Decreased cGMP Level Contributes to Increased Contraction in Arteries From Hypertensive Rats
Role of Phosphodiesterase 1

Fernanda R. Giachini, Victor V. Lima, Fernando S. Carneiro, Rita C. Tostes, R. Clinton Webb

Abstract—Recent evidence suggests that angiotensin II (Ang II) upregulates phosphodiesterase (PDE) 1A expression. We hypothesized that Ang II augmented PDE1 activation, decreasing the bioavailability of cyclic guanosine 3′-5′-monophosphate (cGMP), and contributing to increased vascular contractility. Male Sprague-Dawley rats received mini-osmotic pumps with Ang II (60 ng·min⁻¹) or saline for 14 days. Phenylephrine (PE)-induced contractions were increased in aorta (Eₘₐₓ 168%±8% vs 136%±4%) and small mesenteric arteries (SMA; Eₘₐₓ 170%±6% vs 143%±3%) from Ang II-infused rats compared to control. PDE1 inhibition with vinpocetine (10 μmol/L) reduced PE-induced contraction in aortas from Ang II rats (Eₘₐₓ 94%±12%) but not in controls (154%±7%). Vinpocetine decreased the sensitivity to PE in SMA from Ang II rats compared to vehicle (−log of half maximal effective concentration 5.1±0.1 vs 5.9±0.06), but not in controls (6.0±0.03 vs 6.1±0.04). Sildenafil (10 μmol/L), a PDE5 inhibitor, reduced PE-induced maximal contraction similarly in Ang II and control rats. Arteries were contracted with PE (1 μmol/L), and concentration-dependent relaxation to vinpocetine and sildenafil was evaluated. Aortas from Ang II rats displayed increased relaxation to vinpocetine compared to control (Eₘₐₓ 82%±12% vs 445±5%). SMA from Ang II rats showed greater sensitivity during vinpocetine-induced relaxation compared to control (−log of half maximal effective concentration 6.1±0.3 vs 5.3±0.1). No differences in sildenafil-induced relaxation were observed. PDE1A and PDE1C expressions in aorta and PDE1A expression in SMA were increased in Ang II rats. cGMP production, which is decreased in arteries from Ang II rats, was restored after PDE1 blockade. We conclude that PDE1 activation reduces cGMP bioavailability in arteries from Ang II, contributing to increased contractile responsiveness. (Hypertension. 2011; 57[part 2]:655-663.)

Key Words: angiotensin II ■ cGMP ■ hypertension ■ vinpocetine

Many cellular functions, including mechanical and metabolic events, are regulated by cyclic nucleotides, such as cyclic guanosine 3′-5′-monophosphate (cGMP). Cyclic nucleotides act as second messengers and are responsible for short-term and long-term responses in the smooth muscle cells (SMC).1,2 They are synthesized by different isozymes (cyclases), and cGMP formation causes SMC relaxation by inhibiting Rhoa translocation by lowering intracellular calcium and by activating myosin phosphatase.3–5 Moreover, phosphodiesterases (PDE) regulate the amplitude, duration, degradation, and compartmentalization of intracellular cyclic nucleotide signaling, thereby contributing to vascular tone and cellular proliferation.7 Calcium (Ca²⁺)/calmodulin-dependent PDE isoforms contain 2 Ca²⁺/calmodulin-binding domains, and the binding of both Ca²⁺ and calmodulin is required for full activation of these PDE.8–10 Three different isoforms of calmodulin-dependent PDE isoforms have been reported, PDE1A and PDE1B, which display higher affinity to hydrolyze cGMP compared to cAMP and PDE1C, which has a similar ability to hydrolyze cGMP and cAMP.1,11

Vascular changes in hypertension, including increased contractile activity, are closely associated with humoral factors such as angiotensin II (Ang II).12 Vascular SMC contraction is initiated by a biphasic Ca²⁺ elevation in the cytoplasm. The initial transient increase is attributed to inositol trisphosphate-mediated release of Ca²⁺ from the sarcoplasmic reticulum. The subsequent prolonged increase requires extracellular Ca²⁺ influx through various pathways. When Ca²⁺ is high, PDE1 is activated, resulting in lower levels of cGMP, which theoretically facilitates the SMC contraction.

Recently, it has been reported that chronic infusion of Ang II induces increased PDE1A expression in ventricular tis-
sues. In addition, it has been demonstrated that calmodulin-dependent PDE isoforms mediate hypertrophy in cardiomyocytes. Furthermore, the inhibition of PDE1 in SMC isolated from normal aorta or from atherosclerotic lesions resulted in suppression of SMC proliferation.

In view of this next information regarding the functionality of calmodulin-dependent PDE isoforms, along with the fact that Ang II is able to increase PDE1 expression in vascular SMC, we hypothesized that hypertension induced by chronic infusion of Ang II stimulates PDE1 expression in vascular SMC and results in reduced bioavailability of cGMP and increased vascular contractile responsiveness.

Because Ang II also induces PDE5 expression in vitro, we adopted a comparative strategy between PDE1 and PDE5. These 2 isoforms are expressed in SMC and they have distinct mechanisms of activation. We have used the specific pharmacological inhibitors vinpocetine (a PDE1 inhibitor) and sildenafil (a PDE5 inhibitor) to better-understand the effects of Ang II chronic infusion in the vascular expression and activation of PDE isoforms.

**Materials and Methods**

**Animals and Blood Pressure Measurement**

Ten-week-old male Sprague-Dawley rats (230 to 250 grams; Harlan Laboratories, Indianapolis, IN) maintained on a 12:12-hour light–dark cycle with rat chow and water ad libitum were used in these studies. All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were reviewed and approved by the Institutional Animal Care and Use Committee of the Medical College of Georgia.

Rats were anesthetized with isoflurane via a nose cone for surgical procedures (initially with 5% and then maintained at 2.5% in 100% oxygen). Osmotic mini-pumps (0.5 µL/hr, 14 days; model 2002, Alzet) were implanted subcutaneously. Animals were divided into 2 groups: a control group infused with vehicle only (8.33 µL·min⁻¹) and the other infused with Ang II (60 µg·min⁻¹) for a period of 14 days. Systolic blood pressure was measured in nonanesthetized animals by tail-cuff using a RTBP1001 blood pressure system (Kent Scientific Corporation).

**Vascular Functional Studies**

After euthanasia by CO₂, the mesentery and aorta were rapidly excised and placed in a 4°C cold physiological salt solution containing (mmol): NaCl, 130; NaHCO₃, 14.9; KCl, 4.7; KH₂PO₄, 1.18; MgSO₄·7H₂O, 1.18; CaCl₂·2H₂O, 1.56; EDTA, 0.026; and glucose, 5.5. Second-order branches of mesenteric artery (animals by tail-cuff using a RTBP1001 blood pressure system (Kent Laboratories, Indianapolis, IN) maintained on a 12:12-hour light–dark cycle with rat chow and water ad libitum were used in these studies. All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were reviewed and approved by the Institutional Animal Care and Use Committee of the Medical College of Georgia.

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**Western Blot for Detection of Vascular PDE1A, PDE1B, PDE1C, and PDE5 Isoforms**

Proteins (40 µg) extracted from small mesenteric arteries and aorta were separated by electrophoresis on a 10% polyacrylamide gel and transferred to a nitrocellulose membrane. Nonspecific binding sites were blocked with 5% skim milk in Tris-buffered saline solution with Tween for 1 hour at 24°C. Membranes were incubated with antibodies overnight at 4°C. Selective antibodies for the following were used: PDE1A (1:500; Abcam) predicted size, 61 kDa; multiple bands from 65 to 72 kDa, PDE1B (1:500; Abcam) predicted size, 65 kDa; multiple bands from 65 to 72 kDa, PDE1C (1:500; Abcam) predicted size, 70 kDa; multiple bands from 70 to 87 kDa, PDE5 (1:500; Abcam) predicted size, 105 kDa; multiple bands from 99 kDa and β-actin (1:1000; Sigma) predicted size, 42 kDa. After incubation with secondary antibodies, signals were revealed with chemiluminescence and quantified by density profile extraction and multiple band analysis for entire lanes. Results were normalized to β-actin protein and expressed as arbitrary units. Each value of the experiments from both groups was normalized by the average of the control group, which was assumed as being equal to 1.

**Determination of cGMP Levels**

Aortic rings and small mesenteric arteries were equilibrated for 10 minutes in oxygenated Krebs solution. Vinpocetine (10 µmol/L) or vehicle was added to the preparations for 20 minutes. Arteries were collected immediately and segments were frozen in liquid nitrogen. cGMP was extracted and quantified using a cGMP enzyme immunoassay kit (Cayman Chemical) as previously described. The weights of the dried pellets were used to standardize the different samples.

**Data Analysis**

The results are shown as mean±SEM, where “n” represents the number of rats used in the experiments. Contractions were recorded as changes in the displacement (millinewton) from baseline, normalized by the contraction-induced contraction. Relaxation is expressed as percent change from the PE-contracted levels. Concentration–response curves were fitted using a nonlinear interactive fitting program (Graph Pad Prism 3.0; GraphPad Software). Values of P<0.05 were considered statistically significant difference. Statistical analysis was performed using 2-way ANOVA plus Newman-Keuls post hoc analysis to compare the concentration–response curves between all the groups. Western blot data were analyzed by 1-sample t test comparing control and Ang II, and the probability value was computed from the t ratio and the numbers of degrees of freedom. Values of P<0.05 were considered statistically significant.

**Chemicals**

Acetylcholine chloride, phenylephrine hydrochloride, vinpocetine, and 8-Bromo cGMP were purchased from Sigma Aldrich. Ang II was purchased from Phoenix Pharmaceutical. Sildenafil was kindly provided by Pfizer.
Results

Blood Pressure Data
After 14 days of infusion, Ang II-treated rats displayed increased systolic blood pressure compared to control rats (166±2 vs 130±4 mm Hg, respectively; n=6).

Effects of Chronic Infusion of Ang II on Vascular Contractility
Aortas from Ang II-infused rats displayed increased maximum contraction to PE compared to control values (168%±8% vs 136%±4%, respectively; n=6; P=0.0017; Figure 1A). No differences were observed in the −log of half maximal effective concentration (pD2) values between hypertensive and control rats (Table 1).

Small mesenteric arteries from Ang II-infused rats displayed augmented contraction to PE compared to control values (170%±6% vs 143%±3%, respectively; n=6; P=0.0006; Figure 1B). No differences were observed in the pD2 values between hypertensive and control rats (Table 1).

Effect of PDE1 Inhibition on Vascular Contractility
After vinpocetine incubation (10 μmol/L for 30 minutes), aortas from Ang II-infused rats displayed a reduction in the contractile response to PE (94%±12%; n=6; P<0.0001) when compared to Ang II aortas without PDE1 inhibition (Figure 1A). PE-induced contraction in aortas from control rats were not affected by vinpocetine incubation (154%±7%; Figure 1A). Vinpocetine incubation reduced pD2 values in Ang II rats and control rats compared to their respective vehicle (P<0.0001), indicating that PDE1 inhibition decreases sensitivity to PE-induced contractile response in aorta (Table 1).

In small mesenteric arteries, the PE-induced maximum contraction was reduced by vinpocetine incubation in Ang II (151.7%±5.6%; n=6; P=0.0025), but not in control rats (153.3%±3.6%; n=6; Figure 1B). The pD2 value was reduced in small mesenteric arteries from Ang II rats (P<0.0001), but not in control rats, when compared to their respective vehicle groups (Table 1). These data indicate that PDE1 inhibition decreases sensitivity to PE in small mesenteric arteries from Ang II rats.

Together, these results suggest that PDE1 contribution to increased contractile response is greater in aorta and small mesenteric arteries from Ang II-infused rats compared to control rats. Presumably, these results indicate a greater reduction in CGMP by PDE1 in blood vessels from Ang II hypertensive rats.

Effect of PDE5 Inhibition on Vascular Contractility
After sildenafil incubation (10 μmol/L for 30 minutes), aortas from both Ang II-infused (88.5%±4.3%; n=6; P<0.001) and control rats (79.1%±4.5%; n=6; P<0.001) showed reduction in the maximum contractile response to PE compared to their respective groups without incubation (Figure 2A). Additionally, no differences in PE-induced contraction were observed between Ang II and control aortas after sildenafil treatment.

The pD2 values were decreased after sildenafil incubation, both in Ang II rats (P<0.0001) and in control rats (P<0.0001), indicating that PDE5 similarly contributes to PE sensitivity in aortas from both groups (Table 1).

PE-induced maximum contraction was reduced by sildenafil incubation in small mesenteric arteries from Ang II (117%±9.7%; n=6; P=0.0002) and control rats (109%±10.1%; n=6; P=0.0007) compared to their respective groups without incubation (Figure 2B). The pD2 values were reduced in small mesenteric arteries from Ang II rats.

Table 1. pD2 Values for Phenylephrine-Induced Contraction in the Presence or Absence of Vinpocetine and Sildenafil

<table>
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<th>Aorta</th>
<th>Small Mesenteric Artery</th>
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<tr>
<td></td>
<td>Control Angiotensin II</td>
<td>Control Angiotensin II</td>
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<tr>
<td>Vehicle</td>
<td>8.4±0.1</td>
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<tr>
<td>Vinpocetine</td>
<td>6.2±0.1†</td>
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</tr>
<tr>
<td>Sildenafil</td>
<td>7.05±0.1†</td>
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Concentration–response curves to phenylephrine were performed in aorta and small mesenteric arteries from angiotensin II hypertensive and control rats in the absence or in the presence of vinpocetine or sildenafil (10 μmol/L for 30 minutes). Data are mean±SEM (n=6). *P<0.05 vs respective control group. †P<0.05 vs respective vehicle group.
and control rats ($P<0.0001$), showing that PDE5 similarly contributes to increased sensitivity to PE in small mesenteric arteries in both groups (Table 1). The current results show that PDE5 activation contributes to increased contractile response in arteries from Ang II and control rats.

**Effect of PDE1 Inhibition on Ca$^{2+}$-Induced Contraction**

Aortas from Ang II-infused rats displayed increased maximum contraction to Ca$^{2+}$ ($\text{CaCl}_2$) compared to control values (177%±7.4% vs 155%±5.42%, respectively; n=6; $P=0.037$). Vinpocetine incubation reduced maximum contraction in aorta from Ang II and control rats (15.6%±3.2% vs 32.3%±7.5%, respectively; n=6) and abolished differences between the groups (Figure 3A).

Small mesenteric arteries from Ang II rats displayed similar contraction to Ca$^{2+}$ ($\text{CaCl}_2$) compared to control values (76.6%±6.1% vs 75.6%±3.5%, respectively; n=6). PDE1 inhibition abolished contraction to Ca$^{2+}$ ($\text{CaCl}_2$) in both groups (Figure 3B).

**Effect of PDE1 Inhibition on Vascular Relaxation**

In this set of experiments, concentration–response curves to vinpocetine and sildenafil and PDE1 and PDE5 inhibitors, respectively, were performed in arteries from Ang II and control rats contracted with PE (10 μmol/L, aorta) or U-46619 (10 μmol/L, small mesenteric arteries).

Cumulative concentrations of vinpocetine resulted in greater relaxation response in aortas from Ang II rats compared to control rats (82.3%±6.1% vs 44.5%±5.2%, respectively; n=6; $P<0.0028$; Figure 4A). In this case, no differences were observed in pD$_2$ values between aorta from Ang II and control rats, showing that aorta from both groups display similar sensitivity to vinpocetine (Table 2).

In small mesenteric arteries, no differences in the vinpocetine-induced maximum relaxation response were observed between Ang II (89.9%±3.5%; n=6) and control rats (90.6%±1.5%; n=6; Figure 4A). The pD$_2$ value was increased in small mesenteric arteries from Ang II rats compared to control rats ($P<0.029$), which indicates that small mesenteric arteries from Ang II rats have augmented sensitivity to PDE1 inhibition (on addition of vinpocetine) compared to control rats (Table 2).

**Effect of PDE5 Inhibition on Vascular Relaxation**

When sildenafil relaxation curves were performed in aortas, no differences in the maximum response were observed in Ang II compared to control rats (75.8%±4.3% vs 71.7%±12.5%, respectively; n=6; Figure 4B). The pD$_2$ values were similar between Ang II and control rats (Table 2), indicating that aorta from both groups display similar sensitivity to sildenafil.

In small mesenteric arteries, the sildenafil cumulative curve resulted in a similar relaxation response in Ang II
and Bay41-2272 (Relaxation) Curve to Vinpocetine, Sildenafil, 8-Bromo cGMP, and Ang II hypertensive and control rats. Data are mean ± SEM (n = 6). Symbols represent the results from 2-way ANOVA and express differences in the E₉₀.* P < 0.05 vs control.

Table 2. pD₂ Values for Cumulative Concentration Response (Relaxation) Curve to Vinpocetine, Sildenafil, 8-Bromo cGMP, and Bay41-2272

<table>
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<th>Aorta</th>
<th>Control</th>
<th>Angiotensin II</th>
<th>Aorta</th>
<th>Control</th>
<th>Angiotensin II</th>
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<td>Vinpocetine</td>
<td>4.8 ± 0.1</td>
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<td>5.3 ± 0.1</td>
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<td>Sildenafil</td>
<td>4.3 ± 0.3</td>
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<td>8-Bromo cGMP</td>
<td>4.6 ± 0.2</td>
<td>4.6 ± 0.2</td>
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<tr>
<td>Bay41-2272</td>
<td>5.3 ± 0.008</td>
<td>5.4 ± 0.09</td>
<td>6.8 ± 0.06</td>
<td>6.94 ± 0.11</td>
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Concentration–response curves to vinpocetine, sildenafil, 8-Bromo cGMP, and Bay41-2272 were performed in aortas contracted with phenylephrine (1 μmol/L) or small mesenteric arteries contracted with U-46619 (1 μmol/L) from Ang II hypertensive and control rats. Data are mean ± SEM (n = 6). *P < 0.05 vs respective control group.

Vascular Expression of PDE1 and PDE5 Isoforms
The vascular expression of PDE1 and PDE5 isoforms was accessed by Western blot technique. Aortas from Ang II-infused rats displayed increased expression of PDE1A, PDE1C, and PDE5 isoforms compared to control rats (Figure 5A). Small mesenteric arteries displayed increased expression of PDE1A compared to control rats (Figure 5B).

Effects of PDE1 Inhibition on Vascular cGMP Production
Basal cGMP production was decreased in aortas from Ang II rats compared to control (11.3 ± 0.5 vs 20.0 ± 1.4 pmol/mL, respectively; n = 6; P < 0.0001). When aortas were incubated with vinpocetine (10 μmol/L), cGMP production was increased in Ang II (27.5 ± 1.9 pmol/mL; n = 6; P < 0.0001) as in controls (31.1 ± 1.1 pmol/mL; n = 6; P < 0.0001), and differences between the groups were abolished (Figure 6A).
Similar results were observed in small mesenteric arteries whereas those from Ang II rats had reduced cGMP levels compared to controls (17.4 ± 0.6 vs 25.5 ± 0.8 pmol/mL, respectively; n = 6; P < 0.001). Again, the PDE1 inhibitor increased cGMP levels in small mesenteric arteries from both Ang II (29.7 ± 0.9 pmol/mL; n = 6; P < 0.0001) and control rats (26.8 ± 0.9 pmol/mL; n = 6; P < 0.0001), and differences between the groups were abolished after vinpocetine (Figure 6B).

These data show that augmented activation of PDE1 isoforms in arteries from Ang II rats are contributing to decreased cGMP levels. In addition, PDE1 inhibition was able to abolish differences in cGMP levels between the groups.

**Discussion**

Our major findings are that arteries from Ang II hypertensive rats, compared to control rats, display increased PDE1 expression and activation, resulting in augmented PE-induced contractile maximal response, increased sensitivity to PE stimuli, and impaired cGMP levels. Additionally, PDE1 inhibition abolished differences in the contractile responsiveness between the groups and improved cGMP levels. We found that PDE5 is increased in aorta from hypertensive rats. However, PDE5 inhibition decreased contractile response to PE in arteries from Ang II and control rats in a similar fashion. We speculate that PDE1 may represent a new mechanism by which Ang II enhances vasoconstriction via cGMP-dependent pathways.

Ang II is critical for the regulation of vascular tone, blood pressure, and volume homeostasis. High levels of circulating Ang II lead to increased contractile activity of vascular SMC to agonistic stimuli, vascular growth, migration, apoptosis, and extracellular matrix deposition, which are hallmarks for vascular changes observed during hypertension. Ang II is critical for the regulation of vascular tone, blood pressure, and volume homeostasis. High levels of circulating Ang II lead to increased contractile activity of vascular SMC to agonistic stimuli, vascular growth, migration, apoptosis, and extracellular matrix deposition, which are hallmarks for vascular changes observed during hypertension.22,23

cGMP functions as an antagonist of Ang II actions by counteracting the Ang II signaling pathway at different steps.24,25 For example, cGMP has been shown to block Ang II-stimulated Ca2+ mobilization26 and inhibit several protein kinases that are activated by Ang II.14,27

The functional interplay between Ang II and cGMP are determined by the mutual regulation of Ang II and cGMP signaling pathways at different levels, including nitric oxide (NO) production, guanylyl cyclase activation, cGMP-mediated protein kinase G activation and PDE activation.25

NO directly stimulates guanylyl cyclase, which cause to GTP to be converted into cGMP.28 Additionally, Ang II can stimulate endothelial NO synthase (eNOS) expression.29,30 In vivo experiments showed that Ang II infusion decreases NO production, but this is mainly attributable to the fact that endothelial NO synthase is mainly in the uncoupled form, generating superoxide rather than NO.25,31 Hence, Ang II can negatively mediate guanylyl cyclase expression31 and enzymatic activity,32 because of superoxide-related33 and peroxynitrite-related34 mechanisms. Moreover, Ang II de-
Small-mesenteric arteries

Figure 6. Phosphodiesterase-1 inhibition restores cGMP bioavailability in arteries from angiotensin II (Ang II) hypertensive rats. cGMP production was evaluated in the presence or absence of vinpocetine (10 µmol/L) in (A) aorta and (B) small mesenteric arteries from Ang II hypertensive (black bars) and control (white bars) rats. Data are mean±SEM (n=6). *P<0.05 vs control. †P<0.05 vs respective vehicle group.

cGMP (pmol/mg)

Vinpocetine (10µM)

Control

Ang III

Control

Ang III

Aorta

Small-mesenteric arteries

As mentioned, PDE are important proteins that modulate cGMP bioavailability by hydrolyzing and therefore inactivating the cyclic nucleotide. It has been reported that Ang II is able to increase PDE1 activity and PDE5 expression in vascular SMC, resulting in lower cGMP level. Therefore, it seems that the global reduction of cGMP levels caused by Ang II infusion is attributable to the concomitant activation of PDE and inactivation of guanylyl cyclase, which impairs cGMP signaling pathway.

Our working hypothesis was driven by the observation that Ang II directly modulates PDE1 and its effect on cGMP production. We observed increased expression levels of PDE1A, PDE1C, and PDE5 isoforms in arteries from Ang II rats. However, our functional data supported an important role for PDE1 as a contributor to increased vascular responsiveness and sensitivity to contractile stimuli in the arteries from the hypertensive rats because inhibition of PDE5 decreased the contractile response similarly in both experimental groups.

Considering that these enzymes are coupled to distinct intracellular cGMP-dependent pathways, these results may reflect differential regulatory mechanisms for PDE5 and PDE1. PDE5 is primarily stimulated by NO-induced cGMP, whereas PDE1 is stimulated by increased intracellular Ca2+ and, in this case, PDE1 is hydrolyzing cGMP predominantly during elevations in intracellular Ca2+ in response to agonist stimulation. Our data showed that aortas from Ang II rats displayed increased contractile response to Ca2+, suggesting that this may be a possible mechanism that explains increased PDE1 activity.

Ang II-stimulated Ca2+ signaling is complex and occurs via multiple pathways to elicit an integrated Ca2+ signal. It is well-described that Ang II mediates augmented Ca2+ signaling in vascular SMC, primarily by IP3-induced mobilization of intracellular Ca2+ and secondarily by increasing Ca2+ entry. Considering that PDE1 activation depends on Ca2+, it seems plausible that arteries from Ang II rats may display increased PDE1 activity. It was observed that nitrate relaxation, which is mediated by NO/cGMP, is a process that can be desensitized, leading to nitrate tolerance. This occurs by augmented expression of PDE1A associated with Ca2+ supersensitized cells.

Additionally, we showed that PDE1-specific inhibition was able to increase cGMP levels and to abolish the augmented contractile activity difference between arteries from hypertensive and normotensive rats. These are exciting data if we consider a previous report in which vinpocetine, a PDE inhibitor, in the presence of the inhalated NO was able to enhance pulmonary vasodilation and transpulmonary cGMP without generating a systemic vasodilation. Additionally, 8-methoxymethyl-3-isobutyl-1-methylxanthine, another PDE1 inhibitor, further reduced systemic arterial pressure induced by iloprost, a long-acting prostacyclin analog. Therefore, a combination of current therapies with PDE1 inhibition may be useful. Most recently, it was shown that vinpocetine is able to inhibit inflammation induced by tumor necrosis factor-α by PDE-independent mechanisms. Given the importance of tumor necrosis factor-α in Ang II-induced inflammatory pathways, it seems interesting that a drug that acutely restores vascular contraction during Ang II-induced hypertension may play additional roles in vascular function. For that purpose, chronic studies using PDE1 inhibitors, such as vinpocetine, should be addressed.

It has been shown that SMC mainly express 3 PDE isoforms, including PDE1 and PDE5. Our data show that Ang II increased PDE1A expression in both aorta and small mesenteric arteries, whereas PDE1C was only increased in aorta. PDE1C isoform is closely related to vascular remodeling and proliferation of human vascular SMC, and it was suggested that this enzyme could be a target for treatment of atherosclerosis or restenosis after angioplasty. However, whether Ang II exclusively regulates human SMC proliferations is still unclear. Of importance, differential subcellular localization of the PDE isoforms may account for differential cGMP regulation. Taking this into consideration, this new concept predicts that PDE acts as cyclic nucleotide diffusion barriers through their spatially confined zones of enzymatic activity, contributing to the subcellular compartmentalization of distinct signaling cascades. In this regard, data from our laboratory propose that soluble guanylyl cyclase is compartmentalized in the caveolae and in the cavernosal endothelium, and its spatial organization facilitates NO actions.

In conclusion, we propose that increased PDE1 expression and its activation, as a result of chronic Ang II infusion,
contribute to impaired cGMP levels, resulting in increased vascular response and sensitivity to contractile stimuli.

**Perspectives**

Regardless of the fact that there is a variety of pharmacological preparations available for therapy, a large number of hypertensive patients are refractory to these treatments. Therefore, new strategies to find new targets to treat hypertensive disease should be encouraged. Considering that PDE1 inhibition resulted in reduction of the maximum contraction response occurred in arteries from Ang II hypertensive rats, it seems that this may be an interesting target to improve vascular functionality in hypertensive subjects. Therefore, we aim to investigate how long-term PDE1 inhibition may change during hypertension. In addition, we would like to see whether PDE1 activation can interfere with other signaling pathways that are modified in hypertension, such as mitogen-activated protein kinase activation.

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**Disclosure**

None.

**References**


Decreased cGMP Level Contributes to Increased Contraction in Arteries From Hypertensive Rats: Role of Phosphodiesterase 1
Fernanda R. Giachini, Victor V. Lima, Fernando S. Carneiro, Rita C. Tostes and R. Clinton Webb

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