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ORIGINAL ARTICLE

Vasodilatory activity and antihypertensive profile mediated by inhibition of phosphodiesterase type 1 induced by a novel sulfonamide compound

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ABSTRACT

LASSBio-985 is a sulfonamide compound designed as a simplified structure of a nonselective phosphodiesterase type 4 (PDE-4) inhibitor that promotes vasodilatory activity in vitro. PDE are enzymes responsible for the hydrolysis of cyclic adenosine 3′,5′- monophosphate and cyclic guanosine 3′,5′-monophosphate. Five different isozymes of PDE are found in vascular smooth muscle (PDE1–PDE5). Aortic rings, with or without endothelium, from male normotensive and spontaneously hypertensive rats (SHR) were prepared for isometric tension recording. Blood pressure was measured in Wistar Kyoto (WKY) rats and SHR during intravenous infusion of LASSBio-985 (10 mg/kg/min) during 15 min. LASSBio-985 induced a concentration-dependent vasodilation in aortic rings from normotensive and SHR, which was almost completely inhibited in endothelium-denuded vessels. Vasodilatory activity was also reduced in endothelium-intact aortic rings that had been pretreated with N_{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME), a nitric oxide synthase inhibitor and 1H-[1,2,4]oxadiazolod[4,3-a]quinoxalin-1-one (ODQ), a guanylate cyclase inhibitor. LASSBio-985-induced vasodilation was also inhibited by sildenafil (100 μM) and SQ 22536, a PDE5 inhibitor and adenylate cyclase inhibitor, respectively. To evaluate the involvement of some endothelial receptors, atropine, diphenhydramine, HOE 140, naloxone, propranolol, indomethacin, and wortmannin were tested, but none inhibited the effects of LASSBio-985. The residual effect observed on endothelium-denuded aortic rings was abolished by nicardipine, a voltage-sensitive-Ca^{2+}-channel blocker. Intravenous infusion of LASSBio-985 (10 mg/kg/min) significantly reduced systolic and diastolic pressures in both WKY and SHR. LASSBio-985 is a compound with vasodilatory activity, which could be consequent to PDE1 inhibition and voltage-sensitive-Ca^{2+}-channel blockade.

INTRODUCTION

The cyclic nucleotide phosphodiesterases (PDE) comprise a large family of enzymes that selectively catalyze the hydrolysis of cyclic adenosine 3′,5′-monophosphate (cAMP) and cyclic guanosine 3′,5′-monophosphate (cGMP) into the corresponding 5′-nucleotide monophosphates and play a critical role in maintaining the cellular levels of these cyclic nucleotides [1,2]. There are 11 different families of PDE encompassing more than 50 different isoforms. They modulate several signaling pathways and are useful pharmacological targets in the treatment of diseases [3].

Cyclic adenosine 3′,5′-monophosphate and cGMP are important second messengers involved in cellular signaling and homeostasis. cAMP and cGMP are formed by the catalytic activity of adenylate cyclase (AC) and guanylate cyclase (GC), respectively, and degraded by PDE. Inhibition of PDE raises the levels of cyclic nucleotides that activate cAMP-dependent protein kinases (PKA) and cGMP-dependent protein kinases (PKG) [1]. The protein kinases impact many physiological processes through phosphorylation of a variety of substrates, including transcription factors, ion channels, and signaling proteins [4–6].

Cyclic nucleotides have a crucial role in the maintenance of vascular smooth muscle tone as they are involved in the signaling pathway of both nitric oxide and prostacyclin, which are important endothelium-derived relaxant factors [7]. Consequently, PDE activity directly affects the contractility of smooth muscle.

Five different isozymes of PDE are found in vascular smooth muscle: PDE1, which hydrolyzes preferentially cGMP and is Ca^{2+}/calmodulin-dependent; PDE2, a cGMP-stimulated phosphodiesterase; PDE3 that hydrolyzes cAMP and is inhibited by cGMP; PDE4, cAMP-specific phosphodiesterase; and PDE5 that hydrolyzes cGMP and is insensitive to Ca^{2+}-calmodulin [8,9]. Thus, PDE inhibitors have considerable therapeutic utility for arterial hypertension, pulmonary hypertension, and erectile dysfunction by causing relaxation of smooth muscle [10–13].

Based on the importance of PDE as target for drugs acting on the cardiovascular system, a novel sulfonamide compound named LASSBio-985 (*N*-(3,4-dimethoxyphenethyl)methanesulfonamide, *Figure 1*) was designed as a simplified structure of the nonselective PDE-4 inhibitor 1-(3,4-dimethoxyphenylsulfonyl) indoline [14].

The purpose of this study is to investigate the mechanisms of vasodilation induced by LASSBio-985 and its effect on blood pressure in Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHR).

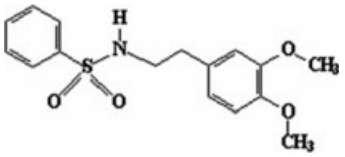

Figure 1 Chemical structure of LASSBio-985.

METHODS

Chemistry

Reaction was routinely monitored by thin-layer chromatography (TLC) in silica gel (F245 Merck plates) and the product visualized with iodine or ultraviolet light (254 and 365 nm). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were determined in $CDCl_3$ using a Bruker AC-200 spectrometer. Peak positions are in parts per million (δ) from the tetramethylsilane internal standard, and coupling constant values (J) are in Hz. Infrared (IR) spectra were obtained using an ABB FTLA2000-100 IR spectrometer. Samples were examined using potassium bromide (KBr) disks. Elemental microanalyses were obtained on an Elemental Analyzer (Flash EA 1112 Series; Thermo Scientific, San Jose, CA, USA) from vacuum-dried samples. The analytical results for C, H, and N were within ± 0.4% of theoretical values. Melting points were determined using a Quimis instrument and are uncorrected. All organic solutions were dried over anhydrous sodium sulfate, and all organic solvents were removed under reduced pressure in rotatory evaporator.

N-(3,4-dimethoxyphenethyl)methanesulfonamide (1)

The 3,4-dimethoxyphenethylamine (8.73 mmol) was added to a solution of methanesulfonamide (8.73 mmol) in 50 mL of methylene chloride and 1.5 mL of triethylamine. The reaction mixture was stirred for approximately 2 h at room temperature. The end of the reaction was observed by TLC. The sulfonamide derivative **1** was isolated by adding 50 mL of methylene chloride and extracting with 10% aq HCl (5 × 25 mL) and brine (3 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated at reduced pressure to give the sulfonamide **1** (yield, 83%).

^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ (ppm): 2.81 (t, 2H, J = 7.1 Hz, $RNHCH_2\underline{CH_2}Ph$); 2.84 (s, 3H, $RNHSO_2\underline{CH_3}$);

3.36 (q, 2H, J = 7.1 Hz and 5.9 Hz, RNHC$\underline{H}_2$CH$_2$Ph); 4.59 (t, 1H, J = 5.9 Hz, RN$\underline{H}$CH$_2$CH$_2$Ph); 6.75 (m, 3H, H2, H5 and H6).

^{13}C NMR (50 MHz, $CDCl_3$, TMS) δ (ppm): 36.05, 40.29, 44.57, 55.93, 111.47, 111.98, 120.84, 130.27, 147.94, 149.14.

IR (υ_{max}, KBr) υ (per centimeter): 3201 (νNH), 3010 (νCH), 1415 (ν C-O), 1301 and 1122 (ν S-N).

Anal. Calcd. for $C_{11}H_{17}NO_4S$: C, 50.95; H, 6.61; N, 5.40. Found: C, 50.91; H, 6.59; N, 5.43.

Biological evaluation

All animal protocols were approved by the Animal Care and Use Committee at Universidade Federal do Rio de Janeiro, DFBCICB 020.

In vitro studies in isolated rat aortic rings

Male Wistar rats and SHR (15–20 weeks old) were anesthetized with ether and then sacrificed by cervical dislocation. The thoracic aorta was immediately dissected. Adherent fat and connective tissues were carefully removed, and the aorta was cut into rings 2–3 mm in length. The aortic rings were placed in a 10-mL vertical chamber containing physiological saline solution composed of (in mM): NaCl, 123; KCl, 4.7; $MgCl_2$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 15.5; $CaCl_2$, 1.2 and glucose, 11.5; maintained at 37.0 ± 0.5 °C, pH 7.4, and continuously oxygenated with carbogen gas (95% O_2/5% CO_2). Isometric tension was measured for each aorta ring mounted between two hooks, one of which attached to a force transducer (Grass mod. FT-03). Signals were conditioned (Cyberamp 380; Axon Instruments, Inc., Union City, CA, USA), displayed, and stored on a computer for future analysis using Axoscope software (Axon Instruments, Inc.). Aortic rings were initially stretched to 1 g and, after 120 min of equilibrium, were contracted with phenylephrine (10 μM) and then relaxed with acetylcholine (10 μM). In some rings, the endothelial layer was mechanically disrupted by gently rubbing the luminal surface with plastic tubing. The endothelium of aortic ring preparations was considered functional when acetylcholine-induced relaxation was >80% of the initial phenylephrine-induced contracture. Removal of functional endothelium was verified by the lack of same acetylcholine-induced relaxation. Vascular tissue from SHR has dysfunction of endothelium cells and as a consequence the endothelium-dependent relaxation induced by acetylcholine is not intense [15]. In experiments with aorta with intact endothelium from SHR, the acetylcholine-induced relaxation is typically 60% of phenylephrine contraction [16]. In our preparations, acetylcholine promoted 55% relaxation in most of the aortic rings used. Thus, it was considered that the endothelium of aortic ring preparations was functional when acetylcholine-induced relaxation was >50% in SHR. The rings were washed with saline solution until a stable resting tone returned. A new phenylephrine contraction was induced, followed by administration of increasing concentrations of LASSBio-985 (0.5–1000 μM).

In some experiments, *N*-nitro-L-arginine methyl ester hydrochloride (L-NAME; 100 μM) [17], 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; 10 μM) [18], Diphenhydramine hydrochloride (10 μM) [19], indomethacin (10 μM) [18], naloxone (100 μM) [20], atropine (10 μM) [21], wortmannin (300 nM) [22], propranolol (10 μM) [23], HOE 140 (1 μM) [24], sildenafil (0.1 and 100 μM) [25], SQ 22536 (100 μM) [17], nicardipine (1 μM) [20] were added to the saline solution in chambers 15–30 min before phenylephrine and LASSBio-985. Results are expressed as the percent relaxation of the maximum contractile tension to phenylephrine after LASSBio-985 concentrations addition.

In some experiments, endothelium-denuded aortic rings were contracted with 40 mM KCl and then exposed to increasing concentrations of LASSBio-985. In experiments with high K^+, the isotonic 40 mM KCl saline solution was prepared by equimolar replacement of NaCl.

In vivo studies

Male WKY rats and SHR (15–20 weeks old) were anesthetized with inhalational sevoflurane (2%) for insertion of a catheter in the right carotid artery under aseptic condition after local anesthesia (1% lidocaine). Another polyethylene catheter was inserted in the jugular vein for intravenous infusion of LASSBio-985. The end of the catheters was exteriorized at the top of the head and after their implantation; rats were allowed 1 h to recover from the surgery prior to blood pressure recording using a calibrated pressure transducer (MLT884; AD Instruments, Sydney, Australia). Three electrodes were placed on the chest for electrocardiogram recording. Blood pressure, electrocardiogram, and heart rate were displayed and stored on a computer for future analysis (Powerlab; ADInstruments) using Lab Chart software (7.0 Version; ADInstruments). After complete recovery from anesthesia, animals were treated with

intravenous infusion of LASSBio-985 through a pump (Harvard pump mod. 1100) for 15 min at a rate of 10 mg/kg/min.

Compounds

Phenylephrine, acetylcholine, L-NAME, ODQ, diphenhydramine hydrochloride, HOE 140, wortmannin from *Penicillium funiculosum*, propranolol, indomethacin, nicardipine, SQ 22536, atropine, and naloxone were purchased from Sigma Chemical Co. (St Louis, MO, USA). Sildenafil was kindly donated by Cristália Produtos Químicos e Farmacêuticos (São Paulo, SP, Brazil). Phenylephrine, acetylcholine, atropine, naloxone, propranolol, L-NAME, diphenhydramine hydrochloride, and HOE 140 were dissolved in distilled water and LASSBio-985, ODQ, indomethacin, wortmannin from *P. funiculosum*, nicardipine, sildenafil, and SQ 22536 were dissolved in dimethylsulfoxide.

Statistical analysis

The effect of LASSBio-985 was expressed as the percent relaxation. Data are shown as the mean ± SEM. One-way analysis of variance (ANOVA) followed by Dunnett tests were used to compare the experimental and control groups. All data were analyzed using Kolmogorov-Smirnov normality test, before the one-way ANOVA. All data have shown a Gaussian distribution. Student's *t*-test was used to compare the response to the same concentration. Differences were considered significant when $P < 0.05$. The concentration–response curve was fit to the following equation: $y = y_{max}+(y_{max}-y_{min})/(1+10^{logEC50 - x * HillSlope})$, where x was the logarithm of concentration and y was the percentage of isometric tension.

RESULTS

Effects of LASSBio-985 on normotensive rat aortic rings

To investigate the effects of LASSBio-985, aortic rings were precontracted with phenylephrine, and increasing concentrations of LASSBio-985 were added to the preparation. LASSBio-985 induced a concentration-dependent relaxation of rat aortic rings (*Figure 2*). Mechanical removal of endothelial cells significantly decreased the effects of LASSBio-985 (*Figure 2a*). The maximum relaxation induced by LASSBio-985 in endothelium-intact rat aortic rings was significantly reduced by endothelium removal, 95.1 ± 1.7% vs. 12.1 ± 1.9%, respectively ($P < 0.05$).

Effects of pretreatment with L-NAME and ODQ

To determine whether nitric oxide (NO)/cGMP pathway was involved in the endothelium-dependent effects of LASSBio-985, concentration–response curves of LASSBio-985 were generated in the absence or presence of L-NAME, a nitric oxide synthase (NOS) inhibitor, or ODQ, a GC inhibitor. Both inhibitors significantly reduced the LASSBio-985 relaxation effect. The maximum relaxation induced by LASSBio-985 (1000 μM) in presence of L-NAME and ODQ was 15.4 ± 3.5 and 18.7 ± 3.9%, respectively, significantly lower than in absence of any inhibitor (*Figure 2b,c*; $P < 0.05$).

Effects of LASSBio-985 on PDE

To evaluate LASSBio-985 effects on cGMP-specific PDE, aortic rings were pre-incubated with sildenafil, a PDE1, PDE5 and PDE6 inhibitor. Sildenafil at 0.1 μM did not

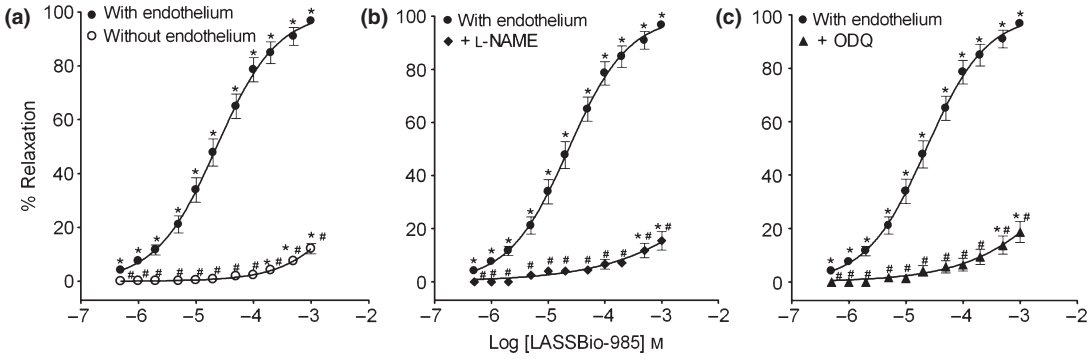

Figure 2 Effects of LASSBio-985 on phenylephrine-precontracted normotensive rat aortic rings. (a) Concentration–response curves for LASSBio-985 in aortic rings with and without endothelium. (b) Concentration–response curves for LASSBio-985 in aortic rings with endothelium in the presence or absence of L-NAME (100 μM). (c) Concentration–response curves for LASSBio-985 in aortic rings with endothelium in the presence or absence of ODQ (10 μM). Values are expressed as mean ± SEM of 8–12 experiments. $^{*}P < 0.05$ compared to control; $^{\#}P < 0.05$ compared to LASSBio-985 with endothelium.

alter the effects of LASSBio-985, but at 100 μM, significantly inhibited LASSBio-985-induced vasodilation (*Figure 3a*). Pretreatment with the higher concentration of sildenafil significantly reduced phenylephrine-induced contraction ($P < 0.05$; *Figure 3b*), but did not promote additional reduction in the presence of LASSBio-985 ($P > 0.05$; *Figure 3b*).

Effects of pretreatment with SQ 22536

To examine the involvement of AC on LASSBio-985 effects, aortic rings were pretreated with SQ 22536, an AC inhibitor. SQ 22536 significantly inhibited LASSBio-985 effect, decreasing maximal relaxation from 98.6 ± 0.8 to 36.4 ± 6.3% ($P < 0.05$; *Figure 4*).

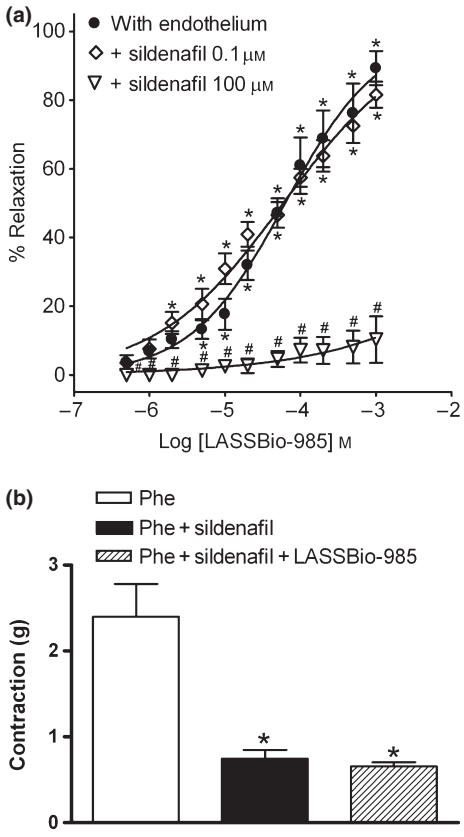

Figure 3 Effect of sildenafil on LASSBio-985 vasodilation and phenylephrine-induced contraction in rat aortic rings. (a) Concentration–response curves for LASSBio-985 in aortic rings with endothelium in the presence or absence of sildenafil (10 and 100 μM). (b) Maximal contraction induced by phenylephrine in rat aortic rings in the presence of sildenafil (100 μM). The concentration of LASSBio-985 was 1 mM. Values are expressed as mean ± SEM of six experiments. (a) $^{*}P < 0.05$ compared to control; $^{\#}P < 0.05$ compared to LASSBio-985 with intact endothelium. (b) $^{*}P < 0.05$ compared to phenylephrine contraction.

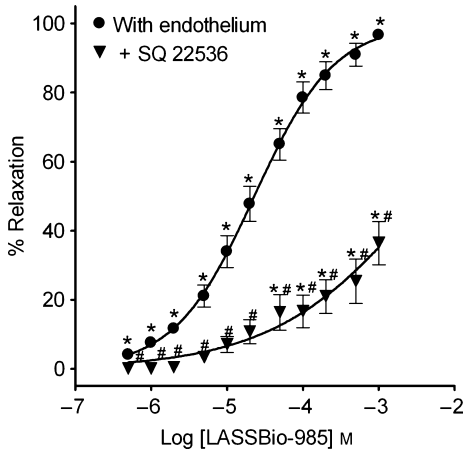

Figure 4 Effect of SQ 22536, an adenylate cyclase inhibitor, on LASSBio-985 vasodilation. Concentration–response curves for LASSBio-985 in aortic rings with endothelium in the presence or absence of SQ 22536 (100 μM). Values are expressed as mean ± SEM of eight experiments. $^{*}P < 0.05$ compared to control; $^{\#}P < 0.05$ compared to LASSBio-985 with endothelium.

Effects of LASSBio-985 on endothelium-denuded aortic rings

To investigate the effects of higher LASSBio-985 concentrations (200–1000 μM) on endothelium-denuded aortic rings, nicardipine, a blocker of voltage-sensitive Ca^{2+}-channels, was incubated with endothelium-denuded aortic preparations. Nicardipine completely inhibited vasodilation induced by LASSBio-985 ($P < 0.05$; *Figure 5*). Endothelium-intact aortic rings were also pretreated with nicardipine but no change was observed at the 50% inhibitory concentration (IC_{50}) or maximal relaxation ($P > 0.05$; *Figure 5*).

Effects of pharmacological agents on LASSBio-985-induced vasodilation

Many receptors in endothelial cells induce NO production. To examine whether LASSBio-985 was binding to one of these receptors, aorta preparations were pretreated with certain receptor antagonists.

To study the involvement of muscarinic M3, histamine H1, bradykinin B2, opioid μ_3, β-adrenergic receptors, we used the respective antagonists atropine, diphenhydramine, HOE 140, naloxone, and propranolol. *Table I* shows the maximum relaxation values for these inhibitors. None of these inhibitors was able to significantly change maximum relaxation of LASSBio-985 ($P > 0.05$).

To analyze the involvement of another endothelial factor, prostacyclin (PGI_2), aortic rings were pretreated

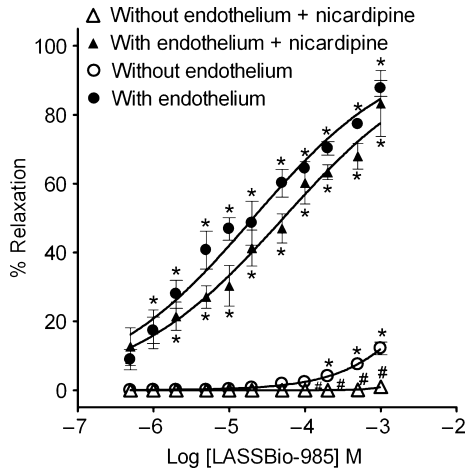

Figure 5 Effect of nicardipine, a voltage-sensitive calcium-channel blocker, on LASSBio-985 vasodilation. Concentration–response curves for LASSBio-985 in aortic rings with endothelium in the presence or absence of nicardipine (1 μM). Values are expressed as mean ± SEM of eight experiments. $^{*}P < 0.05$ compared to control; $^{\#}P < 0.05$ compared to LASSBio-985 without endothelium.

Table I Maximal relaxation of LASSBio-985 in the presence of distinct pharmacological agents.

LASSBio-985	Maximum relaxation (%)
With endothelium	98.6 ± 0.8
+Atropine	96.8 ± 1.4
+Diphenhydramine	94.9 ± 2.7
+HOE 140	100.0 ± 0.0
+Naloxone	96.3 ± 2.9
+Propranolol	88.1 ± 3.6
+Indomethacin	100.0 ± 0.0
+Wortmannin	95.1 ± 3.2

Values are expressed as means ± SEM of 6–12 experiments.

with indomethacin, a cyclooxygenase inhibitor. No significant change in the maximum relaxation induced by LASSBio-985 was observed when using pre-applied indomethacin (*Table I*; $P > 0.05$).

To investigate whether LASSBio-985 could be acting via direct activation of NOS, aortic rings were pre-incubated with wortmannin, a phosphatidylinositol 3′-kinase (PI3K) inhibitor, which also did not inhibit the effect of LASSBio-985 (*Table I*; $P > 0.05$).

Effects of LASSBio-985 on SHR aortic rings

The effects of LASSBio-985 and mechanism of action were also examined on SHR aortic rings. LASSBio-985 induced a concentration-dependent relaxation of SHR aortic rings precontracted with phenylephrine as in normotensive rats (*Figure 6*). An endothelium-dependent effect was also observed (*Figure 6a*), with the maximum relaxation induced by LASSBio-985 in endothelium-intact rat aortic rings being significantly reduced by endothelium removal, 100.0 ± 0.0% vs. 30.0 ± 4.4%, respectively ($P < 0.05$).

To determine whether NO/GMPc pathway is also involved in the endothelium-dependent effects of LASSBio-985, concentration–response curves of LASSBio-985 were constructed in the absence or in the presence of L-NAME or ODQ. Both inhibitors also significantly reduced LASSBio-985 relaxation effect on SHR aortic rings. The maximum relaxation induced by LASSBio-985 (1000 μM) in the presence of L-NAME and ODQ was 35.0 ± 2.6 and 26.1 ± 8.3%, respectively, significantly lower than in the absence of either inhibitor (*Figure 6b,c*; $P < 0.05$).

Effects of LASSBio-985 on SHR endothelium-denuded aortic rings

Additional experiments investigated the effects of LASSBio-985 on K^+-induced contractions. In SHR endothelium-denuded aortic rings, the LASSBio-985-induced relaxation was greater after exposure to high K^+ than to phenylephrine ($P < 0.05$; *Figure 7*). Maximal relaxation on KCl-contracted aortic rings was 54.7 ± 2.2%, significantly higher than that observed on phenylephrine-contracted ones, 30.0 ± 4.4% ($P < 0.05$).

To investigate whether LASSBio-985 impaired extracellular calcium influx through voltage-dependent calcium channels, aortic rings were precontracted with phenylephrine in the presence of nicardipine (1 μM). In the presence of nicardipine, only a partial relaxation was observed at 1000 μM LASSBio-985. At this concentration of LASSBio-985, the maximal relaxation of SHR endothelium-denuded aortic rings in the presence of nicardipine was 6.5 ± 4.8%.

Effects of LASSBio-985 on WKY rats and SHR blood pressure

To investigate whether LASSBio-985 can cause changes on blood pressure, an intravenous infusion of LASSBio-985 was administered to rats. After 15 min of LASSBio-985 infusion (10 mg/kg/min), no significant change was observed in the heart rate of WKY rats or SHR. The control heart rates in WKY rats and SHR were 361.6 ± 23.9 and 388.8 ± 14.5 beats/min, respectively. After 15 min of infusion, heart rates of WKY rats and SHR were 382.8 ± 17.1 and 381.8 ± 9.2 beats/

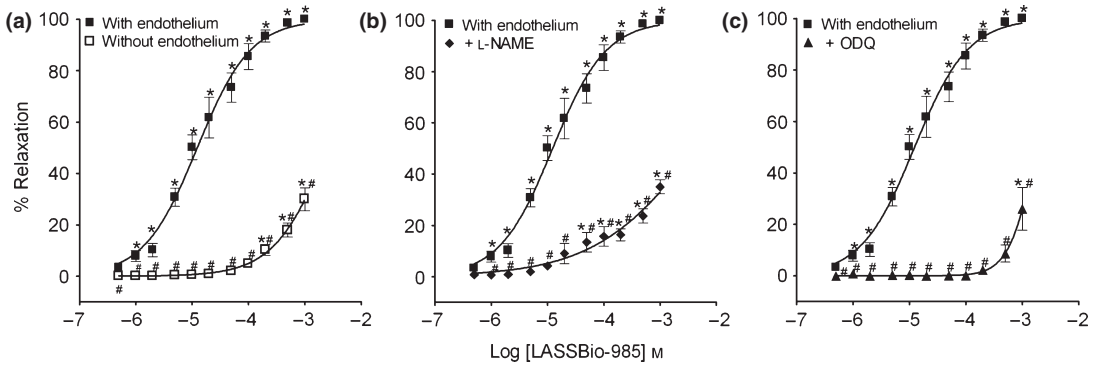

Figure 6 Effects of LASSBio-985 on phenylephrine-precontracted SHR aortic rings. (a) Concentration–response curves for LASSBio-985 in aortic rings with and without endothelium. (b) Concentration–response curves for LASSBio-985 in aortic rings with endothelium in the presence or absence of L-NAME (100 μM). (c) Concentration–response curves for LASSBio-985 in aortic rings with endothelium in the presence or absence of ODQ (10 μM). Values are expressed as mean ± SEM of eight experiments. $^{*}P < 0.05$ compared to control; $^{\#}P < 0.05$ compared to LASSBio-985 with endothelium.

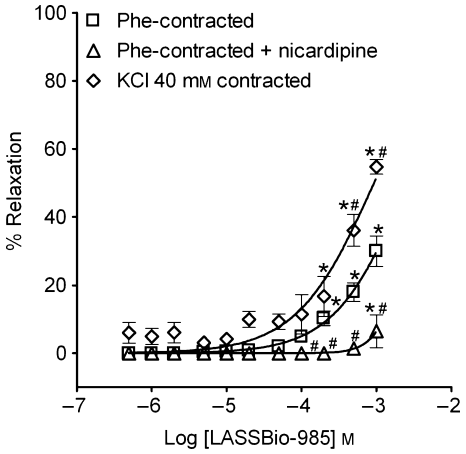

Figure 7 Effects of LASSBio-985 on phenylephrine-precontracted SHR aortic rings without endothelium. Concentration–response curves for LASSBio-985 in phenylephrine-contracted SHR aortic rings without endothelium in the presence or absence of nicardipine (1 μM) and KCl 40 mM contracted SHR aortic rings without endothelium. Values are expressed as mean ± SEM of eight experiments. $^{*}P < 0.05$ compared to control; $^{\#}P < 0.05$ compared to LASSBio-985 phenylephrine-contracted without endothelium.

min, respectively ($P > 0.05$). However, significant reductions in both systolic and diastolic pressures were observed in both strains of rats. In WKY rats, systolic pressure was reduced from 145.5 ± 4.3 to 121.6 ± 3.8 mmHg, and diastolic pressure was reduced from 101.3 ± 4.5 to 69.3 ± 9.2 mmHg ($P < 0.05$) after LASSBio-985 infusion. In SHR, systolic pressure was reduced from 182.6 ± 7.9 to 148.5 ± 7.8 mmHg, and diastolic pressure was also significantly reduced from 129.9 ± 11.5 to 87.8 ± 9.6 mmHg ($P < 0.05$) after 15 min of LASSBio-985 infusion (*Table II*).

DISCUSSION

The present work was designed to investigate the mechanism of action of LASSBio-985-induced relaxation in vascular smooth muscle cells, and its effect when administered in vivo.

Our results showed that LASSBio-985 is a compound that induces vasodilation in normotensive and hypertensive rats, both in vitro and in vivo. LASSBio-985 produced a concentration-dependent relaxation of rat

Table II Effects of LASSBio-985 on Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) blood pressure

Hemodynamic measurements	WKY rats				SHR			
	Control	5′	10′	15′	Control	5′	10′	15′
HR (beats/min)	361.6 ± 23.9	352.3 ± 21.8	380.5 ± 20.1	382.8 ± 17.1	388.8 ± 14.5	388.6 ± 11.9	387.2 ± 12.0	381.8 ± 9.2
SBP (mmHg)	144.5 ± 4.3	131.8 ± 7.1	127.7 ± 5.9	121.6 ± 3.8*	182.6 ± 7.9	164.9 ± 6.9	153.0 ± 7.1*	148.5 ± 7.8*
DBP (mmHg)	101.3 ± 4.5	82.5 ± 9.6	72.4 ± 8.1	69.3 ± 9.2*	129.9 ± 11.5	108.7 ± 12.4	91.4 ± 8.7*	87.8 ± 9.6*

HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure. Values are expressed as means ± SEM of eight experiments. $^{*}P < 0.05$ compared to control.

aortic rings in both normotensive rats and SHR. Mechanical removal of the endothelium significantly reduced the vasodilatory effect of LASSBio-985 in phenylephrine-contracted vessels, indicating that this compound requires the presence of a functional endothelium to exert its maximum effect. Moreover, LASSBio-985 may affect vascular tone by acting on both endothelial and smooth muscle cells because, at higher concentrations, it inhibited the contractile response induced by phenylephrine in endothelium-denuded aorta.

Furchgott and Zawadzki [26], first described endothelium-dependent relaxation, demonstrating that vascular smooth muscle relaxation, induced by acetylcholine, depends on the integrity of endothelium and attributed this effect to an endothelium-derived relaxing factor later identified as NO [27]. Since then, the importance of NO and the endothelial cell layer for vascular homeostasis has been extensively studied. Currently, the role of endothelium in vascular disease has been receiving increased attention as most vascular diseases are primary related to endothelial cell dysfunction [28].

NO released by endothelium diffuses into the vascular smooth muscle cells, binds to, and stimulates GC. Activated GC produces cGMP from guanosine 5′-triphosphate (GTP) that activates PKG. The NO/cGMP/PKG pathway mediates vascular smooth muscle relaxation through several mechanisms [29,30]. Our results demonstrated that both the NOS inhibitor, L-NAME, and the GC inhibitor, ODQ, significantly reduced LASSBio-985-induced relaxation in normotensive rat aortic rings, indicating that the NO/cGMP pathway is involved in this endothelium-dependent effect.

Our hypothesis was that PDE inhibition could be involved in the effects of LASSBio-985 because it was designed as a simplified structure of a nonselective PDE4 inhibitor. There are two types of PDE that hydrolyze cGMP and whose inhibition can promote vasodilation: PDE1, which hydrolyzes cAMP and cGMP, and PDE5 [31]. In the human aorta, PDE1 activity in the presence of Ca^{2+} and calmodulin is more abundant than PDE5 activity [9]. Also, it has been shown that PDE4 and PDE5 inhibitors have endothelium-dependent vasodilatory activity like LASSBio-985 [32]. These observations suggest that LASSBio-985 could be inhibiting PDE1, PDE4, or PDE5. However, additional experiments showed that LASSBio-985 has no effect on tracheal and corpus cavernosum smooth muscle (data not shown), in which the most common PDE are cAMP-specific PDE4 and cGMP-specific PDE5 [33,34], respectively, ruling out the involvement of PDE4 or PDE5 to the activity of LASSBio-985.

LASSBio-985-induced vasodilation was also evaluated in the presence of sildenafil, which is a PDE5 inhibitor at low concentrations, and a PDE1 and PDE6 inhibitor at high concentrations [25,35]. Sildenafil blocked LASSBio-985 effects only at high concentrations, indicating the possible involvement of PDE1, as PDE6 is not found in vascular smooth muscle. Moreover, the AC inhibitor SQ 22536 significantly reduced LASSBio-985-induced vasodilation, demonstrating that AC is also necessary for LASSBio-985 action.

Altogether these observations indicate that LASSBio-985-induced relaxation may be related to PDE1 inhibition because it hydrolyzes cAMP and cGMP [31]. PDE1 is important for hydrolysis of cGMP in vessels, and its inhibition causes hypotension [9,13,36].

To investigate whether LASSBio-985 was also inducing endothelium-dependent vasodilation by binding to a receptor that triggers NO production, muscarinic M3, histamine H1, bradykinin B2, opioid μ_3, and β-adrenergic receptors were blocked with pharmacological agents, but none of them was able to attenuate LASSBio-985 vasodilation, indicating that these receptors are not involved in LASSBio-985 pathways.

Endothelial cells release many other vasorelaxing and contracting factors to modulate vascular tone. One of them is the prostanoid prostacyclin, which activates AC on vascular smooth muscle cells. Subsequent cAMP production and PKA activation result in vasodilation [37]. Preincubation of normotensive rat aortic rings with the cyclooxygenase inhibitor indomethacin did not affect relaxation by LASSBio-985, showing that the production of prostanoids by endothelial cells does not affect LASSBio-985. LASSBio-985 could also be causing endothelium-dependent vasodilation through phosphorylation of eNOS (via PI3K), the enzyme responsible for NOS Ca^{2+}-independent activation. However, pretreatment with wortmannin did not inhibit the effect of LASSBio-985. All these observations suggested that LASSBio-985-induced vasodilation was not mediated by NO release through the activation of membrane receptors but reinforce the hypothesis of the involvement of PDE1. The vasodilatory effect of LASSBio-985 was also studied in SHR aortic rings, as this genetic animal model of essential hypertension shows common characteristics of human hypertension, such as endothelial dysfunction and decreased NO production [15]; it is an attractive model to study new compounds that affect the vasculature.

Our results show that even in SHR aortic rings, which normally have reduced vasodilatory responses to acetylcholine, LASSBio-985 was able to induce concentration-dependent vasodilation and that this effect was almost completely inhibited by removal of endothelium and pretreatment with L-NAME and ODQ, identical to that observed in the normotensive rat aorta. Moreover, LASSBio-985 effects were greater in SHR endothelium-denuded aortic rings.

As SHR has increased Ca^{2+}-channel activity in vascular smooth muscle [38–40], the LASSBio-985 effect was investigated on KCl-contracted aortic rings. High K^+ induces membrane depolarization, which, in turn, opens voltage-sensitive L-type Ca^{2+} channels, leading to increased intracellular concentrations of Ca^{2+} and subsequent contraction [41]. LASSBio-985 showed greater vasodilation on KCl-contracted aorta. Also, its effect was blocked in phenylephrine-contracted denuded aorta that had been pretreated with nicardipine, a blocker of L-type Ca^{2+} channels. These results suggest that the vasodilation induced by LASSBio-985 involves, in part, inhibition of extracellular Ca^{2+} influx. This observation was confirmed on normotensive endothelium-denuded aorta, where a residual vasorelaxant effect was observed at high concentrations and was blocked in the presence of nicardipine.

As expected for vasodilators, intravenous administration of LASSBio-985 decreased blood pressure both in WKY rats and SHR. The hemodynamic effects of LASSBio-985 were more intense and of longer duration in SHR when compared with WKY. In both strains, reduction in blood pressure was not accompanied by a significant increase in heart hate. Recent studies have proposed that PDE1 is a prominent regulator of cGMP hydrolysis in normal and failing human myocardium, where PKG acts as a brake in the heart, working against cAMP-PKA contractile stimulation [42]. Inhibition of voltage-sensitive L-type Ca^{2+} channels, as was suggested for vascular smooth muscle, could also contribute to counteract the tachycardia expected because of LASSBio-induced hypotension.

CONCLUSION

In conclusion, the present work demonstrated that LASSBio-985 has vasodilatory profile that are likely mediated through PDE1 and voltage-sensitive L-type Ca^{2+} channels inhibition, both in normotensive rats and in SHR aortic rings. Also, LASSBio-985 promoted hypotensive effect on normotensive and hypertensive rats.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

ABBREVIATIONS LIST

AC – adenylate cyclase
cAMP – cyclic adenosine 3′,5′-monophosphate
cGMP – cyclic guanosine 3′,5′-monophosphate
DMSO – dimethylsulfoxide
GC – guanylate cyclase
GTP – guanosine 5′-triphosphate
IC_{50} – 50% inhibition concentration
L-NAME – N_{ω}-nitro-L-arginine methyl ester hydrochloride
NO – nitric oxide
NOS – nitric oxide synthase
ODQ – 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one
PDE – phosphodiesterase
PI3K – phosphatidylinositol 3′-kinase
PKA – cAMP-dependent protein kinase
PKG – cGMP-dependent protein kinase
SHR – spontaneously hypertensive rat
WKY – Wistar Kyoto.

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