPLA_2$) and type VI $PLA_2$ ($iPLA_2$) are activated following agonist binding to receptors, which results in the release of arachidonic acid. Type VI $PLA_2$ is considered to be principally involved in the restructuring of membranes by reincorporating free arachidonic acid into phospholipids (36), whereas, type IV $PLA_2$ is generally thought to be the principal enzyme that is involved in agonist-induced arachidonic acid generation.

Accordingly, agonist binding to various cell surface receptors has been shown to lead to the activation of $cPLA_2$ and the generation of arachidonic acid in several different cell types (21,37). Nevertheless, we can rule out the participation of the non-secretory forms of $PLA_2$ (IV and VI $PLA_2$) in the production of the arachidonic acid, since neither the inhibitor of IV $PLA_2$, AATFMK, nor the inhibitor of VI $PLA_2$, MAPF, affected the activity of Phen on the abdominal aorta.

Even if $PLA_2$ is the major pathway of arachidonic acid release, PLC or PLD can also produce this acid. Activation of PLC results in the formation of $IP_3$ and diacylglycerol, and the hydrolysis of the latter by diacylglycerol lipase may serve as a source of arachidonic acid (38,39). PLD can hydrolyze phosphatidylcholine to form phosphatidic acid and choline. The resulting phosphatidic acid can then be acted upon by phosphatidic acid phosphohydrolase to yield diacylglycerol, from which arachidonic acid can be released via the action of diacylglycerol and monoacylglycerol lipases (35).

The total inhibition of Phen-induced contraction by U73122 demonstrates that the activation of PLC plays an effect in both the prostanoid-dependent and -independent contractile response of the rat abdominal aorta to this adrenergic drug. In addition, the inhibition of this effect by RHC80267, which is an inhibitor of diacylglycerol lipase (40), supports the idea that diacylglycerol may be the source of the arachidonic acid that is necessary to synthesize the prostanoids involved in

the contractile response of the rat abdominal aorta to Phen. As a consequence, the arachidonic acid required to synthesize the prostanoids involved in this response to Phen could be obtained from diacylglycerol, which may be a direct or indirect product of PLC. This enzyme directly produces diacylglycerol by acting on phospholipids in the cellular membrane, and may indirectly produce it through the activation of PLD.

## 6. ACKNOWLEDGMENTS

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**Abbreviations:** COX: Cyclooxigenase, COX-1: cyclooxigenase 1, COX-2: cyclooxigenase-2, Phen: phenylephrine, AATFMK: arachidonyl trifluoromethyl ketone, MAPF: methyl arachidonyl fluorophosponate, PLC: phosoholipase C

**Key Words:** Phenyleprhine, Thoracic Aorta, Abdominal Aorta, Cyclooxygenase 2, Cyclooxygenase 1, COX-2, COX-1, Vascular Smooth Muscle

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