The role of endothelium in the vasorelaxant effects of the essential oil of *Ocimum gratissimum* in aorta and mesenteric vascular bed of rats

Alana F. Pires, Socorro V. Frota Madeira, Pedro M.G. Soares, Claudia M. Montenegro, Emmanuel P. Souza, Angela C. Resende, Roberto Soares de Moura, Ana M.S. Assreuy, and David N. Criddle

Abstract: This study investigated the endothelium-dependent vasorelaxant effects of the essential oil of *Ocimum gratissimum* (EOOG) in aortas and mesenteric vascular beds isolated from rats. EOOG (3–300 μg/mL) relaxed the tonic contractions induced by phenylephrine (0.1 μmol/L) in isolated aortas in a concentration-dependent manner in both endothelium-containing and endothelium-denuded preparations. This effect was partially reversed by L-NAME (100 μmol/L) but not by indomethacin (10 μmol/L) or TEA (5 mmol/L). In mesenteric vascular beds, bolus injections of EOOG (30, 50, 100, and 300 ng) decreased the perfusion pressure induced by noradrenaline (6 μmol/L) in endothelium-intact preparations but not in those treated with deoxycholate. L-NAME (300 μmol/L) but not TEA (1 mmol/L) or indomethacin (3 μmol/L) significantly reduced the vasodilatory response to EOOG at all of the doses tested. Our data showed that EOOG exerts a dose-dependent vasodilatory response in the resistance blood vessels of rat mesenteric vascular beds and in the capacitance blood vessel, the rat aorta. This action is completely dependent on endothelial nitric oxide (NO) release in the mesenteric vascular beds but only partially dependent on NO in the aorta. These novel effects of EOOG highlight interesting differences between resistance and capacitance blood vessels.

Key words: Ocimum gratissimum, essential oil, mesenteric vascular bed, aorta, vasodilatation, nitric oxide, endothelium.

Résumé : Cette étude a examiné les effets vasorelaxateurs de l'huile essentielle de *Ocimum gratissimum* (HEOG) sur les lits vasculaires aortiques et mésentériques du rat, en fonction de l'endothélium. L'HEOG (3–300 μg/mL) relaxait les contractions toniques d'aortes isolées induites par la phényléphrine (0,1 μg) et ce, de façon dépendante de la concentration, tant chez les préparations comprenant l'endothélium que chez celles qui en étaient dépourvues. Cet effet était partiellement renversé par le L-NAME (100 μmol/L) contrairement à l'indométhacine (10 μmol/L) ou le TEA (5 mmol/L). Des injections en bolus d'HEOG (30, 50, 100 et 300 ng) diminuaient la pression de perfusion induite par la noradrénaline (6 μmol/L) de préparation de lits vasculaires mésentériques où l'endothélium était intact, mais pas des lits traités au désoxycholate. Le L-NAME (300 μmol/L), mais pas le TEA (1 mmol/L) ni l'indométhacine (3 μmol/L), réduisait significativement la réponse vasodilatatrice à l'HEOG à toutes les doses testées. En résumé, nos données montrent que l'HEOG induit une réponse vasodilatatrice dépendante de la dose sur la résistance des vaisseaux sanguins des lits vasculaires mésentériques du rat et sur la capacitance vasculaire de l'aorte du rat. Cette action est complètement dépendante de la libération de NO endothélial des lits vasculaires mésentériques, mais seulement partiellement dépendante du NO dans l'aorte. Ces nouveaux effets de l'HEOG mettent en lumière des différences intéressantes entre la résistance et la capacitance des vaisseaux sanguins.

Mots-clés : Ocimum gratissimum, huile essentielle, lit vasculaire mésentérique, aorte, vasodilatation, oxyde nitrique, endothélium.

[Traduit par la Rédaction]

Introduction

The plant *Ocimum gratissimum* L. (Labiatae) is widely distributed in northeastern Brazil, where it is popularly known as "Alfavaca-cravo". It is used in a variety of culinary and medicinal applications, including as an antiseptic and in the treatment of cough, fever, and conjunctivitis (Matos 1998).

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A.F. Pires, C.M. Montenegro, and A.M.S. Assreuy. Laboratório de Fisio-Farmacologia da Inflamação – LAFFIN, Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará (UECE), Av. Paranjana 1700, 60740-000, Fortaleza-CE, Brasil.
S.V.F. Madeira, A.C. Resende, and R. Soares de Moura. Departamento de Farmacologia, Centro Biomédico – IB, Universidade do Estado do Rio de Janeiro, Av. 28 de Setembro, 81, 20 551 - Rio de Janeiro, Brasil.
P.M.G. Soares and E.P. Souza. Departamento de Morfologia – Universidade Federal do Ceará (UFC), Delmiro de Farias, S/N, Rodolfo Teófilo, 60.416-030 - Fortaleza – CE, Brasil.
D.N. Criddle. The Physiological Laboratory, University of Liverpool, Crown Street, Liverpool L69 3BX, UK.

Corresponding author: Emmanuel P. Souza (e-mail: emmanuelprata@gmail.com).

Fig. 1. The relaxant effect of EOOG (essential oil of *Ocimum gratissimum*). Data are presented as mean ± SE. (*a*) Noradrenaline (NA)-precontracted mesenteric vascular bed. (*b*) Rat thoracic aorta precontracted with phenylephrine (PE). Comparison is shown between the EOOG relaxant effect on endothelium-intact (E+, open circles) and endothelium-denuded (E–, solid circles) preparations. *, $p < 0.05$ compared with initial tonic contraction (100% of PE- or NA-induced contraction); #, $p < 0.05$ compared with intact endothelium..

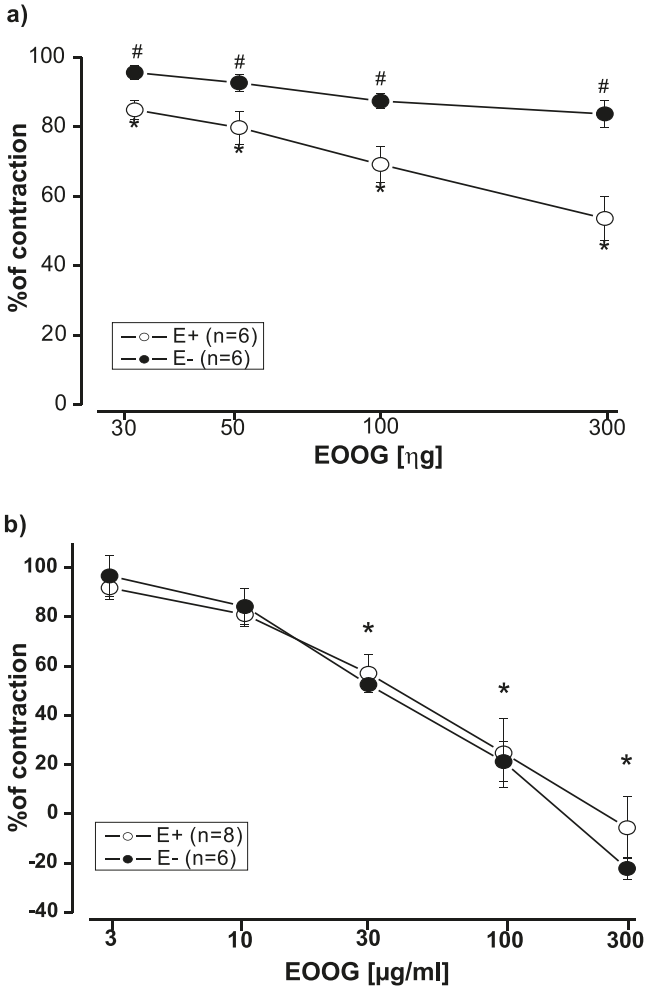
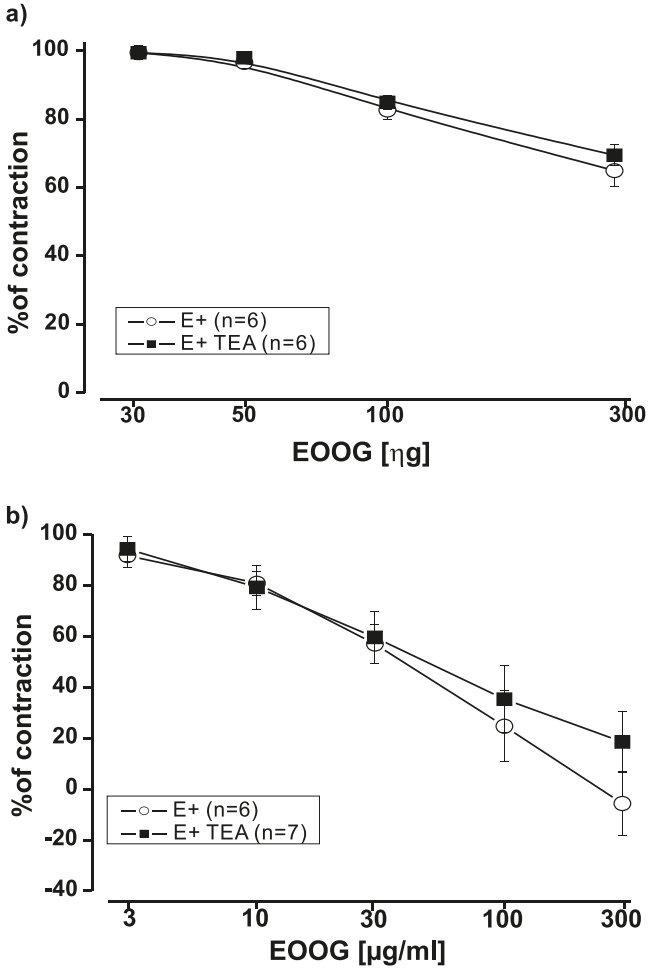


Fig. 2. The relaxant effect of EOOG (essential oil of *Ocimum gratissimum*) in the presence of TEA (tetraethylammonium). Data are presented as mean ± SE. (*a*) Noradrenaline-precontracted mesenteric vascular bed. (*b*) Rat thoracic aorta precontracted with phenylephrine. Comparison between the EOOG relaxant effect in the absence (open circles) and presence (solid squares) of TEA. *, $p < 0.05$ compared with intact endothelium. The data are expressed as percentages of PE- or NA-induced contraction.

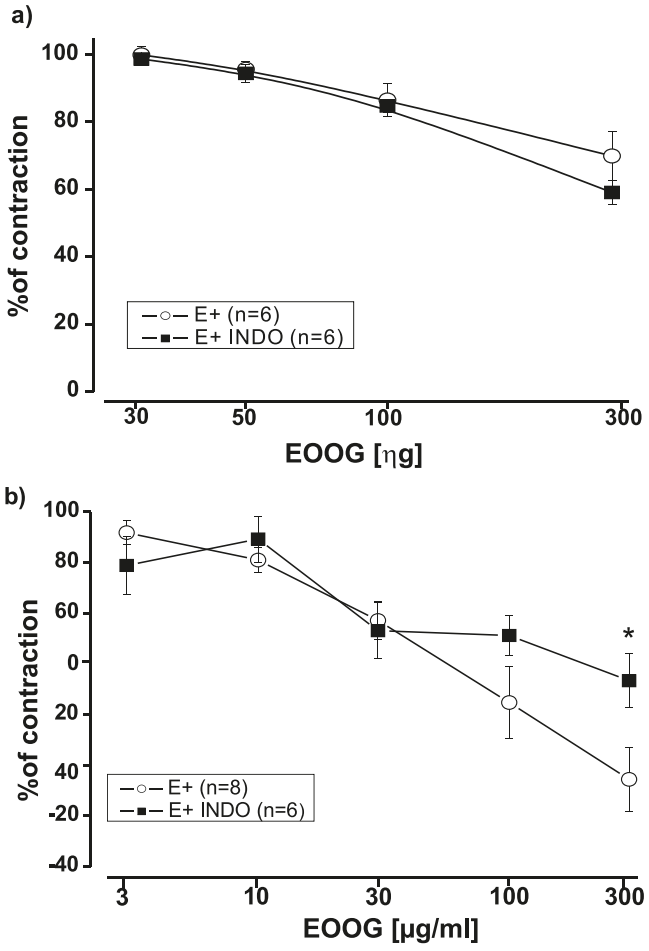


The flowers and leaves of this plant are a rich source of essential oils (de Vasconcelos Silva et al. 1999) and are generally used to prepare infusions or teas (Matos 1998).

The chemical composition of the essential oil of *O. gratissimum* (EOOG) can vary based on geographic location and climate conditions. This variation has been verified in different regions of Brazil. For example, the EOOG from the Amazonian region has *p*-cimene as the major constituent, whereas the oil of the northeastern plant is rich in eugenol (Maia et al. 1988; Matos 1998; de Vasconcelos Silva et al. 1999). However, a higher content of eugenol (98%) has been found in EOOG from plants harvested at 1200 h compared with plants harvested at 0900 h, which have 1,8-cineole as the major constituent; this observation suggests that solar light increases the production of eugenol (de Vasconcelos Silva et al. 1999).

Despite the multiple popular medicinal applications of this plant, few studies have examined its basic pharmacological properties. It has been demonstrated that EOOG possesses anti-inflammatory (Rabelo et al. 2001), antinociceptive (Rabelo et al. 2003), and hypotensive properties (Lahlou et al. 2004) in rodents and relaxant effects on the isolated ilea of guinea pigs (Madeira et al. 2002, 2005) and aortas of deoxycorticosterone acetate (DOCA)–salt hypertensive rats (Interaminense et al. 2007). In the present study, we examined the role of the endothelium in the relaxant action of *O. gratissimum* essential oil on isolated rat aortas, which are capacitance vessels, and in the perfused mesenteric vascular bed,

Fig. 3. The relaxant effect of EOOG (essential oil of *Ocimum gratissimum*) in the presence of indomethacin (INDO). Data are presented as mean ± SE. (*a*) Noradrenaline-precontracted mesenteric vascular bed. (*b*) Rat thoracic aorta precontracted with phenylephrine. Comparison between the EOOG relaxant effect in the absence (open circles) and presence (solid squares) of indomethacin. *, $p < 0.05$ compared with intact endothelium. The data are expressed as percentages of PE- or NA-induced contraction.



in which tone is dependent on resistance vessels. Possible mechanisms of action were explored.

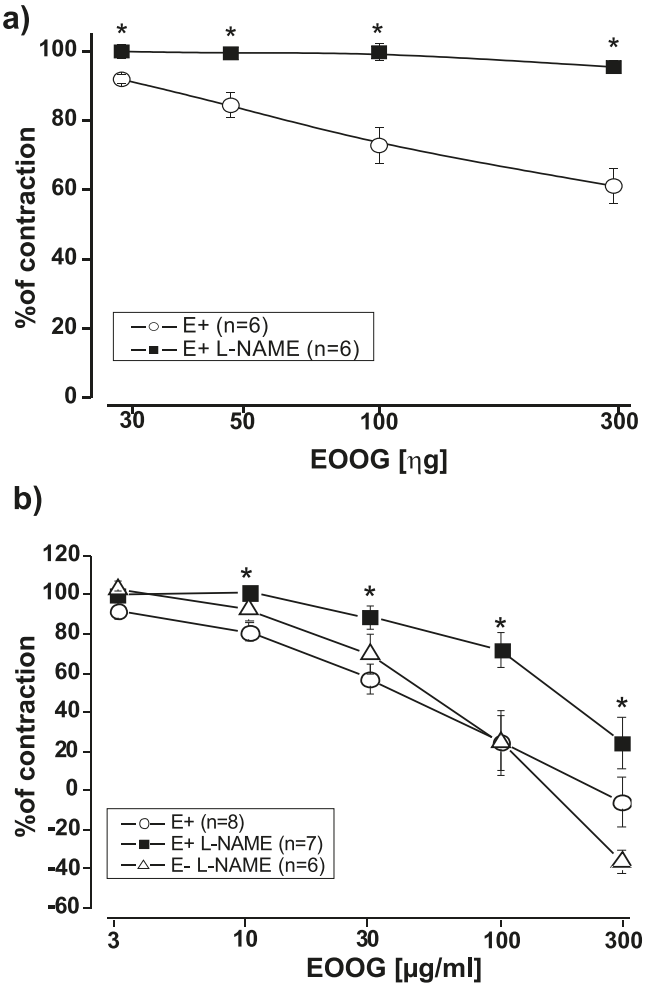
Materials and methods

Plant material

Ocimum gratissimum leaves were harvested at 1200 h from the Medicinal and Aromatic Plants Garden of the Federal University of Ceará (UFC–Fortaleza). A voucher specimen of the plant has been deposited in the Prisco Bezerra Herbarium of the UFC (EAC 14968).

The isolation of the essential oil from the leaves of the plant was carried out by steam distillation according to a previously described method (Craveiro et al. 1976). The composition (%) of the EOOG used in this study was determined by gas chromatography and mass spectrometry: eugenol (52.14%), 1,8-cineole (29.17%), β-selinene (5.56%), transcariophylene (3.35%), ocimene (2.73%), α-selinene (1.58%), β-pinene (1.51%), and unidentified components (3.96).

Fig. 4. The relaxant effect of EOOG (essential oil of *Ocimum gratissimum*) in the presence of L-NAME (N^{ω}-nitro-L-arginine methyl ester). Data are presented as mean ± SE. (*a*) Noradrenaline-precontracted mesenteric vascular bed. (*b*) Rat thoracic aorta. Comparison between the EOOG relaxant effect in the absence (open circles) of L-NAME and in the presence of L-NAME with (solid squares) or without (open triangles) endothelium. *, $p < 0.05$ compared with intact endothelium. The data are expressed as percentages of PE- or NA-induced contraction.



Animals

Male Wistar rats weighing 250–300 g were used. Animals were maintained in rooms with a controlled 12 light : 12 h dark cycle and temperature of 25 °C with free access to food and water. All experimental protocols were approved by the Animal Care and Use Committee of the State University of Ceará (UECE – No. 0559924-4), Fortaleza-CE, Brazil, in accordance with international guidelines.

Thoracic aorta

Rats were sacrificed, and the thoracic aorta was quickly removed, defatted, and cleaned of connective tissue. Aortic ring segments (3–5 mm) were mounted for tension recording (2 g) in 10 mL organ baths filled with modified Tyrode solution (136 mmol/L NaCl, 5 mmol/L KCl, 0.98 mmol/L $MgCl_2$, 2 mmol/L $CaCl_2$, 0.36 mmol/L NaH_2PO_4, 11.9 mmol/L $NaHCO_3$, and 5.5 mmol/L glucose). The rings were main-

tained at 37 °C, gassed with 95% O_2 and 5% CO_2 (pH = 7.4), and equilibrated for 45 min. Contractile responses (isometric tension, in *g*) were measured with an isometric tension transducer (PowerLab, ADinstruments, Australia) and a computerized data acquisition system (Chart 4.2, ADInstruments). In all of the experiments, aortic rings were challenged with KCl (60 mmol/L) after equilibration to assure the contractile condition of the preparation. Aortic rings were precontracted with phenylephrine (PE; 0.1 μmol/L), and once a plateau was attained for 10 min, increasing concentrations of EOOG (3, 10, 30, 100, and 300 μg/mL) were added. The role of the endothelium in the EOOG response was evaluated by adding the oil to both endothelium-intact and endothelium-denuded aortic rings at the same concentrations. The effectiveness of endothelium removal, which was performed by abrading the inner surface with a fine needle, was confirmed by a failure of relaxation in PE-precontracted rings upon the addition of acetylcholine (ACh; 1 μmol/L) (Furchgott and Zawadzki 1980). The involvement of endothelium-derived relaxant factors in the possible vasodilatory effect of EOOG was investigated in endothelium-intact aortic rings preincubated with the antagonists of nitric oxide (NO) synthase (N^{ω}-nitro-L-arginine methyl ester (L-NAME); 100 μmol/L), K^+ channel hyperpolarization (tetraethylammonium (TEA); 5 mmol/L), and prostacyclin (PGI_2) synthesis (indomethacin; 10 μmol/L) for 30 min before PE-induced contraction. To exclude antagonism between compounds, L-NAME was also administered to endothelium-denuded aortic rings. Classically, the adrenergic agonist noradrenaline (NA) has been used to stimulate contractions in the mesenteric vascular bed (MVB) and aorta. However, PE has been used in the aorta to promote sensitivity to relaxation by NO (Auguet et al. 1992). Therefore, NA was used in the MVB and PE in the aorta.

Mesenteric vascular bed (MVB)

MVBs were isolated, mounted at a constant flow of 4 mL/min, and perfused with a modified Krebs–Henseleit solution (11.0 mmol/L glucose, 118.0 mmol/L NaCl, 25.0 mmol/L $NaHCO_3$, 4.7 mmol/L KCl, 1.2 mmol/L KH_2PO_4, 1.2 mmol/L $CaCl_2$, and 1.2 mmol/L $MgSO_4$) gassed with 95% O_2 and 5% CO_2 at 37 °C (pH 7.4). Perfusion pressure changes were monitored using a pressure transducer, and preparations were set up and equilibrated for 30–40 min before the addition of drugs (Criddle et al. 1997). The perfusion pressure was tonically increased by adding 6 μmol/L NA to the perfusion fluid reservoir. When NA-induced vascular tone had plateaued, control responses to bolus injections of EOOG (30, 50, 100, and 300 ng) and ACh (10, 30, and 100 pmol) were measured. The volume of each bolus injection was smaller than 50 μL. After the initial control responses to the tested substances, NA was removed from the perfusion stream by washout, and basal tonus was reestablished.

We examined the role of the endothelium in the response to EOOG by adding the oil to both endothelium-intact and endothelium-denuded MVBs at the same concentrations. Deoxycholate (1 mg/mL) was included in the perfusion stream for 3 min to remove the endothelium from the MVB (Cusma-Pelógia et al. 1993). Subsequently, NA was reapplied to the tissue to augment basal tone, and EOOG and ACh were readded. Similarly, in separate experiments, the possible roles of NO, hyperpolarizing K^+ channels, and prostacyclin were examined by assessing the effects of L-NAME (300 μmol/L), TEA (1 mmol/L), and indomethacin (3 μmol/L), respectively, on the vasodilatory activity of EOOG. These substances were included in the perfusion stream at fixed concentrations and applied to the MVB jointly with the second application of NA after the initial control responses had been obtained.

Drugs

All of the reagents used were of analytical grade. L-NAME, indomethacin, deoxycholate, tetraethylammonium, ACh, and PE were purchased from Sigma Chemical Company (Missouri, USA).

EOOG solutions were prepared directly in Tyrode or Krebs–Henseleit solutions and always sonicated before use.

Data analysis

The results are presented as the mean ± SE of a number of observations (*n*). Values were analyzed using the Student's *t* test or ANOVA as appropriate and were considered to be significantly different when $p < 0.05$.

Results and discussion

The vasodilatory effect of EOOG was partial in the MVB and was observed only in endothelium-intact preparations precontracted with NA (6 μmol/L). Bolus injections of EOOG (30, 50, 100, and 300 ng) induced transient dose-dependent decreases in perfusion pressure, which reached approximately 50% of the induced tone. The vasodilatory effect of EOOG was significantly inhibited after treatment with deoxycholate to remove endothelium at all of the doses tested, although a small (~10%) vasodilatory component remained (Fig. 1*a*).

In contrast, EOOG (3–3000 μg/mL) showed a complete concentration-dependent vasorelaxant effect, which was not significantly different between endothelium-intact or endothelium-denuded aortic rings precontracted with PE (0.1 μmol/L) (Fig. 1*b*). In endothelium-intact preparations, 30, 100, and 300 μg/mL EOOG significantly inhibited the PE-induced contractions by 42.94% ± 7.56%, 75.31% ± 14.06%, and 105.66% ± 12.64%, respectively (Fig. 1). In endothelium-denuded preparations, 30, 100, and 300 μg/mL EOOG significantly reduced the PE-evoked contractions by 47.49% ± 1.47%, 78.75% ± 8.26%, and 122.23% ± 4.31%, respectively (Fig. 1*b*). It is important to note that 300 μg/mL of EOOG relaxed the aorta rings beyond the basal tone.

The observed vasorelaxant effects of EOOG on the MVB and thoracic aorta of the rat are consistent with the relaxant activity previously reported in other smooth muscles, such as its antispasmodic effect on the isolated ileum of guinea pigs (Madeira et al. 2002) and its endothelium-derived effect in DOCA–salt hypertensive rats (Interaminense et al. 2007). The presence of eugenol, the main constituent of EOOG, might contribute to this effect; eugenol has been shown to induce vasorelaxant action in vascular smooth muscle preparations, including rabbit ear artery (Nishijima et al. 1998), thoracic aorta (Damiani et al. 2003; Nishijima et al. 1999), and MVBs (Criddle et al. 2003). Beyond the effect of its oil, aqueous extracts have shown smooth muscle relaxant effects

in stomach preparations (Aziba et al. 1999), justifying the global therapeutic use of this plant.

The removal of the endothelium reduced the EOOG relaxant effect in the MVB but not in the aorta. These data could be explained by the important role played by the endothelium in resistance microvessels, such as mesenteric arteries, compared with capacitance vessels, such as aorta (Burton 1954). Previous reports have also suggested that endothelial factors may affect eugenol activity (Damiani et al. 2003; Nishijima et al. 1998). However, the influence of the endothelium on the aorta cannot be excluded because a mix of the oil constituents may have an antagonistic effect on these vessels.

Therefore, we investigated the role of endothelial-derived relaxant factors in EOOG response. The activation of potassium channels may be responsible for the relaxant action of endothelium-derived hyperpolarizing factor (EDHF) in vascular smooth muscles, which can be blocked by TEA (Edwards et al. 1998). We pre-incubated both tissues (aorta and mesenteric arteries) with TEA, a nonspecific blocker of calcium-dependent potassium channels (Nelson et al. 1990), and observed no alteration in the EOOG response (Figs. 2*a*, 2*b*). These data indicate that the vasodilatory action of EOOG most likely is not mediated by the release of EDHF, which acts by opening plasmalemmal potassium channels (Chen et al. 1988; Feletou and Vanhoutte 1988).

Furthermore, PGI_2, the main product of the cyclooxygenase pathway in endothelial cells, was postulated as a possible modulator of the effects of EOOG. PGI_2 is rapidly released and synthesized in response to increases in intracellular calcium concentration (Moncada et al. 1976; Nilius and Droogmans 2001). PGI_2 induces smooth muscle relaxation via an increase in the intracellular concentration of cAMP, which activates protein kinase A (PKA) (Karaki et al. 1997). The cyclooxygenase inhibitor indomethacin was used to investigate the possible involvement of PGI_2 in the EOOG response. Like TEA, indomethacin did not alter the vascular responses to EOOG in MVB (Fig. 3*a*), excluding the involvement of PGI_2 in its effects, and only partially reduced EOOG-induced relaxation in aortas, from 105.66% ± 13.05% to 66.45% ± 10.37% (Fig. 3*b*) at the highest concentration tested (300 μg/mL), suggesting a minimal participation of PGI_2 in this phenomenon.

The role of NO, the main endothelium-derived relaxant factor, in the effects of EOOG were examined. The decreases in MVB perfusion pressure induced by EOOG were completely inhibited by L-NAME, a nonspecific inhibitor of NO synthase (Rees et al. 1989), at all of the doses tested (Fig. 4*a*), indicating that the vasodilatory activity of EOOG is most likely mediated by the release of NO from mesenteric vascular endothelium. NO is the principal substance responsible for the maintenance of vasomotor tonus, activating the enzyme guanylyl cyclase to increase the concentration of GMPc and consequently activate PKG, which ultimately evokes the relaxation of smooth muscles (Karaki et al. 1997; Moncada et al. 1991).

Moreover, the addition of L-NAME to the aortic rings partially reduced the effects of EOOG at 10, 30, and 100 μg/mL to 0%, 11.53% ± 6.10%, and 28.10% ± 8.64%, respectively, compared with 19.06% ± 4.8%, 42.94% ± 7.56%, and 75.31% ± 14.06% without L-NAME (Fig. 4*b*). Endothelial removal had no effect. The apparent discrepancy of the observed inhibition by L-NAME in the aorta may suggest that inhibition of endothelial NO release by L-NAME was not by itself responsible for the relaxation effects but that the inhibition was rather caused by an increase in basal tension by L-NAME that produced a physiological antagonism of EOOG-induced relaxation. To test this hypothesis, we investigated the effect of L-NAME in endothelium-denuded aorta rings incubated with EOOG. L-NAME did not alter the relaxant effect of EOOG in endothelium-denuded aorta rings, which may suggest that EOOG depends on NO and thus on the presence of endothelium.

It has been reported that eugenol, the main constituent of EOOG, induces a vasodilatory response in the MVB. This effect was shown to be endothelium-dependent but not mediated by NO, PGI_2, or EDHF, suggesting a direct effect on the smooth muscle (Criddle et al. 2003). In contrast, in the aorta, eugenol evoked a relaxant effect that was at least partly modulated by NO (Damiani et al. 2003). These results suggest that eugenol may be the primary component responsible for EOOG-induced relaxation in the aorta and MVB, although other factors may participate.

In summary, our results have shown that EOOG exerts a dose-dependent vasorelaxation effect in both resistance and capacitance blood vessels. In the MVB, this action was completely dependent on intact endothelium to generate NO release. In the rat aorta, however, the endothelium is not completely responsible for the relaxation induced by EOOG, suggesting that EOOG may have both a direct action on the smooth muscle and endothelial-dependent effects. Because this essential oil has been shown to decrease blood pressure in hypertensive rats (Interaminense et al. 2007), further investigation into the relaxant action of EOOG in vascular smooth muscle is warranted.

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