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Cardiovascular effects induced by *N*-(4′-dihydro)-piperoylthiomorpholine in normotensive rats

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Abstract

Objectives We have tested the cardiovascular effects of *N*-(4′-dihydro)-piperoylthiomorpholine (LASSBio 365) on rats using an in-vivo and in-vitro approach.
Methods LASSBio 365 (0.025, 0.05, 0.1, 0.25, 0.5 or 1 mg/kg, randomly injected) was administered to conscious unrestrained rats and the mean arterial pressure and heart rate were measured. The effects of LASSBio 365 (3×10^{-6}–3×10^{-4} M) on rat isolated aortic rings with and without endothelium were investigated.
Key findings LASSBio 365 induced a dose-dependent decrease in mean arterial pressure and heart rate (ED50 = 158 ± 53 μg/kg). The effects evoked by LASSBio 365 (0.5 mg/kg) were inhibited by pretreatment with atropine. In anaesthetized rats, electrocardiogram recordings revealed second/third degree sinoatrial and atrioventricular blockade induced by the compound, which were completely inhibited after cardiac muscarinic blockade or cervical bilateral vagotomy. In rat isolated aortic rings, LASSBio 365 (3×10^{-6}–3×10^{-4} M) was capable of antagonizing the contractile effects induced by phenylephrine (1 μM) or KCl (80 mM) (IC50 = 107 ± 6; 92 ± 6 μM, respectively). This effect was not inhibited after removal of the vascular endothelium (IC50 = 84 ± 4; 92 ± 10 μM, respectively). LASSBio 365 (10^{-6}–10^{-4} M) antagonized $CaCl_2$-induced contractions in a concentration-dependent manner. Furthermore, LASSBio 365 (98 μM) inhibited contractions produced by noradrenaline (1 μM), but not those induced by caffeine (20 mM).
Conclusions These results suggested that LASSBio 365 produced negative chronotropism and reduced peripheral resistance that were probably due to the stimulation of cardiac muscarinic pathways. Peripheral vasodilation was probably linked to voltage-dependent Ca^{2+}-channel blockade and/or specific inhibition of Ca^{2+} release from noradrenaline-sensitive intracellular stores.
Keywords aortic rings; blood pressure; calcium; LASSBio 365

Introduction

The genus *Piper* (Piperaceae) includes approximately two thousand species distributed in the tropical and subtropical regions of the world and they are used medicinally in various manners.[1] Reports of biological activity for this genus emphasize antitumour properties for some species, and its use for the treatment of asthma and arthritic conditions.[2,3] Various amides bearing isobutyl, pyrrolidine, dihydropyridone and piperidine moieties have been isolated from *Piper* species, and they have generated interest due to their potent insecticidal and antifungal properties.[1,4,5]

Considering the importance of these biologically active compounds and their wide occurrence in the genus *Piper*, we reported previously on the isolation, structure elucidation and pharmacological evaluation of amides obtained from *P. tuberculatum* Jacq.[6] The piperamides, piperine and piperdardine, were isolated from the stem of *P. tuberculatum*; however, due to their similar structure, we were not able to isolate them separately in sufficient amounts to perform pharmacological assays. Instead, we decided to synthesize the amides.

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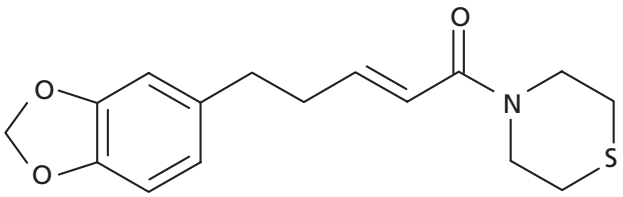

Figure 1 Chemical structure of *N*-(4′-dihydro)-piperoylthiomorpholine (LASSBio 365).

Previously, we reported the synthesis and structural modifications of the natural amides.[7] We synthesized the piperamide *N*-(4′-dihydro)-piperoylpiperidine and two new analogues, designed by replacement of the piperidinyl moiety with the isosteric morpholinyl and thiomorpholinyl units, respectively, to verify the contributions of these substituents on the structure–activity relationship of this class of compounds. In this study we have attempted to characterize and elucidate the mechanisms involved in the cardiovascular effects induced by *N*-(4′-dihydro)-piperoylthiomorpholine (LASSBio 365; Figure 1) in rats, using a combined in-vivo and in-vitro approach.

Materials and Methods

Animals

Male Wistar rats (250–300 g) were used for all experiments. Animals were housed under a controlled temperature (21 ± 1°C) and were exposed to a daily 12-h light–dark cycle. All experimental protocols were approved by the Institutional Animal Care Committee from the Pharmaceutical Technology Laboratory of the Federal University of Paraíba, Brazil.

Drugs

The drugs used were: heparin sodium salt (Roche Diagnostics, Indianapolis, IN, USA), atropine sulfate, acetylcholine chloride, N^G-nitro L-arginine methyl ester (L-NAME) and L-phenylephrine hydrochloride, ethyleneglycol *bis* (β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), caffeine, noradrenaline (norepinephrine) and sodium thiopental (all from Sigma Chemical Co., St Louis, MO, USA). LASSBio 365 was solubilized in a mixture of distilled water / Cremophor and diluted to the desired concentrations with distilled water just before use. The final concentration of Cremophor in the bath never exceeded 0.01% and showed no effect when tested in control preparations.

Measurement of arterial blood pressure in conscious nonanaesthetized rats

Rats were anaesthetized with sodium thiopental (45 mg/kg, i.p.), and polyethylene (PE) catheters were inserted into the lower abdominal aorta via the left femoral artery. Another catheter was inserted into the inferior vena cava via the left femoral vein for the administration of drugs. Both catheters were filled with heparinized saline and led under the skin to exit between the scapulae. After surgery, rats were placed in large individual cages for recordings 24 h later.

The arterial catheter was connected to a precalibrated pressure transducer (Statham P23-ID; Gould, Cleveland, OH, USA). Pressure outputs were fed to an amplifier-recorder (Model TBM-4M, WPI, Sarasota, FL, USA) and to a personal computer equipped with an analogue-to-digital converter board (CIO-DAS16/JR, Computer Boards, Inc., Mansfield, MA, USA). Using CVMS software (WPI, Sarasota, FL, USA), data were sampled at 500 Hz and stored on a CD-ROM. Beat-to-beat time series were generated and processed off-line on another personal computer. For each cardiac cycle, the computer calculated mean arterial pressure (MAP) and pulse interval (referred to as heart rate (HR)). LASSBio 365 (0.025, 0.05, 0.1, 0.25, 0.5 or 1 mg/kg, i.v.) was randomly administered. The time interval between doses was 10 min and changes in MAP and HR were recorded.

Preliminary experiments showed that a submaximal dose of LASSBio 365 (0.5 mg/kg, i.v.) showed responses of similar magnitude when administered repeatedly three to four times. After stabilization of haemodynamic parameters, LASSBio 365 was administered and the effects recorded. After 30 min atropine (2 mg/kg, i.v.) was administered and 10 min later the compound was repeated. Changes in MAP and HR induced by LASSBio 365 were compared before (baseline conditions) and after atropine treatment.

Direct blood pressure measurements and electrocardiogram recordings in anaesthetized and vagotomized rats

The animals were catheterized as previously described, maintained under anaesthesia with sodium thiopental (45 mg/kg; i.v.) under controlled conditions of body temperature through a heater blanket (35 ± 1°C) and separated into two groups. An intra-tracheal probe coupled to an artificial ventilator (Rodent Ventilator, UGO BASILE, VA-Italy) was put in place. The first group was sham operated, while on the second group we performed a bilateral cervical vagotomy (VGX), 2 min before starting the LASSBio 365 administration. The ECG was recorded, using DII derivation, through subcutaneous electrodes implanted in superior and inferior members of the animals, to evaluate electrical cardiac activity changes induced by LASSBio 365. Immediately after the surgical procedure and cardiovascular parameters had stabilized, LASSBio 365 (0.5 mg/kg, i.v.) was administered and MAP, HR and ECG alterations were recorded. Similar recordings were obtained in rats after VGX or after treatment of the rats with atropine (2 mg/kg, i.v., 15 min).

Studies using rat isolated aortic rings

The thoracic aorta was removed and cleaned from connective tissue and fat. Rings (2–4 mm) were obtained and suspended by platinum hooks for isometric tension recording in Kreb's Henseleit solution (pH 7.4) maintained at 37°C and gassed with 95% O_2 and 5% CO_2. The composition of Kreb's Henseleit solution (in mM) was: NaCl 118.0; KCl 4.7; $NaHCO_3$ 25.00; $CaCl_2.2H_2O$ 2.5; glucose 11.1; KH_2PO_4 1.2 and $MgSO_4.7H_2O$ 1.2. The rings were allowed to equilibrate for 1 h under a resting tension of 1 g. During this time, the bathing medium was changed every 15 min to protect against interfering metabolites.[8] Rings without endothelium were obtained by

gently rubbing the intimal surface of the vessel. LASSBio 365 (3×10^{-6}–3×10^{-4} M) was cumulatively applied after the contractile response induced by phenylephrine (1 μM) or KCl (80 mM). Inhibition was calculated by comparing the response of the agonists before and after the addition of the inhibitor or antagonist. The IC50 (concentration required for 50% inhibition) values were calculated by nonlinear regression of individual concentration–response curves. The presence of functional endothelium was assessed by the ability of acetylcholine (1 μM) to induce more than 85% relaxation of vessels precontracted with phenylephrine (1 μM). The absence of relaxation by acetylcholine was taken as evidence that the vessel segments were functionally denuded of endothelium.

The effects of LASSBio 365 on voltage-dependent Ca^{2+}-channels were studied in aortic rings in which functional endothelium was mechanically removed. KCl 50 mM-induced sustained contractions were obtained and the tissues were then washed. In Krebs nominally without Ca^{2+} depolarized (KCl 50 mM) solution, two cumulative concentration–response curves to $CaCl_2$ (5×10^{-7}–10^{-2} M) were obtained, and 30 min before the third $CaCl_2$ curve, LASSBio 365 (10^{-6}–10^{-4} M) was added to the preparations. The maximal contraction obtained with the control concentration–response curves for $CaCl_2$ was taken as 100% and all values were calculated as percentage of the maximal response. Each preparation was exposed to only one LASSBio 365 concentration.

Finally we verified the influence of LASSBio 365 on noradrenaline and caffeine-sensitive intracellular calcium stores.[9] We chose 98 μM LASSBio 365, since this concentration was not significantly different from the IC50 value. After the stabilization period, the tissues were exposed to a 65.4 mM K^+ solution for 3 min. The tissues were then washed with Ca^{2+}-free solution over 2 min followed by the addition of 1 μM noradrenaline or 20 mM caffeine. After washing the tissues with normal Krebs solution, a high potassium solution (KCl, 65.4 mM) was added for 3 min (Ca^{2+} loading period). The preparations were washed with Ca^{2+}-free solution over 2 min, followed again by administration of noradrenaline or caffeine. This procedure was repeated until two similar transient contractions to the agonists had been obtained. The experiment was then repeated with LASSBio 365 being added 2 min before the administration of noradrenaline or caffeine.

Data analysis

Except when otherwise specified, values are expressed as the mean ± SEM. Statistical analysis was performed by means of paired Student's *t*-tests and two-way analysis of variance. Linear regressions were performed by the least square method, using GraphPad Prism software, version 3.02 (GraphPad Software, Inc.).

Results

Effects of LASSBio 365 on MAP and HR in conscious nonanaesthetized rats

In conscious unrestrained rats, LASSBio 365 (0.025, 0.05. 0.1, 0.25, 0.5 or 1 mg/kg, i.v., randomly injected) produced dose-related decreases in MAP (ED50 = 158 ± 53 μg/kg) and HR (Table 1). Furthermore, MAP and HR values obtained with 1 and 0.5 mg/kg (i.v.) LASSBio 365 were not significantly different from each other.

Table 1 Changes in mean arterial pressure and heart rate obtained after LASSBio 365 administration in nonanaesthetized rats

LASSBio 365 (mg/kg, i.v.)	Δ MAP (mmHg)	Δ HR (beats/min)
0.025	−9 ± 1	−20 ± 12
0.05	−27 ± 3	−60 ± 29
0.1	−38 ± 2	−66 ± 10
0.25	−56 ± 5	−235 ± 24
0.5	−64 ± 6	−250 ± 21
1.0	−64 ± 5	−280 ± 17

MAP, mean arterial pressure; HR, heart rate. The values are means ± SEM ($n = 7$).

Effect of a bilateral cervical vagotomy on LASSBio 365-induced response in anaesthetized rats

Anaesthesia with sodium thiopental did not affect the LASSBio 365-induced responses when compared with those induced in nonanaesthetized rats (see values for LASSBio 365, 0.5 mg/kg, i.v., in Tables 1 and 2). Bilateral cervical vagotomy was capable of completely attenuating the decrease in MAP, while the decrease in HR was strongly reduced (Table 2).

Effects of LASSBio 365 on ECG recordings in anaesthetized rats

Analysis of the ECG recordings in pentobarbital-anaesthetized rats revealed sinoatrial (SA) and atrioventricular (AV) blockade after the injection of LASSBio 365 (0.5 mg/kg, i.v.). A third-degree AV block occurred more frequently in response to LASSBio 365. The effects on the ECG, MAP and HR induced by LASSBio 365 were abolished in rats in which we performed a cervical bilateral vagotomy or in rats pretreated with 2 mg/kg (i.v.) atropine (Table 3).

Effects of LASSBio 365 on aortic rings

As illustrated in Figure 2, LASSBio 365 at 3×10^{-6}–3×10^{-4} M antagonized in a significant ($P < 0.05$) and concentration-dependent manner the phenylephrine-induced contractions of the aortic rings (IC50 = 107 ± 6 μM, $n = 6$). Experiments demonstrated that the response induced by LASSBio 365 was not endothelium-dependent, since after removal of aortic endothelial cells the relaxant response induced by increasing concentrations of LASSBio 365 remained unchanged when compared with control values.

Addition of LASSBio 365 (10^{-6}–10^{-4} M) induced an inhibition of the concentration–response curves to $CaCl_2$ in aortic rings (Figure 3). As illustrated in Figure 4, in the Ca^{2+}-free solution, LASSBio 365 (98 μM) inhibited the noradrenaline (1 μM)-induced contraction in a concentration-dependent manner and did not modify the phasic contractile response evoked by caffeine (20 mM).

Discussion

The major finding of this work was that LASSBio 365 produced a dose-related decrease in MAP followed by a

Table 2 Effect of LASSBio 365 on changes in mean arterial pressure and heart rate in sham operated and bilateral cervical vagotomy rats before and after atropine

Parameter	Sham operated	After VGX	After VGX + atropine
Δ MAP (mmHg)	−64 ± 6	−7 ± 1*	−12 ± 2*
Δ HR (beats min^{-1})	−250 ± 17	−88 ± 15*	−51 ± 10*

LASSBio 365 0.5 mg/kg (i.v.); atropine 2 mg/kg (i.v.). MAP, mean arterial pressure; HR, heart rate; VGX, bilateral cervical vagotomy. The values are means ± SEM ($n = 7$). Analysis of variance followed by Bonferroni's multiple comparison test, *$P < 0.05$ vs sham.

Table 3 Effects of LASSBio 365 on the cardiac electrical activity, before (control) and after cervical bilateral vagotomy or muscarinic blockade in anaesthetised rats

Animal number	Control		After muscarinic blockade or cervical bilateral vagotomy	
	SA block	AV block	SA block	AV block
1	–	++	–	–
2	+	++	–	–
3	+	++	–	–
4	+	++	–	–
5	+	++	–	–
6	–	+	–	–
7	+	++	–	–
8	–	+	–	–

LASSBio 365 0.5 mg/kg (i.v.). Control, $n = 8$. Muscarinic blockade obtained using atropine 2 mg/kg (i.v.), $n = 8$. +, Sinoatrial (SA) blockade and first-degree atrioventricular (AV) blockade. ++, Second/third-degree AV blockade. –, absence of effect.

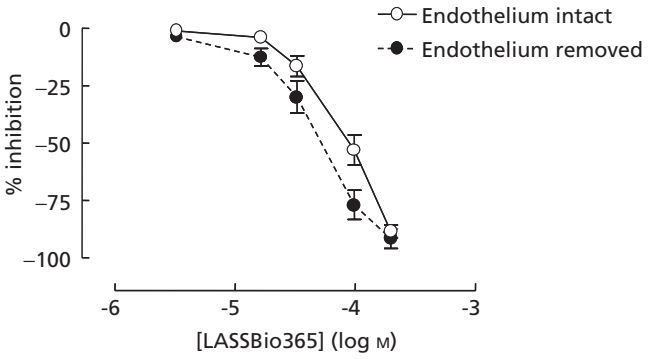

Figure 2 Line plot graph showing the effects of LASSBio 365 on rat isolated aortic rings. LASSBio 365 was used at $3 \times 10^{-6} - 3 \times 10^{-4}$ M. The aortic rings were precontracted with phenylephrine 1 μM ($n = 8$), and were either with intact endothelium or the endothelium had been mechanically removed. Symbols are mean ± SEM.

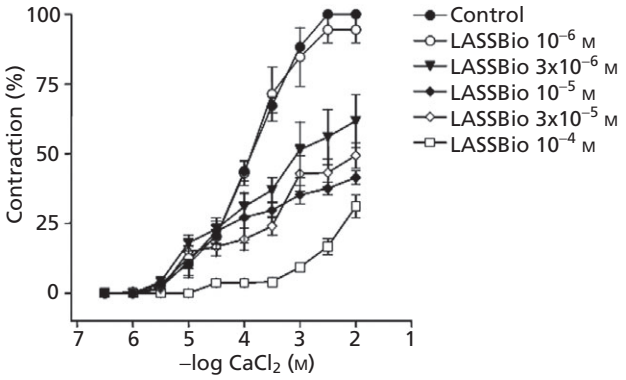

Figure 3 Line plot graph showing the effects of increasing concentrations of LASSBio 365 on concentration–response curves to $CaCl_2$ in medium nominally without Ca^{2+} in rat isolated aortic rings. LASSBio 365 was used at 10^{-6}, 3×10^{-6}, 10^{-5}, 3×10^{-5} and 10^{-4} M. Values are mean ± SEM ($n = 6$).

significant decrease in HR, probably due to a direct cardiac depressant effect and to a decrease in the total peripheral vascular resistances.

It is well established that the primary autonomic regulation of the sinoatrial node function is by vagal action via stimulation of cardiac muscarinic receptors.[10] The stimulation of these receptors induced intense bradycardia followed by hypotension due to the decrease of the cardiac output. These receptors were predominantly M_2 subtype, as confirmed by the localization of M_2 mRNA in the rat heart by in-situ hybridization.[11,12] Although the expression of M_1, M_3 and M_4 subtypes of muscarinic receptor genes in mammalian heart has been reported, they have not yet been associated with any functional response in the atria.[13–16] To evaluate the role of these receptors in the LASSBio 365-induced responses, we performed experiments in the presence of atropine, a nonselective antagonist of muscarinic receptors. In these conditions, both the decrease in MAP and decrease in HR were significantly attenuated and almost completely abolished. Thus, we can suggest that LASSBio 365 would be acting either directly via these receptors or indirectly via vagal activation.

Another explanation for the effects presented by LASSBio 365 could be due to the stimulation of the parasympathetic efferent pathways (vagal) with subsequent acetylcholine release and stimulation of muscarinic receptors.[17] To confirm the participation of the vagus nerve pathway in this effect, we used anaesthetized cervical bilateral vagotomized rats. It is

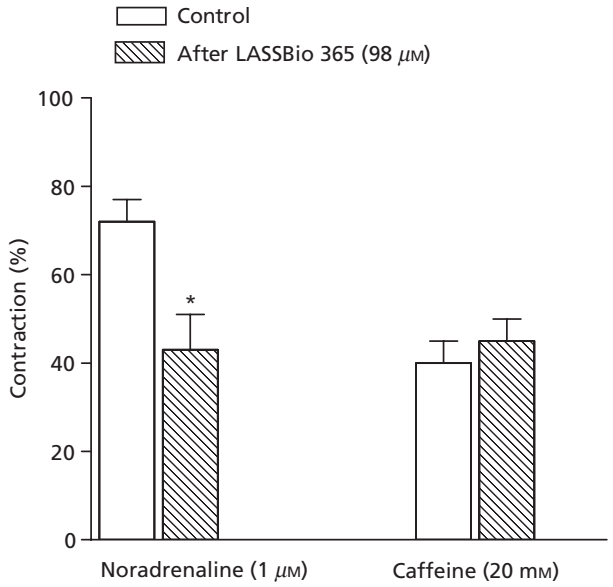

Figure 4 Bar graph showing the effects of LASSBio 365 on noradrenaline or caffeine-induced transient contractions in Ca^{2+}-free media in rat isolated aortic rings. LASSBio 365 98 μM; noradrenaline 1 μM; caffeine 20 mM. Values are mean ± SEM. $*P < 0.05$ compared with control.

important to emphasize that the anaesthesia with sodium thiopental did not affect the LASSBio 365-induced response. In bivagotomized rats, decreases in MAP and HR induced by LASSBio 365 were significantly attenuated, suggesting that the compound probably induced a vagal stimulation. In addition, in these anaesthetized rats we found that LASSBio 365 induced sinoatrial blockade, which was completely abolished by atropine. Taken together, these results suggested that vagal parasympathetic pathways were probably implicated in the cardiac depressant activity induced by LASSBio 365.

The negative chronotropic effect induced by LASSBio 365 was still observed after VGX plus atropine. Calcium channel antagonists, such as verapamil, are known to cause AV block with negative chronotropism and inotropic effect.[18] The negative chronotropic effect of LASSBio 365 may be also the outcome of calcium channel blockade in the cardiac muscle.

The intravenous injection of a small dose of acetylcholine produces a fall in blood pressure due to generalized vasodilatation. Since LASSBio 365 produced a decrease in MAP and HR, we hypothesized an additional relaxant activity of the compound on vascular smooth muscle cells, which was verified using rat isolated aortic rings. In these preparations, LASSBio 365 antagonized the phenylephrine-induced contractions in a concentration-dependent manner. In vascular beds, the stimulation of muscarinic (M_3 subtype) receptors produces an intense dilation, despite the lack of apparent cholinergic innervation of most blood vessels.[19] The muscarinic receptors responsible for relaxation are located on the endothelial cells of the vasculature, and when they are stimulated the endothelial cells release endothelium-derived relaxing factors, mainly nitric oxide (NO), which diffuses to adjacent smooth muscle cells and causes them to relax.[20–22] To determine whether part of the relaxant effect produced by LASSBio 365 in isolated aortic rings could be due to stimulation of endothelial M_3-receptors and consequently the release of NO, we performed experiments in aortic preparations in which the endothelium was mechanically removed.[23] The relaxant effect evoked by LASSBio 365 was not inhibited by removal of the vascular endothelial tissue, indicating that the endothelium did not take part in the smooth muscle relaxant response produced by LASSBio 365.

It is well known that the maintenance of smooth muscle contraction depends upon Ca^{2+} entry from extracellular space through voltage- and/or receptor-operated Ca^{2+} channels.[24–26] It is well reported that an increase of external K^+ concentration (KCl 80 mM) induces smooth muscle contraction through activation of voltage-dependent Ca^{2+} channels and subsequent release of calcium from the sarcoplasmic reticulum, without changing other signal transduction systems including phosphatidylinositol turnover and calcium sensitization.[27,28] LASSBio 365 antagonized in a concentration-dependent manner the contractions induced by both KCl or phenylephrine. KCl elicits contraction by allowing the influx of extracellular Ca^{2+} through voltage-dependent (L- and T-type) Ca^{2+} channels. The membrane potential is essentially clamped by the high K^+ solution, and so the mechanism by which relaxation can be produced is through the blockade of voltage-dependent Ca^{2+} channels, but not by mediators that cause hyperpolarization. Table 4 shows that LASSBio 365 was virtually equieffective against each of the contraction agents used, as can be seen by comparing the IC50 values. That LASSBio 365 relaxed arterial segments precontracted by KCl depolarization therefore suggested that LASSBio 365 blocked Ca^{2+} entry through voltage-dependent Ca^{2+} channels. This is further supported by our findings that LASSBio 365 inhibited, in a concentration-dependent manner, the $CaCl_2$-induced contractions in a depolarizing medium nominally without Ca^{2+}. The antagonism of LASSBio 365 could be reversed after repeated washings. LASSBio 365 seemed to block voltage-operated Ca^{2+} channels, as the contractions induced by potassium were inhibited in a concentration-dependent manner by the compound.

The activation of phosphoinositide turnover by G protein coupling of phospholipase C and α-adrenergic receptors is crucial for the cytoplasmic calcium increase involved in the contraction induced by activation of these receptors and involves calcium from intracellular stores.[29] LASSBio 365 inhibited phenylephrine-induced contractions. This led us to investigate if the compound could exert its nonspecific spasmolytic actions by interfering with the calcium release induced by phosphoinositide production following receptor activation. In this respect we investigated the effect of LASSBio 365 on noradrenaline- and caffeine-induced transient contractions in Ca^{2+}-free media. LASSBio 365 (98 μM) selectively inhibited transient contractions induced by noradrenaline, but not those induced by caffeine. It has been suggested that the noradrenaline-induced release of calcium is attributable to receptor-mediated formation of inositol 1, 4, 5-trisphosphate whereas the caffeine-induced calcium release is due to Ca^{2+}-induced Ca^{2+}-release mechanism.[22] Thus, LASSBio 365 may have relatively selectively inhibited the Ca^{2+} release due to inositol 1,4, 5-trisphosphate in rat aorta.

Furthermore, the fact that the same concentration of LASSBio 365 that inhibited noradrenaline-induced contractions did not inhibit caffeine-induced contractions suggested

Table 4 IC50 values for the relaxant effects of LASSBio 365 in rat aortic rings in different experimental conditions

Agonist	Endothelium intact IC50 (μM)	Endothelium removed IC50 (μM)	r^2
Phenylephrine (1 μM)	107 ± 6	84 ± 10	0.96 ± 0.01
KCl (80 mM)	92 ± 6	92 ± 4	0.91 ± 0.02

r^2, correlation coefficient. IC50, concentration required for 50% inhibition. $n = 8$.

that the inhibitory effect of LASSBio 365 on noradrenaline could not be attributable to direct inhibition of the smooth muscle machinery contractile elements. Thus, LASSBio 365 may have relatively selectively inhibited the Ca^{2+} release due to 1,4,5-trisphosphate activation in rat aorta. Clinically, Ca^{2+} antagonists are used for the treatment of hypertension due to their ability to induce smooth muscle relaxation. Verapamil, the agent blocking the Ca^{2+} channels, inhibited the contraction induced by phenylephrine and KCl, as well as the increase in $[Ca^{2+}]$.[29–31] As in our results, LASSBio 365 also inhibited the contraction induced by phenylephrine and KCl.

Nevertheless, the influence of LASSBio 365 on another step of the event cascade that led to smooth muscle contraction could not be ruled out in this study and further studies are required to confirm the direct relationship between the actions of LASSBio 365 and intracellular Ca^{2+} levels in smooth muscle.

Conclusions

This study, using a combined approach (in-vivo and in-vitro experiments), demonstrated that LASSBio 365 markedly lowered arterial pressure and HR in conscious unrestrained or anaesthetized animals. Whatever could be the underlying additional mechanisms, the results shown here suggested that the hypotensive action of the compound could be a consequence of both a decrease in HR and peripheral vascular resistance. Decrease in HR was probably due to both direct and indirect muscarinic receptor stimulation, a consequence of the vagal parasympathetic stimulating pathway.

The results obtained so far indicated that LASSBio 365 caused relaxation of vascular smooth muscle, probably as a consequence of the inhibition of calcium entry to smooth muscle cells by voltage-dependent Ca^{2+} channels and an interference with the mobilization of intracellular calcium from noradrenaline-sensitive stores, which seemed to be the main mechanisms of its nonspecific spasmolytic action.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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