JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 359–365

Received August 28, 2002
Accepted November 29, 2002
DOI 10.1211/002235702694
ISSN 0022-3573

Endothelium-dependent and -independent vasodilator effects of eugenol in the rat mesenteric vascular bed

David Neil Criddle, Socorro Vanesca Frota Madeira and Roberto Soares de Moura

Abstract

The possible involvement of the endothelium in the vasodilator action of eugenol was investigated in the mesenteric vascular bed (MVB) of the rat. Bolus injections of eugenol (0.2, 2 and 20 μmol) and acetylcholine (ACh; 10, 30 and 100 pmol) induced dose-dependent vasodilator responses in noradrenaline-precontracted beds that were partially inhibited by pretreatment of the MVB with deoxycholate (1 mg mL^{-1}) to remove the endothelium ($\sim$14% and $\sim$30% of the control response remaining at the lowest doses of ACh and eugenol, respectively). The vasodilator effect of glyceryl trinitrate (1 μmol) was unaltered by deoxycholate. In the presence of either N$^{\omega}$-nitro-L-arginine methyl ester (300 μM) or tetraethylammonium (1 mM) the response to ACh was partially reduced, whereas eugenol-induced vasodilation was unaffected. Similarly the vasodilator effect of eugenol was not inhibited by indometacin (3 μM). Under calcium-free conditions the vasoconstrictor response elicited by bolus injections of noradrenaline (10 nmol) was dose-dependently and completely inhibited by eugenol (0.1–1 mM). Additionally, the pressor effects of bolus injections of calcium chloride in potassium-depolarized MVBs were greatly reduced in the presence of eugenol (0.1 mM), with a maximal reduction of $\sim$71% of the control response. Our data showed that eugenol induced dose-dependent, reversible vasodilator responses in the rat MVB, that were partially dependent on the endothelium, although apparently independent of nitric oxide, endothelium-derived hyperpolarizing factor or prostacyclin. Furthermore, an endothelium-independent intracellular site of action seemed likely to participate in its smooth muscle relaxant properties.

Laboratório de Farmacologia dos Canais Iônicos (LAFACI), Departamento de Ciências Fisiológicas, CCS, Universidade Estadual do Ceará, Av. Paranjana 1700, Fortaleza CE 60740-000, Brazil

David Neil Criddle, Socorro Vanesca Frota Madeira

Departamento de Farmacologia, Centro Biomedico – IB, Universidade do Estado do Rio de Janeiro, Av. 28 de Setembro, 81, 20 551 – Rio de Janeiro, Brazil

Roberto Soares de Moura

Correspondence: D. N. Criddle, Laboratório de Farmacologia dos Canais Iônicos (LAFACI), Departamento de Ciências Fisiológicas, Centro de Ciências da Saúde, Universidade Estadual do Ceará, Av. Paranjana 1700, Campus Itaperí, Fortaleza CE 60740-000, Brazil. E-mail: criddle@uece.br

Funding: Socorro Vanesca Frota Madeira was supported by FUNCAP (Brasil).

Introduction

Eugenol is a naturally occurring aromatic substance that is present in a variety of plant essential oils, such as clove oil, and is commonly used as a food flavouring and in fragrances. This aromatic substance has found widespread therapeutic application in dentistry for the treatment of toothache due to its analgesic properties (Shibata et al 1994), its beneficial effect probably being due to inhibition of cyclooxygenase enzyme (Thompson & Eling 1989; Dohi et al 1991). However, many other effects of eugenol have been documented, including activation of calcium and chloride channels in rat dorsal root ganglion cells (Ohkubo & Kitamura 1997), inhibition of guinea-pig cardiac calcium and potassium currents (Sensch et al 2000), nerve-mediated contraction of rat bladder (Patacchini et al 1990) and relaxant actions in vascular and visceral smooth muscle (Reiter & Brandt 1985; Nishijima et al 1998, 1999).

Although the vasodilator property of eugenol has been recognised for some time (Hume 1983), its underlying mechanism of action remains unclear. Interestingly, Nishijima et al (1998) suggested that the vasorelaxation of histamine-precontracted rabbit ear artery induced by eugenol might be influenced by the endothelium. Since the majority of studies addressing the vascular effects of eugenol have been performed in large conduit arteries, in which nitric oxide is the predominant factor released from the endothelium (Nagao et al 1992), we were interested to evaluate the actions of this compound in small resistance vessels. Thus, in this study we have used the perfused mesenteric vascular bed to characterize the vasodilator effects of eugenol, a preparation in which perfusion pressure is governed by resistance vessels with an intact endothelium (Criscione et al 1984). We have assessed the influence of the endothelium

on the action of eugenol, comparing its effects with those of acetylcholine and glyceryl trinitrate, endothelium-dependent and -independent vasodilators, respectively.

Materials and Methods

Mesenteric vascular bed

Institutional approval for the experimental protocols adopted in this study was obtained. Mesenteric vascular beds from male Wistar rats (250–350 g) were isolated and perfused according to Criddle et al (1997) using a modified method of McGregor (1965). Briefly, the mesenteric vascular beds were mounted at a constant flow of 4 mL min^{-1} and perfused with a modified Krebs-Henseleit solution of the following composition (mM); glucose 11.0, NaCl 118.0, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 1.2, $MgSO_4$ 1.2, gassed with 95% O_2, 5% CO_2, at 37 °C (pH 7.4). Perfusion pressure changes were monitored using a pressure transducer. Preparations were set up and left to equilibrate for 30–40 min before the addition of any drugs.

Perfusion pressure was tonically elevated using 6 μM noradrenaline added to the perfusion fluid reservoir. Initially, when noradrenaline-induced vascular tone had stabilized to a plateau level, control responses to bolus injections of eugenol (0.2, 2 or 20 μmol), acetylcholine (ACh; 10, 30 or 100 pmol) and glyceryl trinitrate (1 μmol) directly into the perfusion stream, at a site 2 cm from the mesenteric artery, were obtained. The volume of bolus injections was always < 50 μL. Following the initial control responses to the test substances, noradrenaline was removed from the perfusion stream and basal tonus re-established on washout. We examined the effects of removing the endothelium on the vasorelaxation induced by eugenol and control substances, using the following strategies. Firstly, following a return to resting conditions after the initial control responses had been obtained, deoxycholate (1 mg mL^{-1}) was included in the perfusion stream for 3 min to remove the endothelium from the mesenteric vascular bed. Subsequently noradrenaline was reapplied to the mesenteric vascular bed, to augment basal tone once again, and the bolus doses of eugenol, ACh and glyceryl trinitrate were repeated. Similarly in separate experiments, the possible influence of nitric oxide, potassium channels or prostaglandins was examined by assessing the effects of N^{ω}-nitro-L-arginine methyl ester (L-NAME; 300 μM), tetraethylammonium (TEA; 1 mM) and indometacin (3 μM), respectively, on vasodilations induced by eugenol, ACh and glyceryl trinitrate. After the initial control responses had been obtained, washout of noradrenaline and recovery of baseline perfusion pressure, these inhibitory substances were included in the perfusion stream at fixed concentrations for a 30-min period before reapplication of noradrenaline and vasodilator agents.

Also investigated were the effects of eugenol (0.1–1 mM) on pressor responses induced by noradrenaline (10 nmol) in nominally calcium-free conditions. The protocol adopted consisted of initially obtaining control responses to bolus injections of KCl (120 μmol) and noradrenaline (10 pmol) in normal Krebs-Henseleit solution, after which the preparation was perfused for 2 min with a calcium-free solution of the following composition (mM); glucose 11.0, NaCl 17.0, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 0, $MgSO_4$ 1.2. Responses to KCl (120 μmol) and noradrenaline (10 nmol) were then obtained in calcium-free solution in the absence and presence of eugenol, applied in increasing concentrations (0.1–1 mM), with KCl application employed as a positive control to verify that vasoconstriction under calcium-free conditions was the result of release of intracellular calcium and not dislocation of membrane-bound calcium.

Also performed were experiments to investigate the effects of eugenol (0.1 mM) on pressor responses to calcium chloride in calcium-free potassium-depolarized preparations. Briefly, the mesenteric vascular bed was perfused with a modified Krebs-Henseleit solution of the following composition (mM); glucose 11.0, NaCl 17.0, $NaHCO_3$ 25.0, KCl 100, KH_2PO_4 1.2, $CaCl_2$ 0, $MgSO_4$ 1.2. Bolus injections of $CaCl_2$ (0.1–300 μmol) were applied to the mesenteric vascular bed to induce vasoconstriction. Responses were obtained in the absence and presence of eugenol (0.1 mM), included at steady concentration in the perfusion fluid.

Data analysis

The results were expressed as mean ± s.e. of n observations. Values were analysed using a one-way analysis of variance test, and were considered to differ significantly when $P < 0.05$.

Drugs

All reagents were of analytical grade and eugenol, L-NAME, indometacin, deoxycholate, tetraethylammonium, acetylcholine and glyceryl trinitrate were all purchased from Sigma Chemical Company (MO).

Stock solutions of eugenol were prepared in absolute ethanol and diluted in Krebs-Henseleit solution on the day of the experiment. All solutions were sonicated before use.

Results

Comparative vasodilator effects of eugenol, ACh and glyceryl trinitrate in the rat mesenteric vascular bed

Basal perfusion pressure (27.5 ± 1.7 mmHg) was increased by constant perfusion of noradrenaline (6 μM) to a stable plateau of 127.5 ± 8.4 mmHg (n = 8). Bolus injections of eugenol (0.2, 2 or 20 μmol) elicited rapid transient, dose-dependent decreases of perfusion pressure, indicative of vasodilation, with a reduction of 84.3 ± 2.9% of the noradrenaline-induced pressure at the highest dose tested (Figure 1, n = 8). Similarly ACh (10, 30 or 100 pmol) induced transient, dose-dependent decreases of perfusion pressure with a reduction of 68.2 ± 5.8% at the highest dose utilized (n = 8). Glyceryl trinitrate (1 μmol) elicited a decrease of perfusion pressure of 45.5 ± 4.9% (n = 8) (Figure 1).

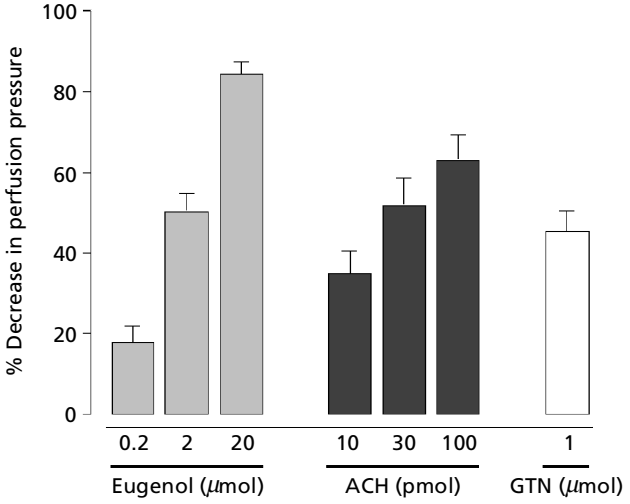

Figure 1 Vasodilator responses of eugenol (0.2, 2 or 20 μmol), acetylcholine (ACh; 10, 30 or 100 pmol) and glyceryl trinitrate (GTN; 1 μmol) on noradrenaline (6 μM)-precontracted rat mesenteric vascular beds. Data are expressed as the mean ± s.e.m. (n = 8).

Actions of deoxycholate on eugenol-, ACh- and glyceryl trinitrate-induced vasodilation of noradrenaline-precontracted mesenteric vascular beds

In separate experiments noradrenaline (6 μM) induced a stable rise in perfusion pressure of 96.0 ± 10.0 mmHg (n = 6). Bolus injections of acetylcholine (10, 30 or 100 pmol), eugenol (0.2, 2 or 20 μmol) and glyceryl trinitrate (1 μmol) induced rapid, transient dose-dependent decreases of perfusion pressure, as previously observed (Figure 2A and C). After deoxycholate (1 mg mL^{-1}) treatment of the mesenteric vascular bed, to remove the endothelial cell layer, subsequent addition of noradrenaline (6 μM) induced a stable rise of 54.6 ± 9.9 mmHg (n = 6). The vasodilator effects of eugenol (0.2, 2 or 20 μmol) were significantly inhibited after deoxycholate treatment at all doses tested, the effect being more pronounced at lower doses of eugenol (Figure 2B). The vasodilator responses of ACh were more markedly and uniformly inhibited after deoxycholate treatment at all doses, although a small vasodilator component still remained (26% of the control response at the highest dose used). In contrast, the effect of a bolus injection of glyceryl trinitrate (1 μmol) remained unaltered after deoxycholate application (Figure 2A, B and C).

Effects of L-NAME and tetraethylammonium (TEA) on eugenol-, ACh- and glyceryl trinitrate-induced vasodilation of noradrenaline-precontracted mesenteric vascular beds

In the presence of L-NAME (300 μM), noradrenaline (6 μM) induced a stable rise of perfusion pressure of 88.0 ± 6.5 mmHg (Figure 3B, n = 6). The vasodilator responses to acetylcholine were significantly reduced by

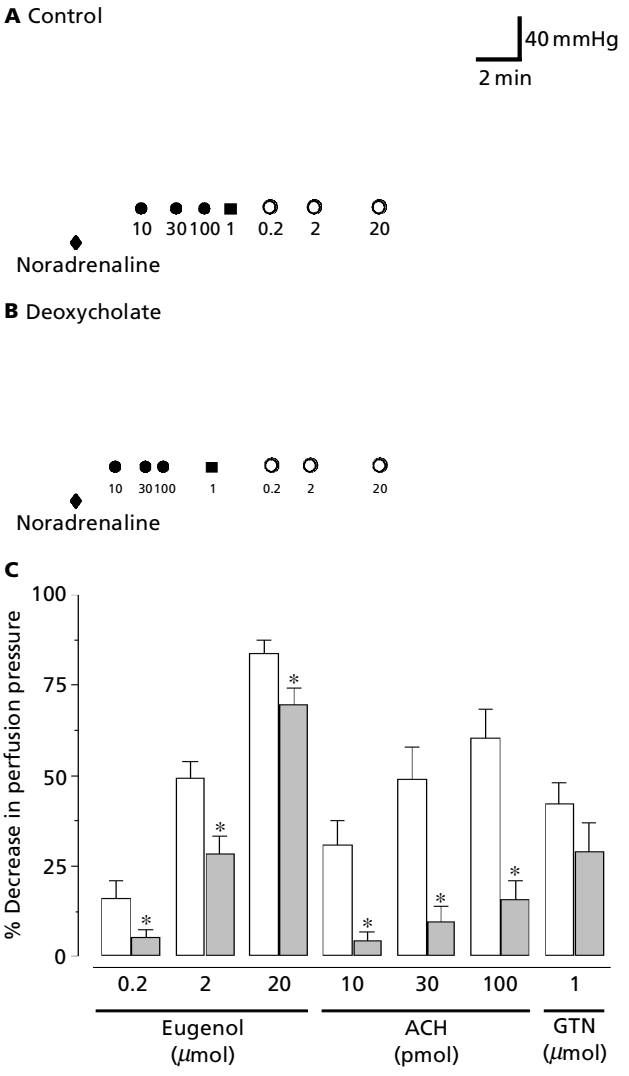

Figure 2 Typical traces showing the vasodilator responses of eugenol (0.2, 2 or 20 μmol; ○), acetylcholine (ACh; 10, 30 or 100 pmol; ●) and glyceryl trinitrate (GTN; 1 μmol; ■) in (A) control and (B) deoxycholate-treated, noradrenaline-precontracted rat mesenteric vascular beds. (C) Mean data from these experiments showing control vasodilator responses (white bars) and those after deoxycholate-treatment (grey bars). Data are expressed as the mean ± s.e.m. (n = 6) (*$P < 0.05$).

L-NAME at all doses, although a considerable vasodilator component still remained. In contrast, neither the effect of eugenol (0.2, 2 or 20 μmol) nor glyceryl trinitrate (1 μmol) was significantly altered in the presence of L-NAME (Figure 3A, B and C, n = 6).

In separate experiments, noradrenaline (6 μM) induced stable rises in perfusion pressure of 93.3 ± 8.7 and 94.0 ± 10.9 mmHg, before and after inclusion of TEA (1 mM) in the perfusion fluid, respectively (n = 6). ACh-induced vasodilator responses were incompletely, but significantly inhibited by TEA, with 71.9% of the control response remaining at the highest dose used (Figure 4). In contrast, neither the effect of eugenol (0.2, 2 or 20 μmol)

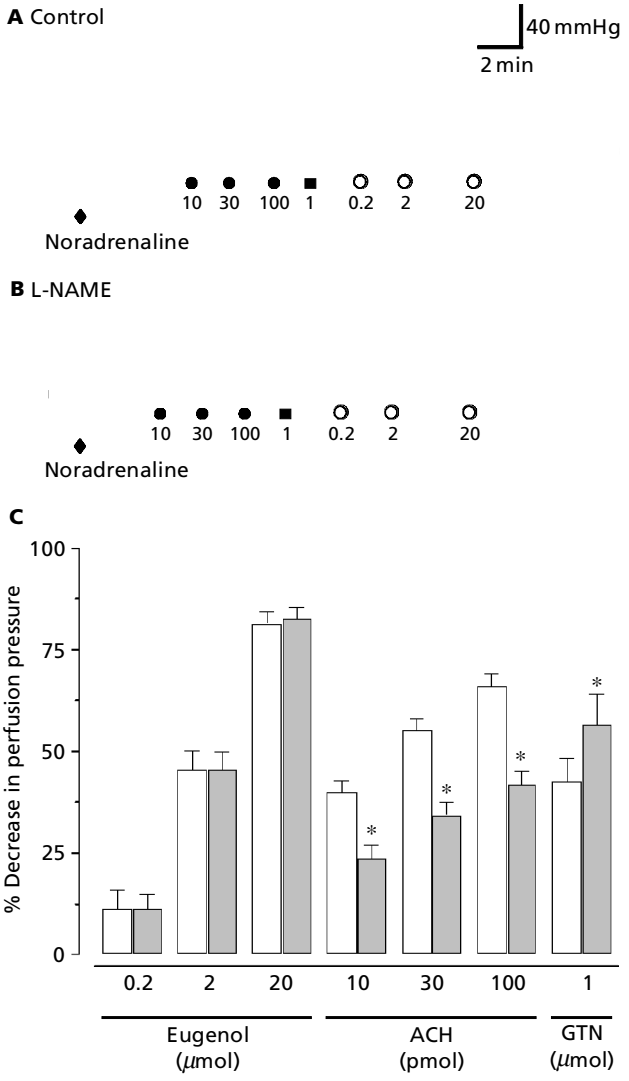

Figure 3 Typical traces showing the vasodilator responses of eugenol (0.2, 2 or 20 μmol; ○), acetylcholine (ACh; 10, 30 or 100 pmol; ●) and glyceryl trinitrate (GTN; 1 μmol; ■) in (A) the absence and (B) presence of L-NAME (300 μM in noradrenaline-precontracted rat mesenteric vascular beds. (C) Mean data from these experiments showing control vasodilator responses (white bars) and those in the presence of L-NAME (grey bars). Data are expressed as the mean ± s.e.m. (n = 6) (*$P < 0.05$).

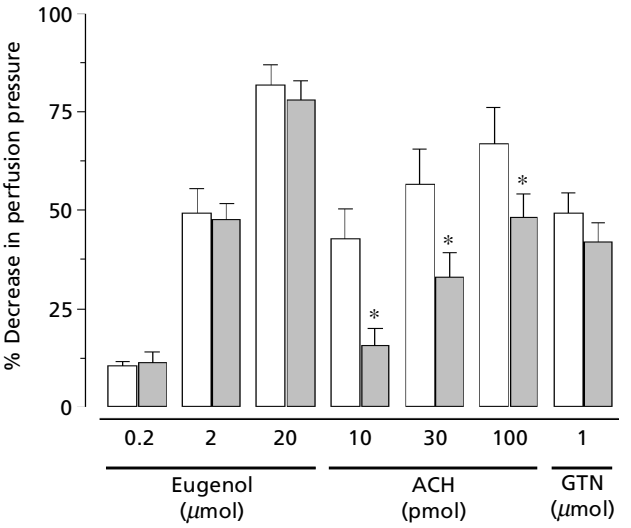

Figure 4 Vasodilator responses of eugenol (0.2, 2 and 20 μmol), acetylcholine (ACh; 10, 30 and 100 pmol) and glyceryl trinitrate (GTN; 1 μmol) in the absence (white bars) and presence (grey bars) of TEA (1 mM) in noradrenaline-precontracted rat mesenteric vascular beds. Data are expressed as the mean ± s.e.m. of six experiments (*$P < 0.05$).

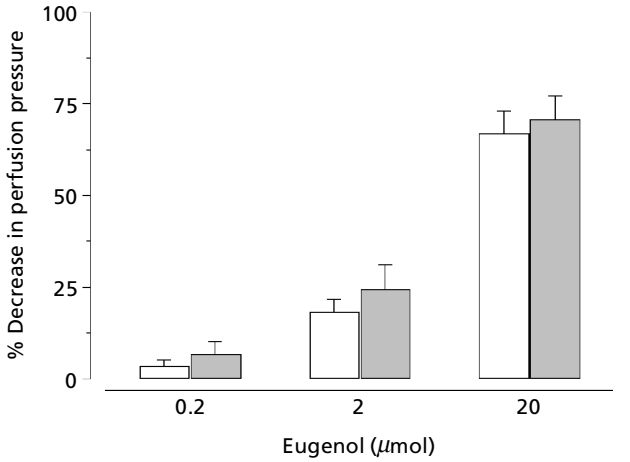

Figure 5 Vasodilator responses of eugenol (0.2, 2 and 20 μmol) in the absence (white bars) and presence (grey bars) of indometacin (3 μM) in noradrenaline-precontracted rat mesenteric vascular beds. Data are expressed as the mean ± s.e.m. (n = 6) ($P < 0.05$).

nor glyceryl trinitrate (1 μmol) was significantly altered in the presence of TEA (Figure 4, n = 6).

Lack of inhibitory effects of indometacin on eugenol-induced vasodilation of noradrenaline-precontracted mesenteric vascular beds

Noradrenaline (6 μM) induced stable rises in perfusion pressure of 106.0 ± 9.3 and 94.6 ± 9.4 mmHg in the absence and presence of indometacin (3 μM), included in the perfusion fluid, respectively (n = 6). The vasodilator responses elicited by eugenol (0.2, 2 or 20 μmol) were not altered in the presence of indomethacin (3 μM) (Figure 5, n = 6).

Inhibitory action of eugenol on the pressor responses to noradrenaline under calcium-free conditions

Bolus injections of noradrenaline (10 nmol) and KCl (120 μmol) elicited rises in perfusion pressure of

117.0 ± 24.9 mmHg (n = 6) and 96.0 ± 10.1 mmHg (n = 6), respectively. Under nominally calcium-free conditions, bolus injection of noradrenaline (10 nmol) induced a mean rise in perfusion pressure of 106.6 ± 11.9 mmHg, that was 90.8% of the control response in normal calcium-containing Krebs-Henseleit solution. In contrast, the vasoconstrictor response to KCl (120 μmol) was completely abolished in the absence of extracellular calcium (Figure 6A). When eugenol (0.1 or 1 mM) was applied in the perfusion fluid the vasoconstrictor response induced by noradrenaline in calcium-free solution was further reduced to 50.6 and 2.1% of the control value, respectively (Figure 6B and C). The response to noradrenaline in time-matched control experiments remained unaltered in the absence of eugenol (Figure 6A and C).

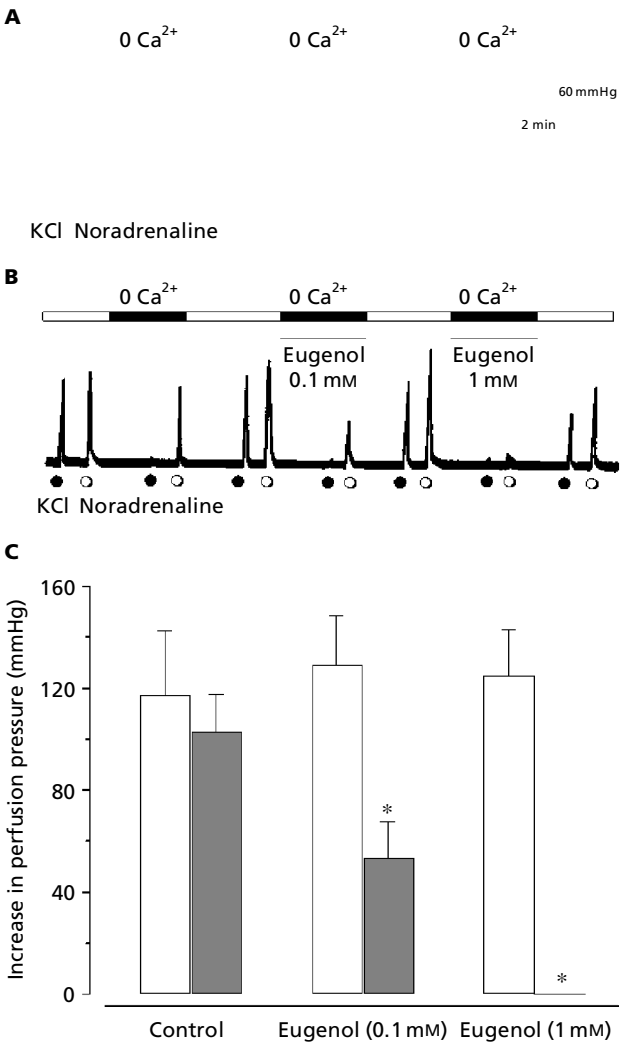

Figure 6 Typical traces showing (A) control responses and (B) the inhibitory effects of eugenol (0.1–1 mM) on noradrenaline (○; 10 nmol)- and KCl (●; 120 μmol)-induced vasoconstrictor responses in calcium-containing (open bar) and calcium-free (filled bar) extracellular solution. (C) Mean data from these experiments showing noradrenaline-induced vasoconstrictor responses in the absence (control time-matched; white bars) and presence of eugenol (grey bars). Data are expressed as the mean ± s.e.m. (n = 6) (*$P < 0.05$).

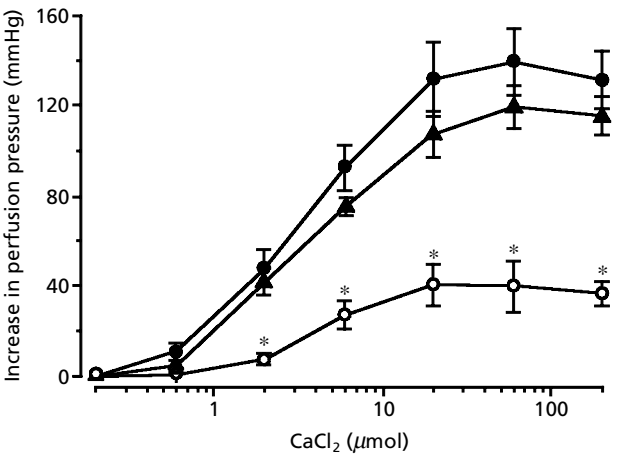

Figure 7 Inhibitory effects of eugenol (0.1 mM) (○; n = 6) on $CaCl_2$-induced vasoconstriction (control ●; n = 12) and response in time-matched control (second curve in the absence of eugenol) experiments (▲; n = 6). Data are expressed as the mean ± s.e.m. (n = 6) (*$P < 0.05$).

Inhibitory action of eugenol on $CaCl_2$-induced vasoconstrictor responses in K^+-depolarized preparations

Under nominally calcium-free conditions in potassium (100 mM)-depolarized preparations, bolus injections of $CaCl_2$ (0.2–200 μmol) induced rapid, dose-related rises of perfusion pressure (Figure 7). These responses were significantly inhibited in the presence of eugenol (0.1 mM), the maximal control increase in perfusion pressure elicited by 60 μmol $CaCl_2$ being reduced by 71.2% (Figure 7). In time-matched control experiments the vasoconstrictor response to calcium did not vary over the experimental period (n = 6, Figure 7).

Discussion

This study has demonstrated for the first time that eugenol exerted dose-dependent, reversible vasodilator effects on the mesenteric vascular bed of the rat. This is consistent with the vasorelaxant action reported previously in other vascular smooth muscle preparations, including the rabbit ear artery (Hume 1983; Nishijima et al 1998) and thoracic aorta (Nishijima et al 1999). The magnitude of the vasodilation induced by eugenol observed in this study was similar to that of acetylcholine and glyceryl trinitrate, delineating the importance of the present findings. In terms of potency, eugenol was similar to glyceryl trinitrate (μmol range) and weaker than ACh (pmol range). Thus, it is possible that eugenol may exert significant vasodilator effects if allowed access to the circulation during therapeutic application.

The use of deoxycholate to remove the endothelial cell layer suggested that the vasodilator effects of eugenol were partially endothelium-dependent, the inhibition by deoxycholate being more pronounced at lower doses of eugenol. The vasodilator action of ACh was even more strongly inhibited after deoxycholate treatment, resulting in approximately 74% decrease in the control response of ACh at the highest dose used. This endothelium-dependency is consistent with previous studies using ACh in this preparation (Moore et al 1990; Parsons et al 1994). Importantly, within this experimental protocol there appeared to be little or no disruption of the smooth muscle of the vascular bed by a detergent action of deoxycholate, since the vasodilator response to glyceryl trinitrate, which occurs via an endothelium-independent elevation of intracellular cyclic GMP in the mesenteric vasculature (Shibata et al 1986; Khan et al 1992), remained unaltered. Thus damage to the vascular preparation as a possible explanation for the reduction in the eugenol vasodilatory response after deoxycholate treatment appeared unlikely from the present findings.

Experiments performed with L-NAME, an inhibitor of nitric oxide synthase (Rees et al 1989), indicated that the vasodilator action of eugenol probably did not occur via the release of nitric oxide from the mesenteric vascular endothelium. In contrast to the lack of inhibition by L-NAME of eugenol-induced effects, the vasodilation elicited by ACh was substantially reduced, although approximately 60% of the response remained in the presence of the synthase inhibitor, in accordance with previous studies in this tissue (Parsons et al 1994; Hansen & Olesen 1997). It should be noted, however, that nitric oxide synthase-independent release of nitric oxide has recently been reported in this preparation (Mendizabal et al 2000), indicating that use of L-NAME in this study may not definitively exclude the involvement of this radical in the vasodilator action of eugenol.

Current evidence suggests that the remaining portion of ACh-induced vasodilation that is resistant to nitric oxide synthase inhibition is likely to be mediated via release of an as yet unidentified endothelium-derived hyperpolarizing factor (EDHF) that acts ultimately by the opening of plasmalemmal potassium channels (Chen et al 1988; Feletou & Vanhoutte 1988; Doughty et al 1999). Indeed in our study the vasodilator effects of ACh were partially inhibited by TEA, a non-specific blocker of calcium-dependent potassium channels (Nelson et al 1990), in agreement with previous data in mesenteric resistance arteries (Hansen & Olesen 1997) and coherent with this hypothesis. However, that the effects of eugenol were not reduced by TEA, would indicate that release of EDHF was not involved in its vasodilator action on mesenteric vessels. As expected, the vasodilator effects of glyceryl trinitrate were resistant to L-NAME and TEA, showing that both agents were selective in their inhibitory actions and thus adequate pharmacological tools within the experimental conditions adopted in this study.

Another possible candidate that might mediate the observed effects of eugenol is prostacyclin, since its stable synthetic analogue iloprost has been shown to elicit vasodilation of the rat mesenteric vascular bed (Yamawaki et al 2000), and noradrenaline has been reported to release prostacyclin in this preparation (Peredo & Adler-Graschinsky 2000). However, in our study we found that the cyclooxygenase blocker indometacin did not alter the vascular responses to eugenol and thus the current data appeared to exclude the involvement of prostaglandins in its mechanism of action.

A recent electrophysiological study has shown inhibitory effects of eugenol on calcium currents in cardiac myocytes similar to those of nifedipine (Sensch et al 2000), which might account for the vasodilator effects encountered in this study. However, this appears unlikely given the physiological characteristics of the rat mesenteric circulation. For example, the mesenteric vasoconstrictor response to noradrenaline has been well characterized, being poorly inhibited (20–30%) by dihydropyridine calcium channel blockers in both in-vitro isolated mesenteric resistance vessels (Cauvin et al 1987, 1988) and in the perfused mesenteric vascular bed (Quast & Baumlin 1991; Criddle et al 1997) of the rat. Thus, since eugenol was able to induce a profound vasodilatory response that was ~84% of the noradrenaline-induced tonic increase in perfusion pressure at the highest dose used (20 μmol), it would appear to be exerting its vascular relaxant effects predominantly via a mechanism(s) independent of voltage-dependent calcium channel inhibition.

This conclusion was further supported by our experiments examining the actions of eugenol on noradrenaline-induced pressor responses under nominally calcium-free conditions. The contribution of extracellular calcium influx into the smooth muscle to noradrenaline-induced vasoconstriction appeared minimal in the mesenteric vascular bed, since the response under calcium-free conditions was approximately 90% of the control effect with calcium present in the perfusion solution. Concentrations of eugenol (0.1 and 1 mM) previously shown to exert relaxant effects in other vascular smooth muscle preparations (Nishijima et al 1998, 1999) dose-dependently and fully inhibited phasic vasoconstrictor responses induced by noradrenaline in calcium-free solution. This would possibly suggest that intracellular calcium release mechanisms were impaired or that eugenol affected contraction at a site distal to this process. The latter alternative appears to be supported by our additional observation that the maximal vasoconstrictor effect of calcium chloride in potassium-depolarized preparations was greatly depressed by eugenol. This was coherent with the findings of Nishijima et al (1999), who suggested that eugenol may suppress the calcium sensitivity of the smooth muscle contractile apparatus, based on simultaneous measurements of contraction and intracellular calcium levels in rabbit thoracic aorta. The possibility exists, however, that eugenol may affect calcium-induced calcium release and at present cannot be excluded on the basis of our current findings.

In conclusion, our data showed that eugenol exerted dose-dependent, reversible vasodilator responses on the resistance blood vessels of the rat mesenteric vascular bed. The action of eugenol was partially dependent on

the endothelium, in agreement with previous observations in histamine-precontracted rabbit ear artery (Nishijima et al 1998), although this does not appear to be mediated via liberation of nitric oxide, EDHF or prostacyclin. Our experiments indicated that an endothelium-independent intracellular site of action seemed likely to participate in its smooth muscle relaxant properties.

References

Cauvin, C., Hwang, O., Yamamoto, M., Van Breemen, C. (1987) Effects of dihydropyridines on tension and calcium-45 influx in isolated mesenteric resistance vessels from spontaneously hypertensive and normotensive rats. *Am. J. Cardiol.* **59**: 116B–122B

Cauvin, C., Weir, S. W., Wallnofer, A., Ruegg, U. (1988) Agonist-induced activation of rat mesenteric resistance vessels: comparison between noradrenaline and vasopressin. *J. Cardiovasc. Pharmacol.* **12** (Suppl. 5): S128–S133

Chen, G., Suzuki, H., Weston, A. H. (1988) Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. *Br. J. Pharmacol.* **95**: 1165–1174

Criddle, D. N., Soares de Moura, R., Greenwood, I. A., Large, W. A. (1997) Inhibitory action of niflumic acid on noradrenaline- and 5-hydroxytryptamine-induced pressor responses in the isolated mesenteric vascular bed of the rat. *Br. J. Pharmacol.* **120**: 813–818

Criscione, L., Muller, K., Forney, M. P. (1984) Endothelial cell loss enhances the pressor response in resistance vessels. *J. Hypertens.* **3** (Suppl. 2): S441–S444

Dohi, T., Anamura, S., Shirakawa, M., Okamoto, H., Tsujimoto, A. (1991) Inhibition of lipoxygenase by phenolic compounds. *Jpn. J. Pharmacol.* **55**: 547–550

Doughty, J. M., Plane, F., Langton, P. D. (1999) Charybdotoxin and apamin block EDHF in rat mesenteric artery if selectively applied to the endothelium. *Am. J. Physiol.* **276**: H1107–H1112

Feletou, M., Vanhoutte, P. M. (1988) Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br. J. Pharmacol.* **93**: 515–524

Hansen, P. R., Olesen, S.-P. (1997) Relaxation of rat resistance arteries by acetylcholine involves a dual mechanism: activation of K^+ channels and formation of nitric oxide. *Pharmacol. Toxicol.* **80**: 280–285

Hume, W. R. (1983) Effect of eugenol on constriction responses in blood vessels of the rabbit ear. *J. Dent. Res.* **62**: 1013–1015

Khan, M. T., Jothianandan, D., Matsunaga, K., Furchgott, R. F. (1992) Vasodilation induced by acetylcholine and by glyceryl trinitrate in rat aortic and mesenteric vasculature. *J. Vasc. Res.* **29**: 20–28

McGregor, D. D. (1965) The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessels of the rat. *J. Physiol.* **177**: 21–30

Mendizabal, V. E., Poblete, I., Lomniczi, A., Rettori, V., Huidobro-Toro, J. P., Adle-Graschinsky, E. (2000) Nitric oxide synthase-independent release of nitric oxide induced by KCl in the perfused mesenteric bed of the rat. *Eur. J. Pharmacol.* **409**: 85–91

Moore, P. K., Al-Swayeh, O. A., Chong, N. W. S., Evans, R. A., Gibson, A. (1990) L-N^G-nitro arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilatation *in vitro*. *Br. J. Pharmacol.* **99**: 408–412

Nagao, T., Illiano, S., Vanhoutte, P. M. (1992) Heterogeneous distribution of endothelium-dependent relaxation resistant to N^G-nitro-L-arginine in rats. *Am. J. Physiol.* **263**: H1090–H1094

Nelson, M. T., Patlak, J. B., Worley, J. F., Standen, N. B. (1990) Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. *Am. J. Physiol.* **259**: C3–C18

Nishijima, H., Uchida, R., Kawakami, N., Shimamura, K., Kitamura, K. (1998) Role of endothelium and adventitia on eugenol-induced relaxation of rabbit ear artery precontracted by histamine. *J. Smooth Muscle Res.* **34**: 123–137

Nishijima, H., Uchida, R., Kameyama, K., Ohkubo, T., Kitamura, K. (1999) Mechanisms mediating vasorelaxing action of eugenol, a pungent oil, on rabbit arterial tissue. *Jpn. J. Pharmacol.* **79**: 327–334

Ohkubo, T., Kitamura, K. (1997) Eugenol activates Ca^{2+}-permeable currents in rat dorsal root ganglion cells. *J. Dent. Res.* **76**: 1737–1744

Parsons, S. J. W., Hill, A., Waldron, G. J., Plane, F., Garland, C. J. (1994) The relative importance of nitric oxide and nitric oxide-independent mechanisms in acetylcholine-evoked dilatation of the rat mesenteric bed. *Br. J. Pharmacol.* **113**: 1275–1280

Patacchini, R., Maggi, C. A., Meli, A. (1990) Capsaicin-like activity of some natural pungent substances on peripheral endings of visceral primary afferents. *Naunyn Schmiedebergs Arch. Pharmacol.* **342**: 72–77

Peredo, H. A., Adler-Graschinsky, E. (2000) Effects of eicosanoids and nitric oxide on the noradrenaline-induce contractions in the rat mesenteric bed. *J. Auton. Pharmacol.* **20**: 163–170

Quast, U., Baumlin, Y. (1991) Cromakalim inhibits contractions of the rat isolated mesenteric bed induced by noradrenaline but not caffeine in Ca^{2+}-free medium: evidence for interference with receptor-mediated Ca^{2+} mobilization. *Eur. J. Pharmacol.* **200**: 239–249

Rees, D. D., Palmer, R. M. J., Hodson, H. F., Moncada, S. (1989) A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. *Br. J. Pharmacol.* **96**: 418–424

Reiter, M., Brandt, W. (1985) Relaxant effects on tracheal and ileal smooth muscles of the guinea pig. *Arzneimittelforschung* **35**: 408–414

Sensch, O., Vierling, W., Brandt, W., Reiter, M. (2000) Effects of inhibition of calcium and potassium currents in guinea-pig cardiac contraction: comparison of β-caryophyllene oxide, eugenol, and nifedipine. *J. Pharmacol.* **131**: 1089–1096

Shibata, T., Ogawa, K., Ito, T., Hashimoto, H., Nakagawa, H., Satake, T. (1986) Role of cyclic GMP of canine vascular smooth muscle in relaxation by organic nitrates. *Jpn Circ. J.* **50**: 1091–1099

Shibata, T., Ohkubo, T., Tsuruda, K., Takahashi, H. (1994) Mode of analgesic action of phenolic dental medicaments through substance P release. *Jpn. J. Oral. Biol.* **36**: 49–59

Thompson, D., Eling, T. (1989) Mechanism of inhibition of prostaglandin H synthase by eugenol and other phenolic peroxidase substrates. *Mol. Pharmacol.* **36**: 809–817

Yamawaki, H., Sato, K., Hori, M., Ozaki, H., Karaki, H. (2000) Platelet-derived growth factor causes endothelium-independent relaxation of rabbit mesenteric artery via the release of a prostanoide. *Br. J. Pharmacol.* **131**: 1546–1552