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Original article

Cryptostrobin and catechin isolated from *Eugenia mattosii* D. Legrand leaves induce endothelium-dependent and independent relaxation in spontaneously hypertensive rat aorta

Giovana Vechi, Rita de Cássia Melo Vilhena de Andrade Fonseca da Silva*, Priscila de Souza, Luísa Mota da Silva, Sérgio Faloni de Andrade, Valdir Cechinel Filho

Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade do Vale do Itajaí (UNIVALI), Itajaí, SC, Brazil

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ABSTRACT

Background: Considering the therapeutic potential of phenolic compounds, the purpose of the present study was to investigate the mechanisms involved in the relaxation induced by cryptostrobin and catechin, isolated from *Eugenia mattosii* D. Legrand leaves, in the aorta of spontaneously hypertensive rats (SHR).

Methods: The thoracic aorta was isolated from SHR and kept in the organ bath system by recording contractile or relaxant responses.

Results: The addition of cumulative concentrations of cryptostrobin and catechin induced endothelium-dependent and-independent relaxation in aorta rings from SHR, as well as both compounds were effective in reducing phenylephrine-induced contraction. Pretreatment of aortic rings with N_{ω}-nitro-L-arginine methylester (L-NAME, an inhibitor of nitric oxide synthase) or 1H-[1,2,4] oxadiazolo[4,3-a] quinoxalin-1-one (ODQ, an inhibitor of soluble guanylate cyclase), resulted in a significant change of relaxant effect induced by catechin, and a slight influence on cryptostrobin-induced relaxation. Muscarinic receptor and potassium channels are involved in catechin-induced relaxation as assessed using atropine (a muscarinic receptor antagonist), tetraethylammonium (a non-selective K^+ channel blocker) and glibenclamide (an ATP-sensitive K^+ channel blocker). Conversely, cryptostrobin, but not catechin, blunted the contraction induced by the addition of phenylephrine in a calcium-free solution. Besides that, cryptostrobin attenuated the contraction of rat aorta rings induced by internal Ca^{2+} release and external Ca^{2+} influx.

Conclusions: These findings indicated that cryptostrobin and catechin alter vascular smooth muscle reactivity, and this effect may be involved, at least in part, by enhancing the endothelium NO/cGMP pathway and potassium channels activation. In addition, cryptostrobin reduced the phenylephrine, KCl and $CaCl_2$-induced contractions in a calcium-free solution.

Introduction

Natural products have been used for thousands of years. Despite this practice has declined in the middle of the last century, in the past few years new attention has been taken to natural products for the development of new therapies to treat many human diseases [1,2]. Natural products provide unique structural diversity in comparison to standard combination chemistry, being one opportunity for the discovery of new molecules with biological activity or molecules susceptible to modifications. Furthermore, only 10% of the world biodiversity has been evaluated for their potential biological activity. Therefore, this natural chemical diversity waits to be accessed [3]. The projects based on natural products are predominantly being studied for treating cancer, anti-infective and in other important areas such as cardiovascular and metabolic diseases [4]. Of these, stand out the preparations based on vegetal species, of which polyphenolics compounds are found in abundant concentrations in a variety of fruits, vegetables, and plant-based beverages [5].

Hypertension is one of the most contributors to the development of cardiovascular events and is one of the main causes of morbidity and mortality around the world [6]. The pathogenesis of

* Corresponding author.
E-mail address: ritacvilhena@hotmail.com (R.d.C.M.V.d.A.F. da Silva).

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hypertension involves many factors, as well as, many other diseases, so a single treatment for a single target often fails to achieve the desired effect. Indeed, patients with hypertension require life-long medication, and although there is a great variety of antihypertensive drugs to reduce blood pressure, the discovery of new agents with higher efficacy and lower toxicity leads the search for new compounds with antihypertensive activity [7]. In hypertension, endogenous nitric oxide (NO) bioavailability is impaired, which compromises the vasodilatation. Indeed, the vascular relaxation is very important for the treatment of hypertension, because of that there is a great interest in discovering compounds that are able to stimulate the synthesis or the release of NO in the biological system [8]. Besides that, inhibition of vascular smooth muscle contractile pathway, in which calcium is a key factor in vasoconstriction [9], is crucial to induce and/or to keep vasorelaxation and consequently contribute to the reduction of blood pressure levels.

Among the natural bioactive, polyphenols are compounds that show a therapeutic effect on the vascular system [10–12]. Polyphenols are secondary metabolites of plants and are usually synthesized as defense mechanisms against stressor as pathogens, and include a thousand structural variants, from simple molecules to highly polymerized compounds [13]. It has been observed that consumption of polyphenols can improve endothelium dysfunction and increases artery dilatation [14,15], and as a consequence reduce blood pressure, an effect that may be due to the improvement of endothelium function and antioxidant activity [16].

Studying biochemical mechanisms underlying vascular dysfunction as well as biological effects of polyphenols may contribute to reveal new therapeutic strategies for the treatment of cardiovascular diseases.The present study therefore aimed to evaluate the effect and the mechanisms involved in the relaxation induced by cryptostrobin and catechin on isolated aorta from spontaneously hypertensive rats (SHR), which is considered a standard animal model of human essential or primary hypertension and frequently used to study cardiovascular diseases [17,18].

Experimental design

Obtainment of cryptostrobin and catechin

The leaves from *Eugenia mattosii* D. Legrand were collected in Itajaí-SC, Brazil (coordinates 26° 94′12″ S; 48° 69′08″ W). The plant material was identified by Prof. Oscar Iza (Universidade do Vale do Itajaí, UNIVALI) and a voucher specimen was deposited at the Barbosa Rodrigues Herbarium under number VCFilho 150, in Itajaí-SC, Brazil.

Fresh leaves (560 g) were minced and subjected to a maceration process with methanol at room temperature for seven days. After filtration, the solvent was removed under reduced pressure to obtain the methanolic extract from leaves of *E. mattosii* (15.34%). Methanolic extract from leaves has been fractioned by chromatography column (CC) allowing the isolation of two compounds identified as cryptostrobin (84 mg) and catechin (19 mg), by direct comparison with authentic samples by Thin-Layer Chromatography (TLC), and Nuclear Magnetic Resonance (NMR) as previously described by Vechi et al. [19].

Animals

The experiments were conducted in male, 28–32 weeks old spontaneously hypertensive rats (SHR). The animals were provided by UNIVALI and kept under conditions of constant temperature (22 ± 2 °C) with a 12 h light/12 h dark cycle with free access to water and food. The studies and all methodologies used were approved by the Ethical Committee for the Care and the Use of animals of UNIVALI (authorization nº055/17p), which adopted all the recommendations of the Guide for the Care and Use of Laboratory Animals.

Drugs and reagents

Phenylephrine hydrochloride (PE), acetylcholine chloride (ACh), N^{ω}-nitro-L-arginine methylester (L-NAME), 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one (ODQ),atropine, tetraethylammonium (TEA), glibenclamide and nifedipine were purchased from Sigma (St. Louis, MO, USA). Stock solutions of glibenclamide and ODQ were dissolved in dimethyl sulfoxide (DMSO). All drugs were freshly prepared in physiological Krebs solution (PSS), with the following composition in mM: NaCl 115.3, KCl 4.9, $CaCl_2{\cdot}2H_2O$ 1.46, KH_2PO_4 1.2, $MgSO_4$ 1.2, D-glucose 11.1, $NaHCO_3$ 25 and pH 7.4.

Statistical analysis

The results show the mean ± SEM of n = 6. The data obtained from different experiments were analyzed by two-way ANOVA followed by Bonferroni's t-test, or Student t-test, when applicable. In vascular reactivity studies, the EC_{50} (half-maximum contractile response) determinations were performed using the non-linear regression method of least squares [20] using GraphPad Prism version 6.0. A *p*-value less than 0.05 was considered statistically significant. The graphs were drawn, and the statistical analyses were performed using GraphPad Prism version 6.0 for Windows (GraphPad Software, La Jolla, CA, USA).

Preparation of rat thoracic aorta rings

The isolation of the thoracic aorta and protocols were conducted as previously described [21]. Briefly, thoracic aorta was isolated, cleaned of connective tissues and cut into rings (3–4 mm length). In some preparations, the endothelium layer was mechanically removed by gently rubbing the luminal surface. The aorta rings were then kept in organ baths containing 3 mL of Krebs solution, under a resting tension of 1 g, maintained at 37 °C and continuously aerated with 95% O_2 and 5% CO_2. Following the equilibration period, rings were contracted with KCl (60 mM) for 10 min to test their responsiveness. To confirm the presence of functional-endothelium, each ring was contracted with PE (an α1-adrenoreceptor agonist, 1 μM) and, on the maximal contraction, the preparation was exposed to ACh (a muscarinic agonist receptor; 1 μM). Only rings that presented more than 80% in relaxation were considered with functional endothelium.

Experimental protocol

Effect of cryptostrobin and catechin on vascular reactivity

To investigate whether cryptostrobin and catechin can induce relaxation on aorta rings, the preparations were pre-contracted with PE (1 μM), and on the tonic phase of contraction, cumulative concentrations of cryptostrobin and catechin (0.1–300 μg/mL) were added to the bath.

Effect of cryptostrobin and catechin on PE(α1-adrenoreceptor agonist)-induced contractile response

To study the effect of cryptostrobin and catechin on PE-induced contractions, preparations with functional endothelium were pre-incubated with cryptostrobin and catechin (300 μg/mL) 30 min before cumulative concentration-response curve with PE (1 nM-10 μM) be added to the baths.

Effects of NO/cGMP pathway inhibition, muscarinic receptor and K^+ channel blockade in the relaxation induced by cryptostrobin and catechin

Cumulative concentration curve were performed by cryptostrobin and catechin (0.1–300 μg/mL), in the presence and in the absence of a non-selective inhibitor of nitric oxide synthase (L-NAME; 100 μM), or an inhibitor of the soluble guanylate cyclase (ODQ; 10 μM), or a muscarinic receptor antagonist (atropine; 1 μM), which were added 30 min prior the PE (1 μM)-induced contraction. To evaluate if K^+ channels were involved in the vasorelaxation induced by cryptostrobin and catechin, a non-selective K^+ channel blocker (TEA; 10 mM), or an ATP-sensitive K^+ channel blocker (glibenclamide; 10 μM) were added to the baths 30 min prior the PE (1 μM) induced contraction. On the tonic phase of PE-induced contraction, cumulative concentrations of cryptostrobin and catechin (0.1–300 μg/mL) were added to the bath.

Effects of cryptostrobin on the contraction induced by intracellular calcium release and extracellular calcium influx

First, to study intracellular calcium evaluation, the rings were washed several times with calcium-free Krebs solution and, in the presence of cryptostrobin (300 μg/mL), it was induced a single contraction with PE (1 μM) or KCl (60 mM).

Second, aorta ring was washed with calcium-free Krebs solution, containing ethylenediaminetetraacetic acid (EDTA) (~ 50 mM), which acts as a chelating agent. PE (1 μM) was successively added until no further contraction was triggered. The rings were washed several times with calcium-free Krebs solution and different preparations were then incubated with cryptostrobin (300 μg/mL), nifedipine (1 μM) or vehicle for at least 30 min. Thereafter, 2 approaches were performed: A new PE-induced contraction was obtained and 5 min later, calcium chloride ($CaCl_2$; 10 μM-100 mM) was added to the baths; or a high K^+ (60 mM)-induced contraction was obtained and 5 min later calcium chloride ($CaCl_2$; 10 μM-100 mM) was added to the baths.

Results

Endothelium preserved (E+) and denuded (E-) relaxation induced by catechin and cryptostrobin in SHR aorta

Compounds, catechin and cryptostrobin induced a significantly concentration-dependent vasodilator effect on E + and E- aorta rings of SHR (Figs. 1A and B, respectively), with E_{max} values of 79.87 ± 7.9% and 88.95 ± 8.6% respectively for catechin-induced E + and E- relaxation; and values of 81.66 ± 7.7% and 72.37 ± 6.6% respectively for cryptostrobin-induced E + and E- relaxation.

Effect of cryptostrobin and catechin on PE-induced aorta contraction

The maximal contractile effect induced by PE was not altered by catechin but the EC_{50} was increased from 0.00234 μM (0.000175 to

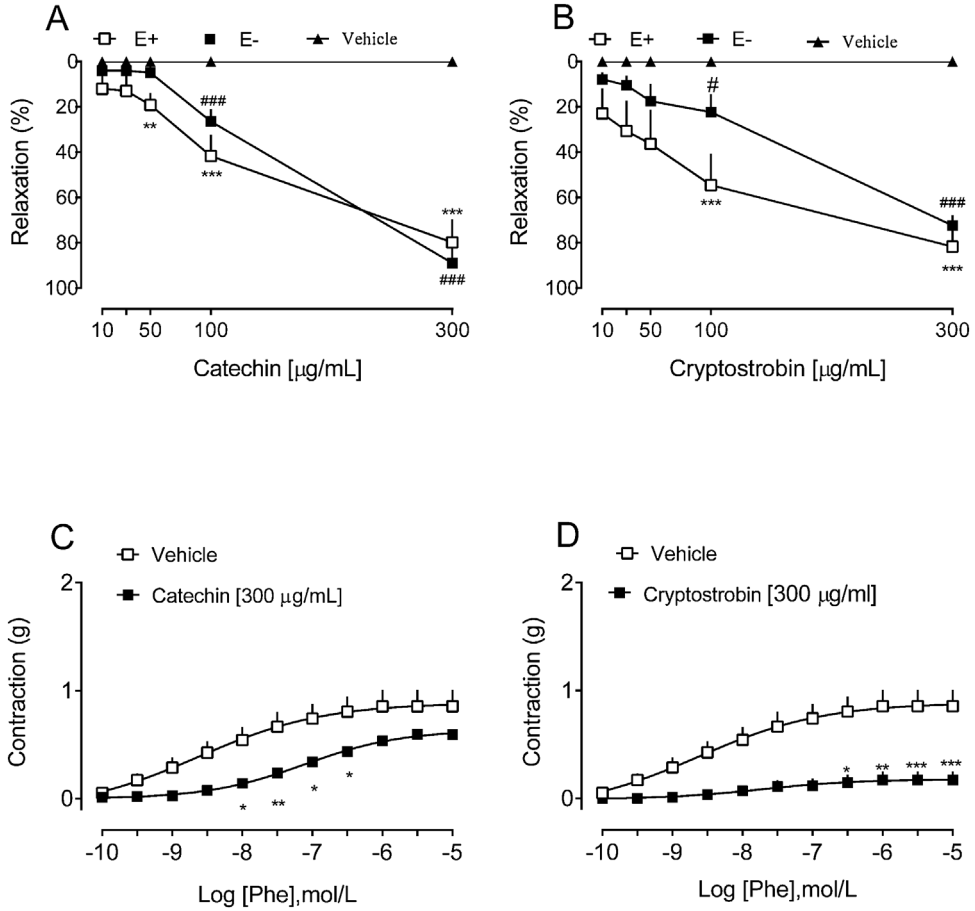

Fig. 1. Relaxation and effect on contractile responses of rat aortic rings induced by phenylephrine (PE) of catechin and cryptostrobin. Responses obtained in endothelium intact, indicated by E+, and endothelium denuded by E-. Cumulative concentrations of (A) catechin or (B) cryptostrobin were added in PE-contracted rat aortic rings. Concentration-responses curves to PE in the absence or after incubation of (C) catechin or (D) cryptostrobin. The results show the mean ± SEM of n = 6. The data obtained from different experiments were analyzed by two-way ANOVA, followed by Bonferroni's *post-hoc* test. * and # indicate $p < 0.05$, ** and ## $p < 0.01$ and *** and ### $p < 0.001$ when compared to vehicle group.

0.0313 μM) in vehicle to 0.0773 μM (0.0411 to 0.145 μM) in catechin-exposed ring. Pre-incubation with cryptostrobin significantly reduced the maximal contractile effect (E_{max} 0.63 ± 0.04 g against E_{max}1.25 ± 0.05 g in the vehicle-treated aorta) induced by PE (Figs. 1C and D, respectively).

Contribution of NO/GMPc pathway in the relaxation induced by cryptostrobin and catechin

As we can see in Figs. 2B and D, in the aorta rings previously treated with L-NAME and ODQ, the relaxation induced by catechin was totally blocked. On the other hand, in the presence of L-NAME and ODQ the relaxation induced by cryptostrobin was reduced about 50 and 35%, respectively (Figs. 2A and C).

Involvement of muscarinic receptors and potassium channels in the relaxation induced by cryptostrobin and catechin

The presence of atropine reduced about 25% of the relaxation induced by cryptostrobin (90.1 ± 5.8–69.5 ± 5.1%), and 51% the relaxation induced by catechin (84.3 ± 7.6–41 ± 4.8%) (Figs. 3A and B). The non-selective potassium channels blocker (TEA) had no significant effect on the relaxation induced by cryptostrobin (Fig. 3C). However, TEA and glibenclamide pretreatment significantly altered the relaxation induced by catechin (about 53 and 39%, respectively) (Figs. 3D and E).

Effects of cryptostrobin on the contraction induced by intracellular calcium release and extracellular calcium influx

As shown in Fig. 4A, in the calcium-free Krebs, the presence of cryptostrobin attenuated PE-induced contraction (0.53 ± 0.04 to 0.22 ± 0.03 g). The contraction induced by PE is due to the activation of α_1-adrenoreceptor with the release of intracellular Ca^{2+}, besides activating calcium channels on the cellular membrane. In addition, the contractile response induced by cumulative addition of $CaCl_2$ (Fig. 4C) was also reduced in the presence of cryptostrobin (E_{max} 0.91 ± 0.03 to 0.64 ± 0.01 g). In Fig. 4B, the response to KCl-induced contraction was also blunted in the presence of cryptostrobin in calcium-free Krebs solution. The contraction induced by extracellular calcium mobilization ($CaCl_2$) on the contraction induced by KCl was also reduced by the presence of cryptostrobin (E_{max} 1.45 ± 0.05 to 0.71 ± 0.03 g) (Fig. 4D).

Discussion

The present study shows that cryptostrobin and catechin have a concentration-dependent vasorelaxant effect in spontaneous hypertensive rats (SHR) aorta rings, by both endothelium-dependent and independent manner. Considering the high prevalence hypertension nowadays, its high mortality index and a modifiable risk factor for cardiovascular disease, studies that

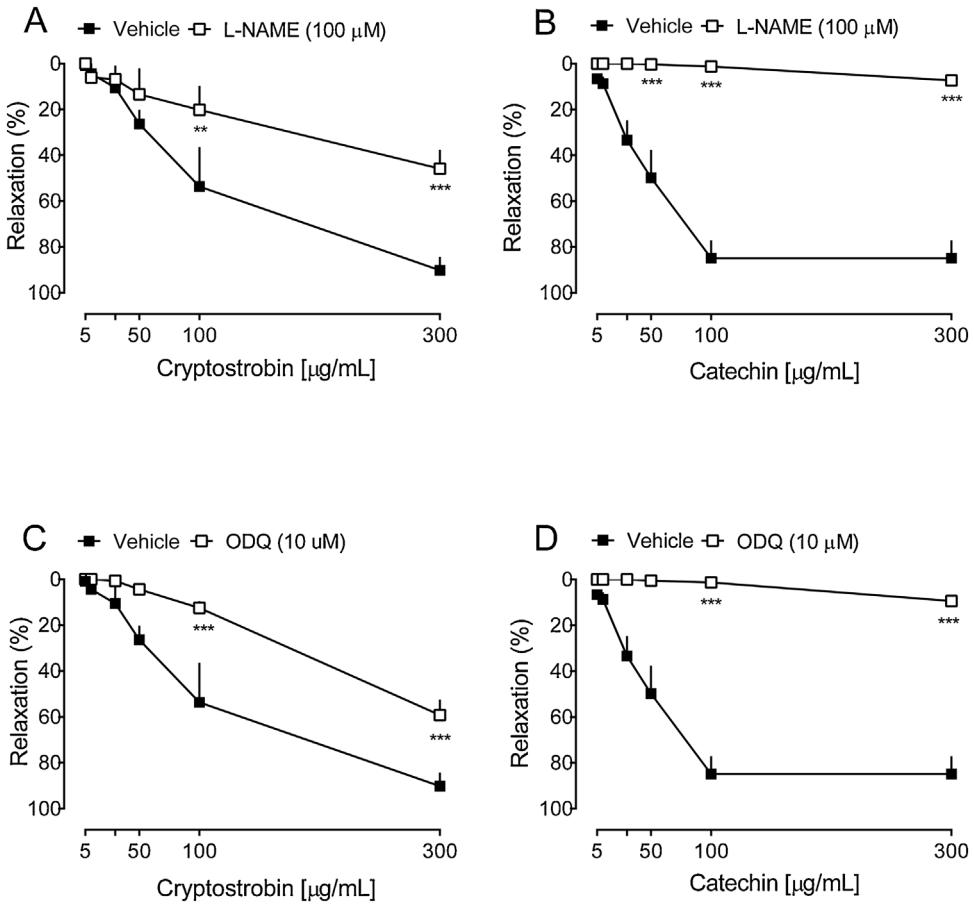

Fig. 2. Cryptostrobin and catechin-induced relaxation in the presence of L-NAME and ODQ. Cryptostrobin-induced relaxation was partially reduced in the presence of L-NAME (A) and ODQ (C). Blockage of catechin-induced relaxation in the presence of L-NAME (B) and ODQ (D). The results show the mean of ± SEM of n = 6. The data obtained from different experiments were analyzed by two-way ANOVA, followed by Bonferroni's *post-hoc* test. *Indicates $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to vehicle group.

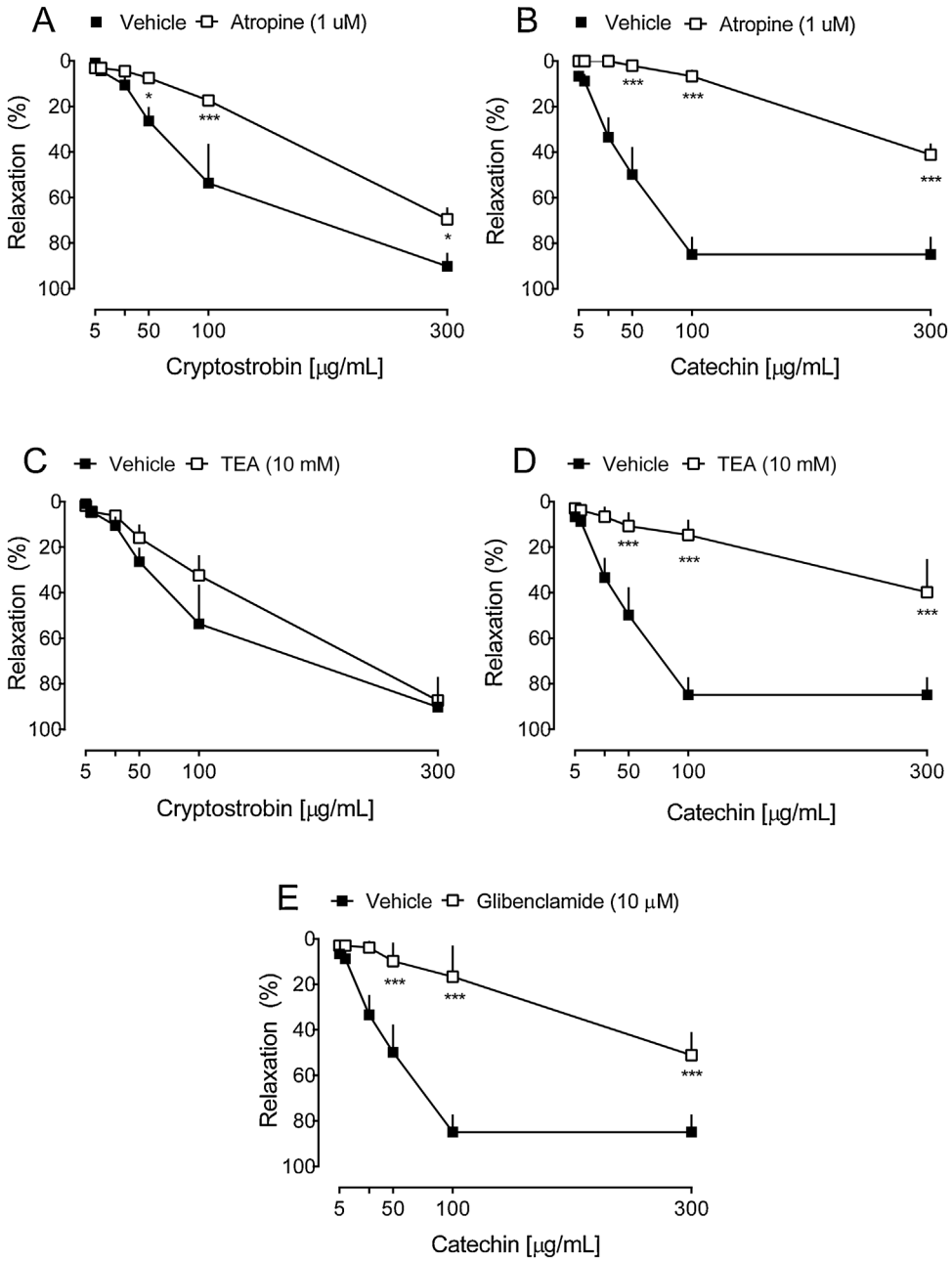

Fig. 3. Effect of membrane receptor antagonist and K^+ channels blockers against cryptostrobin and catechin-induced relaxation. The presence of atropine promoted a slight reduction in relaxation induced by cryptostrobin (A), and the presence of K^+ channel blocker TEA (C) did not reduce cryptostrobin-induced relaxation. Reduction in the relaxation induced by catechin in the presence of atropine (B), TEA (D) and glibenclamide (E). The results show the mean of ± SEM of n = 6. The data obtained from different experiments were analyzed by two-way ANOVA, followed by Bonferroni's *post-hoc* test. *Indicates $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to vehicle group.

address new strategies for the better control of high blood pressure levels, including those directly related to vascular tone, are extremely important.

SHR is characterized by endothelial dysfunction, which many studies have described abnormal endothelium-dependent responses in hypertensive blood vessels because the endothelial cells become dysfunctional in pathological situations [22]. The hypertensive process in the SHR model may result from a greater production of vasoconstrictors from the vascular endothelium cells [23], being reported a large production of vasoconstrictor cyclooxygenase-dependent endoperoxides and endothelin [23]. Luscher and Vanhoutte demonstrated that in SHR arteries high concentrations of acetylcholine besides releasing of endothelium-derived relaxing factors simultaneously release the endothelium-derived contracting substance(s). Since indomethacin abolished the endothelium-dependent contractions to acetylcholine, the authors suggested the involvement of arachidonic acid or one of its metabolites in this process [24]. However, when the aorta from SHR is endothelium-denuded the response to various vasoconstrictors is blunted [25]. In addition, Gendron et al. showed that in aortas of SHR deprived of endothelium the response to vasoconstrictors such as phenylephrine and KCl is weaker when compared to normotensive ones. SHR thoracic aorta presented hyperplasia and hypertrophy during its development, and these morphological alterations compromise aortic distensibility [26]. The mechanism involving the production and release of vasoactive substances by the vascular endothelium is complex is far from being fully understood [27].

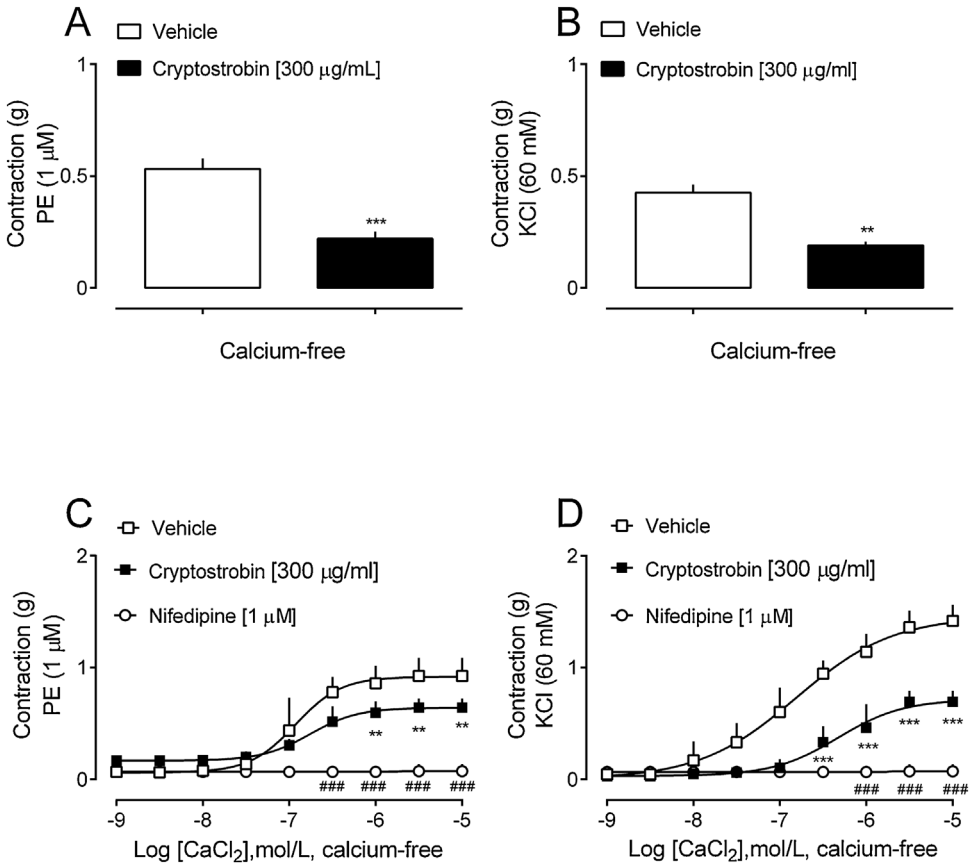

Fig. 4. Study of calcium in contractile responses to PE, KCl and $CaCl_2$ in the presence of cryptostrobin. Contractile responses to PE (A) and KCl (B) in the absence or after incubation of cryptostrobin in calcium-free Krebs solution. $CaCl_2$ induced-contraction after incubation of vehicle, cryptostrobin,and nifedipine on a previously induced contraction by PE (C) or KCl (D). The results show the mean of ± SEM of n = 6. Statistical comparisons were performed using Student *t* test (A and B) or two-way ANOVA, followed by Bonferroni's *post-hoc* test (C and D). **Indicates $p < 0.01$ and *** $p < 0.001$ when compared to vehicle group; ##Indicates $p < 0.01$ and ###$p < 0.001$ when compared to cryptostrobin group.

The relaxing effects found in this study could be related to the NO/cGMP pathway since L-NAME, a nitric oxide synthase inhibitor or ODQ, an inhibitor of soluble guanylate cyclase (sGC), significantly inhibited catechin-induced relaxation. These data suggest that one mechanism responsible for catechin-induced relaxation is by activating the NO/guanosine 3′,5′–cyclic monophosphate (cGMP) pathway. It is well known that the vasorelaxation can be endothelium-dependent and -independent. The endothelium-dependent relaxation involves the production and secretion of the NO, prostacyclin and endothelium-derived hyperpolarizing factors (EDHF) [28–30]. NO is formed from the metabolism of L-arginine by 3 isoforms of NO synthase (NOS) in response to several stimuli [31]. The vasorelaxation induced by NO involves, at least in part, activation of sGC in smooth muscle cells leading to an increase in cGMP, which stimulates cGMP-dependent protein kinases (PKG). PKG phosphorylates a number of protein targets that are implicated in vascular smooth muscle relaxation [32,33]. NO synthesized by endothelial NOS has been considered to the most abundant and important endothelial mediator for regulating vasoreactivity [34].

Besides that, the production and release of NO from endothelial cells induced by catechin may involve activation of muscarinic receptors (type M_3), since atropine (muscarinic receptor antagonist) significantly reduced the relaxation induced by catechin. Muscarinic receptors are present in the blood vessels, the M_3 receptors are G_q α-protein-coupled receptors, in which they mediated vasorelaxation effect through stimulating the cascade signaling pathways within the endothelium. Therefore, the non-selective muscarinic receptor atropine has been widely used to study this pathway. When atropine was bound to the M_3 receptor, the subsequent vasodilatation exerted by the compounds would be decreased. The pre-incubation of atropine at concentrations enough to significantly reduce the relaxation induced by ACh (data not shown) also changed the vasorelaxant effect of catechin-induced vasorelaxation in PE-contracted aortic rings. This data suggests that catechin in a manner not yet fully understood, at least in part, depends on the NO/cGMP pathway activation to induce the vasorelaxation effect disclosed herein.

Endothelium-dependent vascular relaxation is always accompanied by hyperpolarization mediated by activation of the K^+ channels and reflects part of the relaxation mechanism induced by NO [35]. K^+ channels play an essential role in membrane potential and are involved in the regulation of contractile tone being a potential target for anti-hypertensive drugs. Certain vasodilators, as well as protein kinases, can control or modulate K^+ channels, among them, we can highlight the NO. Activators of sGC can hyperpolarize smooth muscle by activating K^+ channels [36]. Byun reported that polyphenols played a crucial role in promoting hyperpolarization *via* K^+ channels activation as well as an increase in NO production [37]. Functional studies confirm that such activation can contribute to vasodilatation [35,38]. Indeed, the pre-incubation with TEA (a non-selective potassium channel blocker) and glibenclamide (an ATP-sensitive K^+ channel blockers) reduced the relaxation-induced by catechin, suggesting the activation of the NO pathway with subsequent activation of the K^+ channels. On the other hand, L-NAME, ODQ, and atropine had little effect on the

relaxation induced by cryptostrobin, while TEA did not alter its relaxation. These findings suggested that cryptostrobin could in part induce relaxation by other routes, such as inhibition of extra or intracellular calcium, which was explored following this study.

Pharmacological properties from some natural products like Ca^{2+} channels blockers have a great appeal for therapeutic use in cardiovascular disorders. Thus, we studied whether pre-incubation with cryptostrobin or catechin decreases the contractile response induced by PE. These results showed that cryptostrobin, but not catechin, reduced the maximal contraction induced by PE. In fact, cryptostrobin was able to decrease the contractile response to the cumulative addition of $CaCl_2$ in aortic rings with preserved endothelium, pre-contracted with PE or KCl in a Ca^{2+}-free Krebs. Moreover, the presence of cryptostrobin decreases the contractile response to PE and KCl.

Intracellular Ca^{2+}concentration is considered an important factor in PE-induced contraction [39]. The interaction of PE with the α_1-adrenergic agonist receptor results in activation of phospholipase C (PLC), which in turn can stimulate the intracellular Ca^{2+} release through activation of IP_3 receptor [40]. The increase in intracellular Ca^{2+} concentration activates the Ca^{2+}-induced Ca^{2+} release through a ryanodine-like receptor in the reticulum and simultaneously activates Ca^{2+} influx through a Ca^{2+} channel operated by stock concentration [41]. Besides that, diacylglycerol (DAG), activates protein kinase C (PKC) in the presence of Ca^{2+}, inducing vascular smooth muscle contraction by activation of Ca^{2+} influx *via* plasma membrane Ca^{2+}channels [42,43].

The KCl solution is a depolarizing agent and does not use a membrane receptor like PE does to induce its effects. KCl induces-contraction on vascular smooth muscle by opening the Ca^{2+} L-type channels located in the cell membrane, and by increasing concentrations of free Ca^{2+} in the cytoplasm. This effect may be eliminated in the presence of Ca^{2+} channels blocker, like nifedipine [44]. Nifedipine was more effective on the inhibition of $CaCl_2$-induced contractions than cryptostrobin. Based on this experimental finding, we considered the possibility that cryptostrobin-induced responses could be related to its ability to block voltage-gated Ca^{2+} channels, and in an unenlightened way modulate the release of intracellular Ca^{2+} and therefore reduces the contraction induced by phenylephrine. Further studies with specific pharmacological tools should be made to clarify how cryptostrobin acts in this cascade of calcium signaling pathway.

Conclusions

In summary, the present results show, for the first time, that cryptostrobin and catechin induce endothelium-dependent and independent relaxation in the aorta of spontaneously hypertensive rats. The vasorelaxant effect of catechin may be mediated, at least in part, by activation of the NO/cGMP pathway, while the vasorelaxation effect of cryptostrobin is partially related to the involvement of endothelial mediators and partially to the inhibition of intra- and extracellular calcium. These data suggest that both compounds may modify the vascular biology and contribute to the effects of medicinal plants useful for the treatment of cardiovascular disorders, which the phenolic compounds are highlighted.

Conflict of interest

On behalf of all authors the corresponding author states that there is no conflict of interest.

Author contributions

Giovana Vechi and Valdir Cechinel Filho phytochemical studies. Priscila de Souza, Luísa M. da Silva, Sérgio F. de Andrade and Rita de C. M. V. A. F. da Silva conceived, designed the study and conducted the experiments. Rita C. M. V. A. F. da Silva wrote the manuscript. All authors read and approved the manuscript.

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