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Vasorelaxant effects of eugenol on rat thoracic aorta

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Abstract

Eugenol is a natural pungent substance and the main component of clove oil, with vasorelaxant action. To elucidate some of the possible mechanisms involved in this action isometric tension was measured in aortic rings from male Wistar rats precontracted with phenylephrine (PHE, 10^{-7} M) or KCl (75 mM). Responses to increasing concentrations of eugenol (10^{-6}–10^{-2} M) were obtained in the presence and absence of endothelium. In the presence of eugenol, dose–response curves to PHE (10^{-9} to 10^{-4} M) and KCl (5–125 mM) were displaced downwards. Concentration-dependent relaxation was observed in rings precontracted with PHE (10^{-7} M) and KCl (75 mM). The tension increment produced by increasing external calcium concentration (0.25–3 mM) was also reduced by eugenol (300 μM) treatment. The inhibitory effects of eugenol (300 μM) were compared to those induced by nifedipine (0.01 μM), a selective Ca^{2+} channel blocker, producing similar relaxant effects. Two other protocols were performed. After precontraction with PHE (10^{-7} M), increasing concentrations of eugenol (10^{-6}–10^{-2} M) were used before and after N^{w}-nitro-L-arginine (L-NAME, 10^{-4} M) and methylene blue (10^{-5} M) treatment. Eugenol-induced relaxation was reduced by endothelial damage (rubbing), L-NAME and methylene blue treatments. Results suggested that eugenol produces smooth muscle relaxation resulting from the blockade of both voltage-sensitive and receptor-operated channels that are modulated by endothelial-generated nitric oxide.

Keywords: Eugenol; Vascular reactivity; Phenylephrine; Endothelium; Nitric oxide; L-NAME

1. Introduction

Essential oils are aromatic substances found in many plants with pharmacological activity and are of fluid occurrence (although some are solid at room temperature), oily and volatile. Eugenol, an essential oil and the chief component of clove oil, is commonly used in the food industry, in aromatherapy and as a therapeutic agent in dentistry (Leal-Cardoso et al., 1994). Otherwise, despite its commercial use, eugenol may be found in large amounts among the essential oils of many plants most of them widely used in folk medicine (Leal-Cardoso et al., 1994).

Although the action of eugenol is thought to be analgesic (Feng and Lipton, 1987; Thompson and Eling, 1989), authors reported that eugenol appears to have other actions (Sticht and Smith, 1971; Brodin and Roed, 1984). This compound reduces arterial blood pressure in dogs after intravenous injections, and increases blood flow after both intra-arterial and intravenous injections (Sticht and Smith, 1971), suggesting that the action of eugenol on the cardiovascular system might be on blood vessels. It was also reported that methyleugenol, an analogue of the phenolic compound eugenol, relaxes the isolated ileum and inhibits contractions induced by stimulation of voltage-dependent and receptor-operated channels (Lima et al., 2000).

Regarding the mechanisms mediating the vasodilating effect of eugenol, Hume (1983) suggested that this agent exerts a direct inhibitory action in the rabbit ear artery acting as a Ca^{2+} antagonist. However, other authors (Magalhães et al., 1998; Nishijima et al., 1998; 1999; Huang et al., 1999) suggested that eugenol and some other derivatives of hindered phenol, such as methyleugenol and eugenodilol, did not simply act as a Ca^{2+} antagonist but also as a metabolic inhibitor. Thus, despite these studies, the mechanisms of action of this compound are still unclear.

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The aim of this study was to elucidate some of the possible mechanisms involved in the eugenol-induced vasorelaxation by performing experiments using rings from rat thoracic aorta.

2. Materials and methods

Male Wistar rats, weighing 250–300 g, were used. The care and use of animals were in accordance with NIH guidelines. Rats were anesthetized with sodium pentobarbital (35 mg/kg ip) and were exsanguinated. The thoracic aorta was quickly removed, cleaned of adhering fat and connective tissue. Ring segments (3–5 mm) were mounted between two stainless steel wires in 10-ml organ baths filled with modified Krebs solution, under passive tension of 1 g for 45 min. The Krebs solution had the following composition (in mM): NaCl 118; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.17; $NaHCO_3$ 20; EDTA 0.01; and glucose 11. The rings were maintained at 37 °C and gassed with a 95% O_2 and 5% CO_2 mixture (pH=7.4). The contractile response (isometric tension, in g) was measured by a force transducer (Grass Instrument; model FT03, Quincy, MA, USA) and coupled to a data acquisition system (Biopac System; model MP100, Sta. Barbara, CA, USA). In all experiments, after 45 min of equilibration, aortic rings were challenged with KCl (75 mM) to assure the good contractile condition of the preparation.

2.1. Protocols

2.1.1. Effects of eugenol in aortic rings

To evaluate the effects of eugenol on the contractions of aortic rings, experiments were performed using rings with intact endothelium. Two different experimental designs were used.

In the first study, after 45 min of equilibration in Krebs solution, control concentration–response curves to phenylephrine (PHE) (10^{-9}–10^{-5} M) or to KCl (5–125 mM) were performed. Eugenol (300 μM) was added to the bath after 45 min equilibration in Krebs solution and, after 20 min, the same concentration–response curves were repeated in the presence of eugenol.

In the second study, after 45 min equilibration, aortic rings were precontracted with PHE (10^{-7} M) or KCl (75 mM), and once the plateau was attained, concentration-dependent curves of eugenol-induced relaxation (10^{-6}–10^{-2} M) were obtained.

2.1.2. Effects of eugenol on extracellular Ca^{2+}-induced contraction activated by PHE

After 45 min of equilibration, experiments were carried out with aortic rings under calcium-free Krebs solution plus 1 mM EGTA. After adding PHE (10^{-6} M), cumulative concentrations of $CaCl_2$ (0.25, 0.5, 0.75, 1, 1.5, 2, 2.5 and 3 mM) were performed (Control). The same protocol was repeated in another group of aortic rings after treatment with eugenol (300 μM) for 20 min. The stepwise increments in tension represented the vasoconstriction dependent on extracellular Ca^{2+} influx induced by PHE.

2.1.3. Possible action of eugenol on the sarcoplasmic reticulum calcium release induced by PHE

To assure sarcoplasmic reticulum full Ca^{2+} storage, the rings were washed with Krebs solution and, after a resting period of 20 min, contractions were induced by high K^+ solution (75 mM). Once the plateau was attained, rings were washed with Krebs solution and the baseline tension was recovered. The rings were washed three times with Ca^{2+}-free Krebs solution plus 1 mM EGTA and allowed to rest for 20 min in this solution. PHE (10^{-6} M) was then added to the bath and the transient contraction was determined to estimate the amount of Ca^{2+} stored in the sarcoplasmic reticulum (Control). To test the effects of eugenol, a similar protocol was performed and the preparations were allowed to rest for 20 min equilibration in the presence of eugenol (300 μM). PHE (10^{-6} M) was added to the bath and the transient contraction was compared to the control.

2.1.4. Comparison of the inhibitory effects of eugenol and nifedipine

This protocol was performed to verify similarities of actions between eugenol and known calcium channel blockers. The inhibitory effects of eugenol (300 μM) were then compared to those induced by nifedipine (0.01 μM), a selective Ca^{2+} channel blocker. Contractions were induced by KCl (75 mM) and the plateau contraction was taken as the control. Rings were washed with normal Krebs solution and the baseline tension was recovered. Eugenol (300 μM) or nifedipine (0.01 μM) was then added to the bath and after 20 min, contractions were induced by KCl (75 mM). These K^+-induced contractions of aortic rings treated with eugenol or nifedipine were compared to their respective controls. The same protocol was also performed in another group of aortic rings using PHE (10^{-7} M)-induced contractions.

To verify whether the effects of eugenol and nifedipine were additive or not, we performed another protocol. After 45 min equilibration in normal Krebs solution, aortic rings were treated with eugenol (300 μM) plus nifedipine (0.01 μM) for 20 min and contractions were induced either by KCl (75 mM) or PHE (10^{-7} M).

2.1.5. Effects of eugenol on the vascular tone: dependence on endothelium

To evaluate if the endothelium plays a role in the eugenol relaxing response, eugenol was added in a cumulative manner (10^{-6}–10^{-2} M) during the tonic contraction phase induced by PHE (10^{-7} M), in both endothelium-intact and endothelium-denuded aortic rings. The endothelium was removed by rubbing the inner surface with a fine needle. The effectiveness of endothelium removal was confirmed by

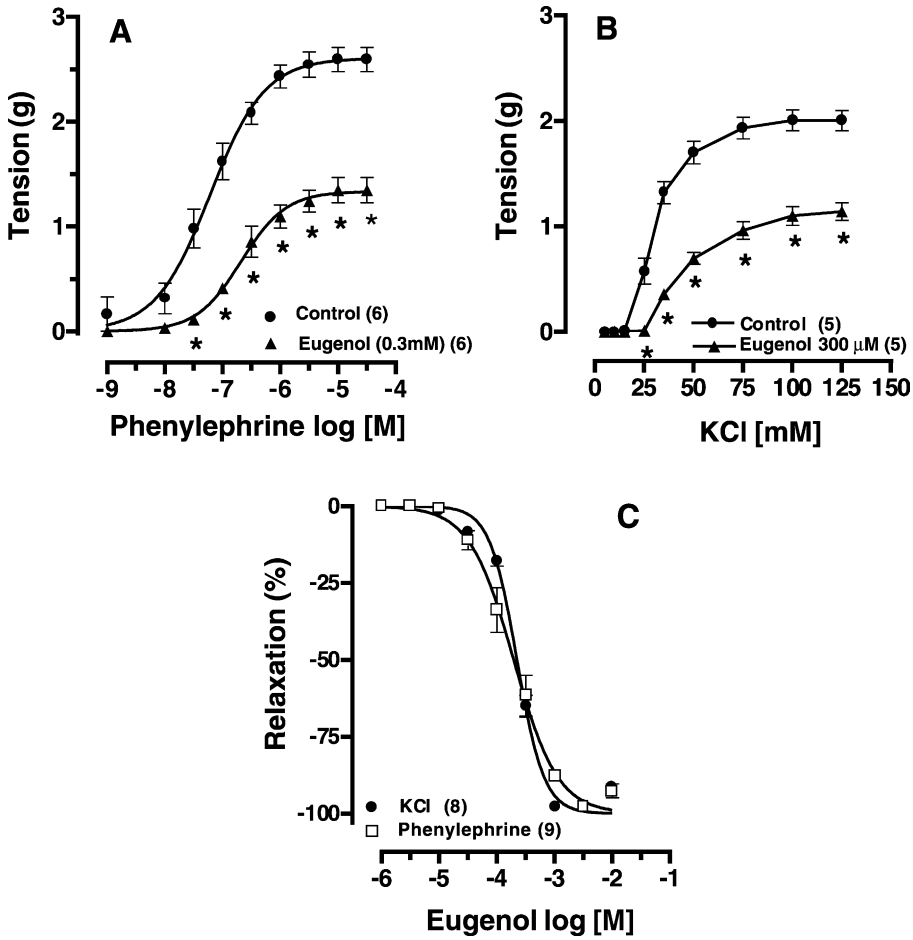

Fig. 1. Inhibitory effects of eugenol (300 μM) (A) on the contraction induced by PHE (10^{-9}–10^{-5} M) (B) and KCl (5–125 mM) on isometric tension from rat thoracic aorta rings. (C) Vasorelaxant effects of eugenol (10^{-6}–10^{-2} M) on endothelium-intact thoracic aorta rings precontracted with PHE (10^{-7} M) and KCl (75 mM). Results are presented as mean±S.E.M.; *P<.05; Eugenol vs. control (A and B); ANOVA followed by Tukey's test. Numbers of rats in parentheses.

the absence of relaxation induced by acetylcholine (10^{-6} M) in aortic rings precontracted with PHE (10^{-7} M).

2.1.6. Effects of endothelial mediators on eugenol-induced relaxation

To investigate the possible involvement of nitric oxide (NO) and guanylyl cyclase in the vasorelaxing effects of eugenol, endothelium-intact aortic rings were preincubated separately with the NO synthase inhibitor N^{w}-nitro-L-arginine (L-NAME, 10^{-4} M) and the guanylyl cyclase inhibitor methylene blue (10^{-5} M) for 15 min. Cumulative concentrations of eugenol (10^{-6}–10^{-2} M) were then applied during the sustained (tonic) phase of PHE (10^{-7} M)-induced contraction. The effects of these inhibitors were studied by comparing the amount of relaxation induced by eugenol in the absence or presence of these inhibitors as well as with endothelium-denuded preparations.

2.2. Drugs

The drugs used in the present experiments were: eugenol; L-PHE hydrochoride, acetylcholine choride; L-NAME; Tween 80, methylene blue and nifedipine (all from Sigma, St. Louis, MO, USA); sodium pentobarbital (Fontoveter, SP, SP, BR). Stock solution of eugenol was prepared in deionized water and, if necessary, with the addition of Tween 80 (5%). The final concentration of Tween 80 was kept at less than 0.05% and this was confirmed to have no effect on tension development. Since nifedipine has low solubility in Krebs solution, it was first diluted in ethanol 95% to be added to the bath. The final

Table 1
Inhibitory effects of eugenol on the PHE- and KCl-induced contractions

	Control		Eugenol	
	pD_2	E_{max} (%)	pD_2	E_{max} (%)
PHE	7.32±0.15	2.59±0.11	6.54±0.03*	1.38±0.08*
KCl	30.43±1.33	2.00±0.19	44.24±1.68*	1.14±0.08*

E_{max} and pD_2 values from rat thoracic aorta rings concentration–response curves to PHE (10^{-9}–10^{-5} M) and KCl (5–125 mM), in the absence (Control) and in the presence of eugenol (300 μM). Results are presented as mean±S.E.M.

* P<.05, eugenol vs. control; ANOVA followed by Tukey's test.

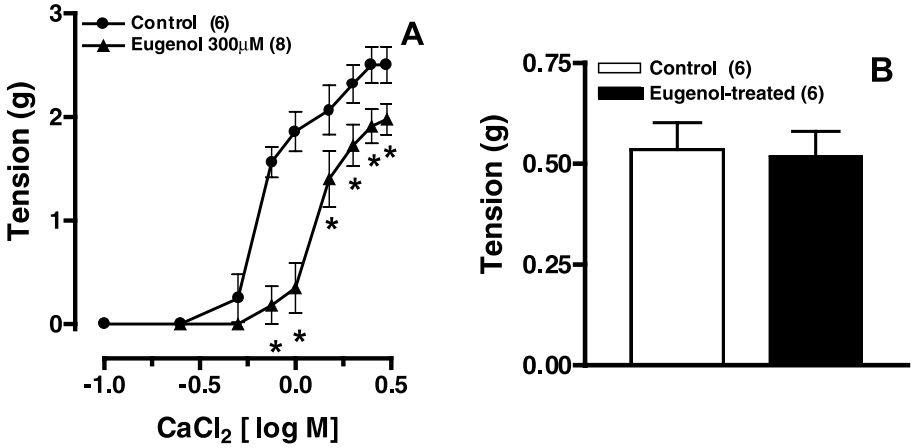

Fig. 2. (A) Inhibitory effects of eugenol (10^{-6}–10^{-2} M) on the cumulative contraction curve dependent on extracellular Ca^{2+} influx (0.25, 0.5, 0.75, 1, 1.5, 2, 2.5 and 3 mM) induced by PHE (10^{-7} M) in Ca^{2+}-free solution of endothelium-intact thoracic aorta rings. (B) Similar contractions to PHE (10^{-6} M) for aortic rings bathed with Ca^{2+}-free solution in the absence and presence of eugenol (300 μM). Results are presented as mean±S.E.M.; *P<.05; Eugenol vs. control; ANOVA followed by Tukey's test. Number of rats in parentheses.

ethanol concentration caused no damage to the preparations.

2.3. Statistical analysis

The −log concentration (pD_2) and the EC_{50} of the drug required to produce 50% of the maximal response were determined by computer-assisted interactive nonlinear regression analysis (GraphPad Prism, San Diego, CA, USA). The maximal response (E_{max}) produced by PHE, KCl and $CaCl_2$ was expressed in grams and those by eugenol and nifedipine were expressed as the percentage relaxation of the agonist-induced contraction. Data are presented as mean±standard error of the mean (S.E.M.). Statistical evaluations were performed using ANOVA and Student's unpaired t test when applicable. Tukey's post hoc test was used to compare means; P<.05 was taken as significant.

3. Results

3.1. Effects of eugenol on aortic rings tension development

In endothelium-intact preparations, either E_{max} or pD_2 of cumulative concentration–effect curves to PHE (10^{-9}–10^{-4} M) or to KCl (5–125 mM) were depressed by eugenol (300 μM) (Fig. 1A and B) (Table 1). The vasorelaxing effect of eugenol (10^{-6}–10^{-2} M) on PHE (10^{-7} M) and KCl (75 mM) precontracted aortic rings was similar, showing no significant difference of either maximal response or sensitivity (Fig. 1C).

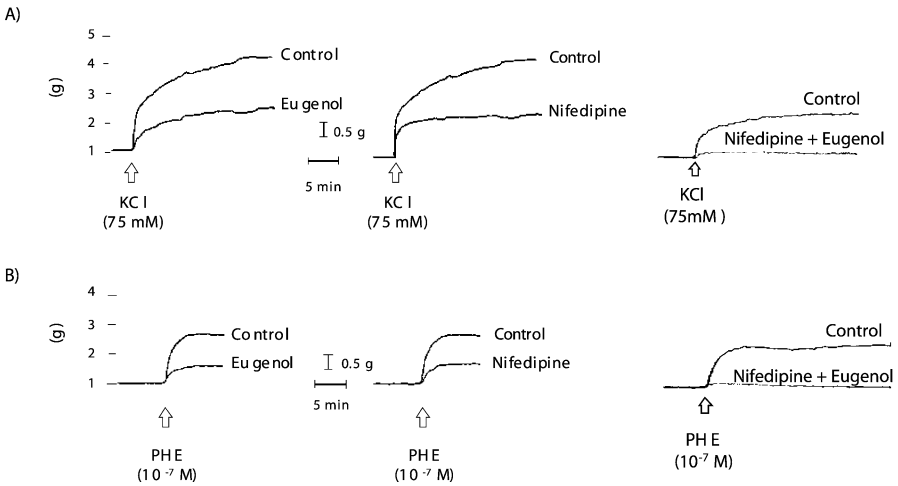

Fig. 3. (A) Typical records of eugenol (300 μM) and nifedipine (0.01 μM) inhibitory actions on the contractions induced by KCl (75 mM) (B) and phenylephrine (10^{-7} M) in rat thoracic aorta rings (n=6); and records of these inhibitory actions when the rings were incubated with eugenol plus nifedipine.

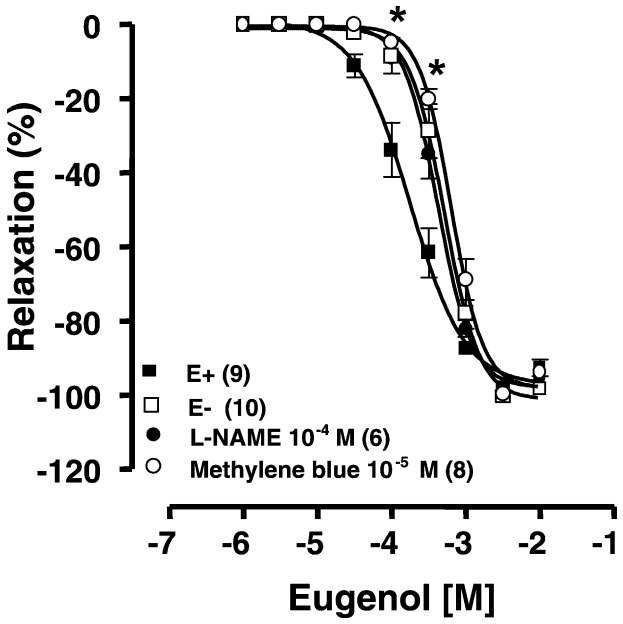

Fig. 4. Effects of L-NAME and methylene blue treatments on eugenol (10^{-6}–10^{-2} M)-induced relaxation in endothelium-intact (E+) thoracic aorta rings. Endothelium-denuded (E−) preparations were also compared. Aortic rings were precontracted with PHE (10^{-7} M), and the change in tension is expressed as a percentage of the active tension generated by phenylephrine (E+). Results are presented as mean±S.E.M.; *P<.05; L-NAME, MB, E− vs. E+; ANOVA followed by Tukey's test. Number of rats in parentheses.

3.2. Effects of eugenol on extracellular Ca^{2+}-induced contraction activated by PHE

In the Ca^{2+}-free solution plus 10^{-6} M PHE, cumulative addition of $CaCl_2$ (0.25–3.0 mM) induced a stepwise tension increment of aortic rings (Fig. 2A). The E_{max} attained at 3 mM Ca^{2+} was 2.50±0.17 g and the sensitivity (pD_2) was 0.15±0.06. When the aortic rings were treated with eugenol (300 μM), the $CaCl_2$-induced contractions were attenuated, suggesting that Ca^{2+} influx was probably reduced by eugenol; E_{max} value attained at 3 mM Ca^{2+} was 2±0.16 g (P<.05, eugenol vs. control) and pD_2 0.10±0.05 (P<.05, eugenol vs. control) (Fig. 2A).

3.3. Possible action of eugenol on sarcoplasmic reticulum calcium release induced by PHE

When extracellular Ca^{2+} was removed by using a Ca^{2+}-free solution, PHE (10^{-6} M) induced a transient contraction due to the release of intracellular Ca^{2+}. In our experiments, aortic rings in Ca^{2+}-free solution showed similar PHE (10^{-6} M)-induced contractions either in the absence or in the presence of eugenol (300 μM) (eugenol 0.52±0.06 g vs. control 0.53±0.07 g, n=6), suggesting that eugenol does not alter the sarcoplasmic reticulum function (Fig. 2B).

3.4. Comparison of the inhibitory effects of eugenol and nifedipine on K^+- and PHE-induced contractions

KCl (75 mM)- and PHE (10^{-7} M)-induced contractions were reduced when the aortic rings were treated either by eugenol (300 μM) or by nifedipine (0.01 μM) (Fig. 3A and B). Despite the great difference between the used concentrations, which denotes the higher capacity of nifedipine to block Ca^{2+} channels, the percentage reduction of tension induced by eugenol was similar to that induced by nifedipine, (%, KCl: eugenol −41.69±3.93 and nifedipine −57.11±5.30 vs. control) and (%, PHE: eugenol −54.09±6.36 and nifedipine −46.35±5.04 vs. control), suggesting similar actions. However, when the aortic rings were treated with eugenol (300 μM) plus nifedipine (0.01 μM), the inhibitory effect was enhanced (%, KCl −80.81±1.45 and PHE −83.94±3.74 vs. control).

3.5. Endothelial dependence of the effects of eugenol

To investigate whether eugenol-induced relaxation was endothelium dependent or not, experiments were performed in both endothelium-intact and -denuded aortic rings precontracted by PHE (10^{-7} M). Concentration–response curves for cumulative eugenol (10^{-6}–10^{-2} M) treatment (Fig. 4) showed no difference in the maximal response. However, endothelial damage reduced the sensitivity (Table 2). Thus, it appears that the vasorelaxation caused by eugenol was, in part, endothelium-dependent.

3.6. Effects of endothelial mediators in eugenol-induced vasorelaxation

To investigate the role of NO or other endothelial-derived guanylyl cyclase stimulator contraction–response curves for cumulative eugenol (10^{-6}–10^{-2} M) treatment in endothelium-intact aortic rings precontracted with PHE (10^{-7} M) were performed before and after treatment with L-NAME (10^{-4} M) or methylene blue (10^{-5} M). The results indicated that treatment with these inhibitors did not affect the maximal relaxation effect. However, both L-NAME and methylene blue (Fig. 4 and Table 2) signific-

Table 2
Effects of endothelial mediators on eugenol-induced vasorelaxation

	pD_2	E_{max} (%)	n
E+	3.78±0.11	−97.87±1.53	9
E−	3.26±0.10*	−100.0±1.05	10
L-NAME	3.38±0.05*	−98.21±1.17	6
MB	3.20±0.05*	−99.48±0.95	8

E_{max} and pD_2 values of the eugenol-induced concentration-dependent (10^{-6}–10^{-2} M) relaxation in endothelium-intact (E+) aortic rings precontracted with PHE (10^{-7} M) compared to L-NAME (10^{-4} M) and methylene blue (MB) (10^{-5} M) treatment, and endothelium-denuded (E−) aortic rings. Results are presented as mean±S.E.M.

* P<0.05, L-NAME; MB and E− vs. E+; ANOVA followed by Tukey's test.

antly reduced the sensitivity. These results were very similar to those obtained with endothelium-denuded preparations, suggesting that inhibitory actions of eugenol affected by removal of endothelium were enhanced by NO (Fig. 4 and Table 2). The same results were obtained when the aortic rings were precontracted by KCl (75 mM) (data not shown).

4. Discussion

This study demonstrates that eugenol acts in aortic rings producing a concentration-dependent depressant effect on KCl- and PHE-induced contractions. These effects suggest that eugenol might be acting as a Ca^{2+} antagonist. Our results also showed that the inhibitory effects of eugenol were attenuated by endothelial denudation of aortic rings. Treatments with L-NAME or methylene blue also suggested that eugenol induced the release of an endothelium-derived vasodilator factor that acts via the NO–guanylyl cyclase pathway. These findings suggest an endothelial modulation occurring mainly at small concentrations of eugenol.

Smooth muscle contracts in response to the activation of voltage-dependent and receptor-operated Ca^{2+} channels (Rüegg et al., 1989; Horowitz et al., 1996; Taggart et al., 1997; Karaki et al., 1997). Activator Ca^{2+} is also released from intracellular stores upon activation (Horowitz et al., 1996; Savineau and Marthan, 2000). In the present study, eugenol inhibited either the PHE (10^{-7} M)- or the KCl (75 mM)-induced contractions. These observations suggested that eugenol might interfere with both voltage- and receptor-operated Ca^{2+} channels reducing the Ca^{2+} influx, and consequently, contraction. These results are in accordance to those found by Nishijima et al. (1998, 1999) using eugenol concentration higher than 0.1 mM, in rabbit aortic rings precontracted by high K^+ solution, and with those found by Lin et al. (1999) using isoeugenolol (a eugenol isomer), in rat thoracic aorta precontracted by PHE and high K^+ solution.

Our experiments showed that preincubation with eugenol could effectively antagonize, in a concentration-dependent manner, Ca^{2+}-induced contractions suggesting that eugenol reduced Ca^{2+} influx through voltage-operated Ca^{2+} channels in the isolated aortic smooth muscle. These observations are consistent with reports suggesting that eugenol and other phenolic compounds may act as a calcium antagonist (Nishijima et al., 1998, 1999).

Since eugenol inhibitory effects were similar to those elicited by Ca^{2+} channel blockers, we compared its antagonist action to the one produced by nifedipine, a known Ca^{2+} channel blocker (Sensch et al., 2000). The Ca^{2+} channel blockers are selective inhibitors of the L-type Ca^{2+} channel (Godfraind et al., 1986). In various types of smooth muscle, Ca^{2+} channel blockers strongly inhibit the high K^+-induced increase in $[Ca^{2+}]_i$ (De Feo and Morgan, 1985; Muraki et al., 1993). Eugenol, like nifedipine, inhibited high K^+-induced contraction suggesting that it may block voltage-dependent Ca^{2+} channels. Our results are in accordance with those observed with methyleugenol which, like nifedipine, also inhibited KCl-induced contractions (60 mM) of guinea-pig isolated ileum (Lima et al., 2000). However, Ca^{2+} influx in smooth muscle cells is not only due to the L-type Ca^{2+} channel activation, which is sensitive to Ca^{2+} channel blockers, but also due to Ca^{2+} release and Ca^{2+} influx through non-L-type Ca^{2+} channel (Benham et al., 1986; Karaki et al., 1997). Contractions induced by α_1-adrenoceptor are less sensitive to Ca^{2+} channel blockers than is the high K^+-induced contraction (Karaki et al., 1997). Our results showed that eugenol reduced the contraction resulting from α_1-adrenoceptor stimulation, indicating that this compound also reduces the Ca^{2+} influx through receptor-operated Ca^{2+} channels. Reinforcing these suggested eugenol-antagonist actions, when the aortic rings were incubated with nifedipine plus eugenol, the tension was dramatically reduced, more than when these agents were used separately. Moreover, in the presence of nifedipine, the influence of voltage-dependent Ca^{2+} entry was eliminated (Mekata, 1974) and the association with eugenol enhanced relaxation by an additional Ca^{2+} channel antagonism. So, it seems that eugenol acts as a Ca^{2+} channel antagonist either through voltage-dependent or through receptor-operated Ca^{2+} channels.

Since PHE was the vasoconstrictor used, eugenol inhibitory actions could also be due to the blockade of sarcoplasmic reticulum Ca^{2+} channels activated by IP_3 (Islam et al., 1996). To investigate this issue, experiments were performed in a Ca^{2+}-free Krebs solution. In such condition tension development activated by PHE should result only from Ca^{2+} released upon activation of IP_3-sensitive Ca^{2+} channels. Our results showed that the PHE-induced transient contractions from eugenol-treated aortic rings were similar to control suggesting that eugenol inhibitory actions are restricted to the sarcolemmal Ca^{2+} channels.

We also investigated the putative participation of the endothelium modulating eugenol actions. Previous reports (Nishijima et al., 1998) already suggested that endothelial factors might affect the effects of eugenol. Our experiments showed that eugenol inhibited PHE-induced contractions either in endothelium-denuded or in endothelium-intact aortic rings. However, despite the final eugenol-induced relaxation to be similar in both preparations, we should stress that the endothelial modulation occurs mainly at small concentrations of eugenol.

In 1980, Furchgott and Zawadski discovered that endothelial cells might release a potent relaxing factor named endothelium-derived relaxing factor (EDRF) that modulates the vascular response. Chemical identity of EDRF is now widely recognized as NO (Moncada et al., 1991). The relaxation of rat aorta in response to NO either generated by endothelium, in response to the stimulation with vasoactive substances, or liberated spontaneously from NO

donors, is mediated by an increase of cGMP contents in vascular smooth cells as a result of activation of soluble guanylyl cyclase (Andreopoulos and Papapetropoulos, 2000). To address this question, L-NAME and methylene blue were used. They have been reported to inactivate the NO system or inhibit the activation of guanylyl cyclase, respectively (Thorin et al., 1998). These results indicated that the maximal vasorelaxing actions of eugenol were not affected even in the presence of L-NAME or methylene blue. However, the sensitivity to eugenol was significantly reduced by L-NAME and methylene blue treatments reproducing the same behavior observed in endothelium-denuded aortic rings. Altogether, these results suggest that the vasorelaxation caused by eugenol is partially endothelium-dependent, mediated by the NO–guanylyl cyclase pathway. The endothelial dependence is seen at small concentrations of eugenol, however, at higher concentrations, the Ca^{2+} channel blocker action prevails.

One last aspect to be considered is related to the very high concentrations of eugenol used in this investigation. The concentrations which produce effects against PHE- and Ca^{2+}-induced contractions are in the mM range, a concentration similar to the ones used by other authors (Nishijima et al., 1999). Even though, until now eugenol or related compounds are not considered as a vasodilator that could be used as a pharmacological agent. However, toxic effects are already described (Wright et al., 1995) including one following ingestion of clove oil (Brown et al., 1992), facts that bring relevance to the knowledge for eugenol effects even at high concentrations.

In summary, results presented here suggest that eugenol acts as a calcium antagonist inhibiting the contractions induced by activation of voltage- and receptor-operated Ca^{2+} channel; its inhibitory action might be, at least in part, modulated by an endothelium-derived factor via a NO–guanylyl cyclase pathway.

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