Clopidogrel, independent of the vascular P2Y$_{12}$ receptor, improves arterial function in small mesenteric arteries from AngII-hypertensive rats

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Abstract

The P2Y$_{12}$ receptor antagonist clopidogrel blocks platelet aggregation, improves systemic endothelial nitric oxide bioavailability and has anti-inflammatory effects. Since P2Y$_{12}$ receptors have been identified in the vasculature, we hypothesized that clopidogrel ameliorates AngII (angiotensin II)-induced vascular functional changes by blockade of P2Y$_{12}$ receptors in the vasculature. Male Sprague–Dawley rats were infused with AngII (60 ng/min) or vehicle for 14 days. The animals were treated with clopidogrel (10 mg·kg$^{-1}$·day$^{-1}$) or vehicle. Vascular reactivity was evaluated in second-order mesenteric arteries. Clopidogrel treatment did not change systolic blood pressure [(mmHg) control-vehicle, 117 ± 7.1 versus control-clopidogrel, 125 ± 4.2; AngII–vehicle, 197 ± 10.7 versus AngII–clopidogrel, 198 ± 5.2], but it normalized increased phenylephrine-induced vascular contractions [(%KCl) vehicle-treated, 182.2 ± 18 % versus clopidogrel, 133 ± 14 %], as well as impaired vasodilation to acetylcholine [(%) vehicle-treated, 71.7 ± 2.2 versus clopidogrel, 85.3 ± 2.8] in AngII-treated animals. Vascular expression of P2Y$_{12}$ receptor was determined by Western blot. Pharmacological characterization of vascular P2Y$_{12}$ was performed with the P2Y$_{12}$ agonist 2-MeS-ADP [2-(methylthio) adenosine 5′-trihydrogen diphosphate trisodium]. Although 2-MeS-ADP induced endothelium-dependent relaxation [(Emax %) = 71 ± 12 %] as well as contractile vascular responses (Emax % = 83 ± 12 %), these actions are not mediated by P2Y$_{12}$ receptor activation. 2-MeS-ADP produced similar vascular responses in control and AngII rats. These results indicate potential effects of clopidogrel, such as improvement of hypertension-related vascular functional changes that are not associated with direct actions of clopidogrel in the vasculature, supporting the concept that activated platelets contribute to endothelial dysfunction, possibly via impaired nitric oxide bioavailability.

Introduction

ADP has been established for a long time as an aggregating agent [1,2]. Activation of platelets by ADP leads to rapid Ca$^{2+}$ entry, mobilization of intracellular Ca$^{2+}$ stores and activates platelet P2Y$_{1}$ and P2Y$_{12}$ purinoceptors to inhibit adenylyl cyclase. Extracellular ADP, via those receptors, elicit these physiological responses [3].

There are two principal classes of nucleotide receptors: P2X receptors, which are ligand-gated ion channels, and...
and P2Y receptors, which belong to the G-protein-coupled receptor family [4,5]. Extracellular nucleotides, such as ADP, activate P2Y purinoreceptors located both in vascular smooth muscle and endothelial cells, generating vasoconstriction and endothelium-dependent vasodilatation respectively [6–8].

P2Y_{12} receptors were identified in human blood vessels, where they induce contraction [9]. Controversially, P2Y_{12} receptors do not play a role in vascular function in aorta from rats [7], although no data regarding the function of P2Y_{12} receptors in resistance vasculature are available.

The first clinical application of P2 receptor antagonism involved the use of thienopyridines, such as the P2Y_{12} ADP receptor antagonist clopidogrel, a platelet aggregation inhibitor [8]. Besides the important role of these drugs in preventing platelet aggregation, recent studies identified direct effects of thienopyridines on endothelial function. Clopidogrel treatment improves endothelial NO (nitric oxide) bioavailability and decreases proinflammatory and prothrombotic-related events in humans and in experimental animals [10–14].

Complications during coronary heart disease, ischaemic stroke and peripheral vascular disease are related to thrombosis rather than haemorrhage. Some complications related to elevated blood pressure, heart failure and atrial fibrillation are also associated with thromboembolism. Thus, it seems plausible that antithrombotic therapy may be particularly useful in preventing thrombosis-related vascular complications of elevated blood pressure [15]. However, whether these beneficial effects of clopidogrel are all related to prevention of platelet aggregation or if there is a direct effect in the vasculature is still unknown.

We hypothesized that treatment with clopidogrel ameliorates hypertension-associated vascular dysfunction, in part, by blocking vascular P2Y_{12} receptors. To test our hypothesis, we assessed the effects of clopidogrel treatment on vascular reactivity in control and AngII (angiotensin II)-treated rats. Pharmacological and molecular approaches were used to establish the presence and functionality of vascular P2Y_{12} receptors in mesenteric resistance arteries from these animals.

Part of this work was presented at the 5th U.S./Japan International Symposium on Molecular and Cellular Aspects of Vascular Smooth Muscle, held in Kailua-Kona on 7–9 January 2007, and subsequently published in abstract form [15a].

**MATERIALS AND METHODS**

**Animals and blood pressure measurement**

Eight-week-old male Sprague–Dawley rats (230–250 g; Harlan Laboratories), maintained on a 12:12-h 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 anaesthetized with a mixture of ketamine (80 mg/kg of body weight) and xylazine (10 mg/kg of body weight) and osmotic mini-pumps (0.5 μl/h, 14 days, model 2022; Alzet) were implanted subcutaneously. Animals were divided into two groups: a control group infused with saline only and the other infused with AngII (60 ng/min) for a period of 14 days. Both groups were simultaneously treated either with clopidogrel (Plavix®; 10 mg · kg⁻¹ of body weight · day⁻¹) or vehicle (peanut butter, 1 g) for 14 days. At day 0 (before experimental procedure) and day 14, SBP (systolic blood pressure) was measured by tail cuff plethysmography in conscious rats. Weight gain was also evaluated by measurement of the body weight at days 0 and 14. The efficacy of treatment with clopidogrel was evaluated by determination of bleeding time, as previously described [16]. Briefly, after 14 days of treatment with clopidogrel or vehicle, rats were placed in individual restrainers, and the tip of the tail (3 mm) was cut, and blood drops were collected on filter paper. The duration of bleeding was recorded.

**Vascular functional studies**

After killing, the mesentery was rapidly excised and placed in a 4 °C ice-cold PSS (physiological salt solution), containing (mM): NaCl, 130; NaHCO₃, 14.9; KCl, 4.7; KH₂PO₄, 1.18; MgSO₄·7H₂O 1.18; CaCl₂·2H₂O, 1.56, EDTA, 0.026, glucose 5.5. Second-order branches of mesenteric artery (ø 2 mm in length with internal diameter ø 150 to 250 μm) were carefully dissected and mounted as ring preparations on two stainless steel wires. The second-order mesenteric arteries were mounted in an isometric Mulvany–Hallpern small-vessel myograph (40 μm diameter; model 610 DMT-USA) and data were recorded by a PowerLab 8/SP data acquisition system (ADInstruments). One wire was attached to a force transducer and the other to a micrometer. Both dissection and mounting of the vessels were carried out in ice-cold (4 °C) PSS. The second-order mesenteric arteries were adjusted to maintain a passive force of 3 mN. Arteries were equilibrated for 45 min in PSS at 37 °C, and continuously bubbled with 5% CO₂ and 95% O₂. Arterial integrity was assessed first by stimulation of vessels with 120 mM KCl and, after washing and a new stabilization time, by contracting the segments with PE (phenylephrine; 10 μM) followed by relaxation with ACh (acetylcholine; 10 μM). Endothelium-dependent relaxation was assessed by measuring the dilatory response to ACh (1 nM to 10 μM) in PE-contracted vessels (3 μM). ACh responses were also evaluated after a
Effects of clopidogrel treatment on systolic blood pressure, bleeding time and weight gain in control and AngII-treated rats

*P < 0.05 versus respective control; †P < 0.05 versus vehicle-treated (n = 16 in each group).

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control + vehicle</th>
<th>Control + clopidogrel</th>
<th>Ang II + vehicle</th>
<th>Ang II + clopidogrel</th>
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<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>117 ± 7.1</td>
<td>125 ± 4.2</td>
<td>197 ± 10.7*</td>
<td>198 ± 5.2*</td>
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<td>Bleeding time (s)</td>
<td>424 ± 31</td>
<td>&gt;1200†</td>
<td>470 ± 42</td>
<td>&gt;1200†</td>
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<tr>
<td>Weight gain (g)</td>
<td>32 ± 8</td>
<td>34 ± 9</td>
<td>21 ± 5</td>
<td>26 ± 5</td>
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30-min incubation with vehicle or with the NO synthase inhibitor l-NAME (N\(^{\text{G}}\)-nitro-l-arginine methyl ester; 100 \(\mu\)M) plus indomethacin (10 \(\mu\)M), an inhibitor of prostanoid synthesis. A concentration–response curve to PE (1 nM to 100 \(\mu\)M) was performed to evaluate vascular contractility. The response to 2-MeS-ADP [2-(methylthio) adenosine 5’-trihydrogen diphosphate trisodium; 0.1 to 100 \(\mu\)M] was evaluated in arteries on basal tonus and after PE-induced (3 \(\mu\)M) contraction. To avoid the possibility of tachyphylactic responses, concentration–response curves to 2-MeS-ADP were performed by testing only one concentration of 2-MeS-ADP in each vascular preparation. Therefore, various vascular preparations from one animal were stimulated with only one concentration of 2-MeS-ADP in this study (0.01 to 100 \(\mu\)M) to construct the concentration–response curve. Responses to 2-MeS-ADP (100 \(\mu\)M) were also evaluated in arteries after incubation with l-NAME (100 \(\mu\)M) plus indomethacin (10 \(\mu\)M), both on basal tonus and after PE-induced (3 \(\mu\)M) contraction. In addition, 2-MeS-ADP-induced responses (both contraction and relaxation) were determined in the presence of selective antagonists for P2Y\(_1\), P2Y\(_{12}\) and P2Y\(_{13}\) receptors: MRS-2179, MRS-2395 and MRS-2211 respectively.

Western blot for detection of vascular P2Y\(_1\), P2Y\(_{12}\) and P2Y\(_{13}\) receptors

Proteins (40 \(\mu\)g) extracted from small mesenteric arteries were separated by electrophoresis on a 10% polyacrylamide gel and transferred to a nitrocellulose membrane. Nonspecific binding sites were blocked with 5% skimmed milk in Tris-buffered saline with Tween for 1 h at 24°C. Membranes were incubated with antibodies overnight at 4°C. Antibodies were as follows: P2Y\(_1\), P2Y\(_{12}\), P2Y\(_{13}\) (1:200; Alomone Labs) and \(\beta\)-actin (1:1000; Sigma). After incubation with secondary antibodies, signals were revealed with chemiluminescence, visualized by autoradiography and quantified densitometrically. Results are normalized to \(\beta\)-actin protein and expressed as arbitrary units.

Data analysis

The results are shown as mean ± S.E.M. where \(n\) represents the number of rats used in the experiments. Contractions were recorded as changes in the displacement (mN) from baseline and normalized by KCl contraction and are represented as percentage of KCl-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). Values of \(P < 0.05\) were considered a statistically significant difference. Statistical analysis was performed using two-way ANOVA plus Newman–Keuls post-hoc analysis to compare the concentration–response curves between all the groups. The other analyses were performed with one-way ANOVA plus Newman–Keuls post-hoc analysis.

Chemicals

ACh chloride, AngII, indomethacin, l-NAME, 2-MeS-ADP, MRS-2395 (2,2-dimethyl-propionic acid 3-(2-chloro-6-methylaminopurin-9-yl)-2-(2,2-dimethyl-propionyloxymethyl)-propyl ester), SNP (sodium nitroprusside) and phenylephrine hydrochloride were purchased from Sigma. MRS-2179 tetrakisodium salt [2-deoxy-N\(^{\text{6}}\)-methyladenosine 3’5’-bisphosphate tetrasodium salt] and MRS-2211 2 disodium salt {[2-(chloro-5-nitrophenyl)azo]-5-hydroxy-6-methyl-3-[(phosphonoxy)methyl]-4-pyridinecarboxaldehyde} were purchased from Tocris.

RESULTS

Physiological parameters and bleeding time

SBP and body weight were similar in all groups of animals at the beginning of the study. AngII significantly increased SBP, whereas clopidogrel treatment did not change SBP either in control or AngII-hypertensive rats (Table 1). The bleeding time of rats treated with clopidogrel was significantly prolonged in both normotensive and hypertensive groups, showing efficacy of treatment (Table 1). AngII-treated animals displayed a tendency to reduced weight gain, and clopidogrel treatment had no effect on body weight gain (Table 1).
Clopidogrel improves ACh-induced relaxation and prevents increased responses to PE in arteries from AngII-treated rats

Figure 1

Concentration–response curves to ACh in second-order mesenteric arteries (A) in the absence or (B) in the presence of L-NAME (100 μM) plus indomethacin (10 μM), and concentration–response curves to PE (C) in the absence or (D) in the presence of L-NAME (100 μM) plus indomethacin (10 μM) in arteries from (O) control+vehicle, (□) control + clopidogrel, (●) AngII + vehicle and (■) AngII+clopidogrel rats. Experimental values of the relaxation induced by ACh were calculated relative to the maximal changes from the contraction produced by PE in each tissue, which was taken as 100% (% of relaxation). Experimental values for PE-induced contraction are represented as a percentage of the KCl-induced response. Results are presented as means ± S.E.M. of n = 6 in each experimental group. *P < 0.05 compared with other groups.

Vascular reactivity studies

Mesenteric resistance arteries (Figure 1A) from AngII-treated animals displayed impaired relaxation in response to ACh (71.8 ± 2.5% versus 86.1 ± 2.5%, control), which was significantly improved in vessels from Ang-II infused rats treated with clopidogrel (85.4 ± 2.5%). Clopidogrel treatment had no effects on ACh responses in vessels from control animals (85.5 ± 2% versus 86.1 ± 2.5%, vehicle). Incubation of mesenteric resistance arteries with L-NAME plus indomethacin abolished the differences in ACh-induced relaxation between the groups (Figure 1B). SNP-induced relaxation was similar amongst mesenteric arteries from all groups (results not shown).

Mesenteric resistance arteries from AngII-hypertensive rats showed increased contractile responses to PE (182.2 ± 18%) when compared to that in arteries from control animals (132.2 ± 8%, Figure 1C). Treatment with clopidogrel normalized PE-induced contraction in arteries from AngII hypertensive rats (133 ± 14%) but did not change the response in arteries from control animals (136 ± 8%). In addition, incubation of mesenteric resistance arteries with L-NAME plus indomethacin abolished the differences in ACh-induced relaxation between the groups (Figure 1D).

This first set of results indicates that treatment with clopidogrel prevents dysfunction associated with Ang-II hypertension. In addition, the results also suggest that effects of clopidogrel are possibly associated with increased NO bioavailability and prostanoid synthesis.

2-MeS-ADP-induced vascular responses

Pharmacological characterization of vascular P2Y12 receptors was performed with 2-MeS-ADP. 2-MeS-ADP is the most potent P2Y12 receptor agonist available.

Relaxant action

Treatment of mesenteric resistance arteries on passive basal tonus with 2-MeS-ADP did not produce changes in force levels. After contraction with PE (3 μM), a significant relaxation in response to 2-MeS-ADP (10 μM) was observed (Figure 2A). Concentration–response curves to 2-MeS-ADP (0.01 to 100 μM) showed that maximum relaxation in mesenteric arteries (71% ± 12) is obtained with 100 μM 2-MeS-ADP (Figure 2B).

Vascular responses to 2-MeS-ADP, after constriction with PE (3 μM), were determined in all experimental groups, and no statistical differences were observed. Figure 2(C) illustrates relaxant responses to
Figure 2 Effects of 2-MeS-ADP in endothelium-intact second-order mesenteric arteries from control and AngII-hypertensive rats

(A) Representative tracings showing that, upon stimulation with 2-MeS-ADP (10 μM), mesenteric arteries in basal tonus display no changes in force levels, whereas PE-contracted (3 μM) arteries exhibit a relaxant response. (B) Concentration–response curve to 2-MeS-ADP (0.1–100 μM) in PE-contracted (3 μM) arteries from control rats. (C) 2-MeS-ADP (100 μM) induced similar relaxation in mesenteric arteries from control and AngII-treated rats treated with vehicle or clopidogrel. (D) Relaxant responses to 2-MeS-ADP (100 μM) were determined in arteries incubated with: MRS-2179 (0.1 μM), a P2Y1 receptor antagonist; MRS-2395 (0.1 μM), a P2Y12 receptor antagonist; MRS-2211 (1 μM), a P2Y13 receptor antagonist or with the combination of these antagonist. Then, they were stimulated with PE (3 μM), and after contractile responses were obtained, 100 μM 2-MeS-ADP was added to the bath. Experimental values of the relaxation induced by 2-MeS-ADP were calculated relative to the maximal changes from the contraction produced by PE in each tissue, which was taken as 100%. Results are presented as means ± S.E.M. of n = 5 in each experimental group. *P < 0.05 versus vehicle in their respective group.

2-MeS-ADP (100 μM) in mesenteric arteries from the four experimental groups.

Although the adenosine diphosphate analogue 2-MeS-ADP is the most potent P2Y12 receptor agonist available, this drug can also activate P2Y1 and P2Y13 receptors [22]. Therefore, after determining the effects of the 2-MeS-ADP in mesenteric resistance arteries, we performed a functional characterization of the receptor subtype(s) that mediate 2-MeS-ADP responses in rat second-order mesenteric arteries, by using MRS-2179, a P2Y1 antagonist; MRS-2395, a P2Y12 antagonist, and MRS-2211, a P2Y13 antagonist. The effects of 2-MeS-ADP were tested after incubation of the vessels with different concentrations of each antagonist (0.01, 0.1 and 1.0 μM), for 30 min. The concentrations chosen for the experiments were 1 μM (MRS-2211) and 0.1 μM (MRS-2179 and MRS-2395), since no further inhibitory effects were observed with greater concentrations of the P2Y1 or P2Y12 antagonists (0.01, 0.1 and 1.0 μM).

In second-order mesenteric arteries from control normotensive rats, blockade of P2Y1 receptors, with MRS-2179 (0.1 μM), and P2Y12 receptors, with MRS-2211 (1 μM), produced approximately a 50% and 40% inhibition of 2-MeS-ADP-induced relaxation.
Effects of 2-MeS-ADP in endothelium-intact second-order mesenteric arteries, incubated with L-NAME plus indomethacin, from control and AngII-hypertensive rats

(A) Representative tracings showing that, upon stimulation with 2-MeS-ADP (10 μM), mesenteric arteries, incubated with L-NAME (100 μM) plus indomethacin (10 μM), display no vascular responses, whereas 2-MeS-ADP (100 μM) induces contraction in PE (3 μM)-stimulated vessels. (B) Concentration–response curve to 2-MeS-ADP (0.1–100 μM) in PE-contracted (3 μM) arteries incubated with L-NAME plus indomethacin. (C) 2-MeS-ADP (100 μM) induced similar contraction in mesenteric arteries from control and AngII-treated rats treated with vehicle or clopidogrel, after incubation with L-NAME plus indomethacin. (D) Contractile responses to 2-MeS-ADP (100 μM) were determined in arteries incubated with: MRS-2179 (0.1 μM), a P2Y<sub>12</sub> receptor antagonist; MRS-2395 (0.1 μM), a P2Y<sub>12</sub> receptor antagonist; MRS-2211 (1 μM), a P2Y<sub>13</sub> receptor antagonist or with a combination of these antagonists. Then, they were stimulated with PE (3 μM), and after contractile responses were obtained, 100 μM 2-MeS-ADP was added to the bath. Experimental values for 2-MeS-ADP-induced contraction are represented as a percentage of PE-induced contraction. Results are presented as means ± S.E.M. of n = 5 in each experimental group. *P < 0.05 versus respective control group.

respectively (Figure 2D). In contrast, blockade of P2Y<sub>12</sub> receptors, with MRS-2395 (0.1 μM), did not interfere with 2-MeS-ADP-induced relaxation. The simultaneous incubation with both P2Y<sub>1</sub> and P2Y<sub>12</sub> or P2Y<sub>13</sub> and P2Y<sub>12</sub> receptor antagonists did not produce greater inhibition of 2-MeS-ADP-induced relaxation (Figure 2D). However, P2Y<sub>1</sub> and P2Y<sub>13</sub> receptor antagonists resulted in almost 75% inhibition of 2-MeS-ADP-induced relaxation. The same profile of results was found in mesenteric arteries from AngII-induced hypertensive rats, except that concomitant blockade of P2Y<sub>1</sub> and P2Y<sub>13</sub> receptor antagonists did not produce greater inhibition of 2-MeS-ADP-induced relaxation in hypertensive rats (Figure 2D).

Contractile action

Similar to what was observed in non-treated mesenteric arteries, 2-MeS-ADP did not induce changes in level of force in endothelium-intact mesenteric resistance arteries treated with L-NAME (100 μM) plus indomethacin (10 μM). However, in the presence of L-NAME plus indomethacin, it further increased tension development when tested in PE (3 μM)-contracted vessels (Figure 3A). Concentration–response curves to 2-MeS-ADP (0.01 to
Vascular dysfunction and P2Y12 receptor

100 μM), after preincubation with l-NAME (100 μM) plus indomethacin (10 μM), were performed in PE-constricted mesenteric arteries. The maximum contractile response in mesenteric arteries (83 % ± 12) from control rats was obtained with 100 μM 2-MeS-ADP (Figure 3B).

Vascular responses to 2-MeS-ADP, in vessels incubated with l-NAME (100 μM) plus indomethacin (10 μM), after constriction with PE (3 μM) were determined in all experimental groups. Figure 3(C) illustrates that contractile responses to 2-MeS-ADP (100 μM) were not different among the groups.

The blockade of P2Y1 receptors with MRS-2179 or P2Y13 receptors with MRS-2211 partially inhibited 2-MeS-ADP-induced contraction in second-order mesenteric arteries, whereas blockade of P2Y12 receptors with MRS-2395 produced no significant effect in the response to 2-MeS-ADP. The concomitant blockade of P2Y1 and P2Y12 or P2Y13 and P2Y12 did not produce further inhibition of 2-MeS-ADP-induced contraction (Figure 3D), whereas simultaneous incubation of P2Y1 and P2Y13 resulted in reduction of 2-MeS-ADP-induced contraction. The same profile of results was found in mesenteric arteries from AngII-induced hypertensive rats, but again, simultaneous blockade of P2Y1 and P2Y13 receptor antagonists did not produce greater inhibition of 2-MeS-ADP-induced contraction (Figure 2D).

These data from functional experiments suggest that in mesenteric arteries, P2Y1 and possibly P2Y13 are involved in both relaxant and contractile responses induced by 2-MeS-ADP.

**Protein expression of vascular P2Y1, P2Y12 and P2Y13 receptors**

Protein expression of P2Y12 receptor was determined in mesenteric arteries from control and AngII-hypertensive animals. Expression of P2Y1 and P2Y13 receptors was also determined because the functional data suggest that the actions of the most selective P2Y12 agonist available are mediated by P2Y1 and P2Y13.

Protein expression of P2Y1, P2Y12 and P2Y13 receptors was observed in mesenteric arteries. No differences in P2Y1 and P2Y12 protein expression were observed between mesenteric arteries from control and AngII-hypertensive animals (Figure 4A). Interestingly, expression levels of P2Y13 receptors were very low in mesenteric arteries from AngII-hypertensive animals (Figure 4B).

**DISCUSSION**

Clopidogrel, an anti-platelet drug, is prescribed to prevent coronary artery disease and thrombotic events. In patients with elevated blood pressure, anti-platelet therapy is recommended for secondary prevention because the magnitude of the absolute benefit in vascular alterations is many times greater [15]. More recently, clopidogrel treatment has been shown to improve endothelial NO bioavailability and ameliorate proinflammatory and prothrombotic-related events [10–12]. Additionally, P2Y12 receptor polymorphisms are more frequently present in patients with vascular disease than in healthy people [17].

Because of these additional effects of clopidogrel on endothelial function, and considering that very few studies have addressed the effects of clopidogrel on vascular reactivity, we hypothesized that clopidogrel, by direct actions on the vasculature, is able to attenuate hypertension-related vascular functional changes. The AngII-induced hypertension model was chosen due to well-characterized changes in vascular reactivity and due to the vasoconstrictor, progrowth and proinflammatory actions of AngII [18].

PE-induced contraction and ACh-induced relaxation were impaired in mesenteric resistance arteries from rats infused with AngII. Clopidogrel treatment completely
prevented the effects of AngII. Since the differences in the ACh-induced relaxation were abolished among the groups after inhibition of NO and prostaglandin production, AngII-induced impaired vascular reactivity is associated with altered endothelial function. These results also indicate that the improvement generated by clopidogrel is partially endothelium-dependent. Additionally, it is well established that AngII decreases NO bioavailability [19] and that the preservation of NO bioavailability is essential for endothelium function [20].

Heitzer and colleagues [10] showed that ADP receptor blockade by clopidogrel improves endothelium-dependent vasodilation in response to ACh and vascular bioavailability of NO in the human forearm of patients with symptomatic coronary artery disease. In addition, clopidogrel reduced inflammatory and oxidative parameters [10]. Our findings support the concept that activated platelets contribute to endothelial dysfunction and impaired NO bioavailability. However, considering that P2Y12 receptors have been described in vascular tissue, it is possible that some of the beneficial effects of clopidogrel are due to blockade of vascular P2Y12 receptors and may not reflect only its antiplatelet effects.

The functional characterization of receptors mediating the vascular responses to 2-MeS-ADP suggested the involvement of P2Y1 and P2Y13, but not P2Y12, receptors both in vascular smooth muscle and endothelial cells. As mentioned, responses of mesenteric resistance arteries to 2-MeS-ADP were not different between the control and hypertensive groups. Furthermore, our functional studies show that in small mesenteric arteries, responses mediated by P2Y1 and P2Y13 receptors are not altered in vessels from AngII-hypertensive rats.

In summary, our results indicate that potentially beneficial effects of clopidogrel, such as improvement of hypertension-related vascular functional changes, are not due to direct effects in the vasculature, and support the
concept that activated platelets contribute to endothelial dysfunction, possibly via impaired NO bioavailability and prostanoid synthesis.

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