Resveratrol induces vasorelaxation of mesenteric and uterine arteries from female guinea-pigs

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ABSTRACT

Naturally occurring hydroxystilbenes have been shown to induce vasorelaxation. Here, we studied the mechanism of resveratrol-induced vasorelaxation in different types of blood vessels, namely mesenteric (resistance) and main uterine (conductance) arteries, from female guinea-pigs on day 7 and day 15 of the oestrous cycle. Resveratrol (5–70 μmol/l) induced concentration-dependent relaxation of both mesenteric and uterine arteries preconstricted with either noradrenaline (NA; 10 μmol/l) or KCl (125 mmol/l). Resveratrol was 2-fold more potent in inducing relaxation of mesenteric arteries than of uterine arteries. Its effects on uterine arteries from both day-7 and day-15 guinea-pigs were similar, irrespective of the constrictor used, but it was significantly (P < 0.01) more potent in inducing relaxation of mesenteric arteries contracted with NA compared with those constricted with KCl. In day-7 arteries precontracted with NA, N⁶-nitro-l-arginine methyl ester (l-NAME; 10 μmol/l) had no effects on the time course of resveratrol-induced vasorelaxation in either mesenteric or uterine arteries. However, indomethacin (50 μmol/l) significantly (P < 0.05) potentiated resveratrol’s effect on mesenteric, but not uterine, arteries. Indomethacin had no effect on resveratrol-induced vasorelaxation of arteries contracted with KCl, whereas l-NAME significantly (P < 0.05) reduced the effects of resveratrol on uterine, but not on mesenteric, arteries. In day-15 arteries, l-NAME significantly (P < 0.01) attenuated the effects of resveratrol on mesenteric arteries contracted with NA. Indomethacin had no effect on resveratrol activity. This study indicates that: (a) the effect of resveratrol on resistance arteries is greater than that on conductance arteries; (b) the effects of resveratrol are not mediated via prostanoids, but NO may play a role; and (c) the stage of the oestrous cycle has no influence on resveratrol-induced vasorelaxation.

INTRODUCTION

The trihydroxystilbene, resveratrol, is a naturally occurring phenolic substance present in a variety of plants, such as Yucca schidigera [1], a South African medicinal plant Erythrophleum lasianthum [2], and grapes [3,4]. Interest in resveratrol has expanded in recent years, focusing on its potentially beneficial effects on the cardiovascular system, as well as its anti-cancer effects. Trihydroxystilbenes are lipophilic compounds [5] which become incorporated into blood cells [6]. They are thought to protect against atherosclerosis and coronary heart disease through various mechanisms [5,7] that may include vasorelaxation [8–11] and anti-platelet effects [7]. The mechanisms by which resveratrol causes vasodilatation are uncertain, but may include inhibition of arachidonic acid metabolism [8,12] and induction of NO synthesis [11,13].

Key words: conductive arteries, female guinea-pigs, resistance arteries, resveratrol.
Abbreviations: ACh, acetylcholine; MA, mesenteric artery; NA, noradrenaline; l-NAME, N⁶-nitro-l-arginine methyl ester; PSS, physiological salt solution; KPSS, high-potassium PSS; UA, uterine artery.
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To elucidate further the mechanisms of resveratrol-induced vasorelaxation, we have studied different arteries of similar calibre, but with different properties, namely the mesenteric artery (MA; a resistance artery) and the main uterine artery (UA; a conductive artery) from female guinea-pigs. To eliminate any possible involvement of the oestrous cycle through changing plasma levels of oestradiol and progesterone that could modulate vascular reactivity, we used arteries from animals at both day 7 (when the plasma progesterone concentration is at its highest) and day 15 (when the plasma oestradiol concentration is maximal) of the oestrous cycle [14]. We also studied the roles of NO and prostanoids in resveratrol-induced vasorelaxation.

METHODS

Experimental procedures
Female guinea-pigs (Dunkin–Hartley; 350–450 g) were examined daily and a vaginal smear was taken when the vagina was open. Day 1 of the oestrous cycle was the day preceding the post-ovulatory influx of leucocytes when cornification was at a maximum. All guinea-pigs had exhibited three cycles of normal length before being used on day 7 or day 15 of the oestrous cycle.

Each guinea-pig was killed by CO2 gas inhalation. Segments of 2 mm length of a second-order MA (~ 330 μm diameter) and of the main UA (~ 320 μm diameter) from each guinea-pig were carefully dissected and freed from connective tissue and fat. Two vessels from each type of artery were mounted on two 40-μm-diameter stainless-steel wires in an automated myograph (Cambustion, Cambridge, U.K.), based on the principle of the Mulvany myograph [15], which measured isometric tension generated in response to the various stimuli. The vessels were incubated in a 5-ml organ bath containing physiological salt solution [PSS; composition (mmol/l): NaCl 119, KCl 4.7, CaCl2 2.5, MgSO4 1.17, NaHCO3 25, KH2PO4 1.18, EDTA 0.026 and glucose 5.5] gassed with 95% O2/5% CO2 at 37 °C.

After a 30 min equilibration, the length–tension characteristics for each vessel were determined using the law of Laplace (P = T/r, where P is the transmural pressure, T is the tension and r is the vessel radius). Each vessel was then set to its normalized diameter, i.e. that which it would achieve at rest in vivo under a transmural pressure of 100 mmHg. This has been shown to be the diameter at which the greatest force is generated [16]. The computer calculated the target tension that each vessel should develop in response to a maximal stimulus.

Arteries were allowed a further 30 min to equilibrate before being depolarized twice with high-potassium PSS (KPSS; 125 mmol/l KCl), in which NaCl in normal PSS was replaced by an equimolar concentration of KCl. In experiment 1, arteries were then contracted with either noradrenaline (NA; 10 μmol/l) or KCl (125 mmol/l). When contractions reached a plateau, relaxation response curves to resveratrol (5–50 μmol/l) were carried out. In experiment 2, we investigated the possible dependence of the vasodilator action of resveratrol on prostanoids or NO. Resveratrol at 50 μmol/l was added to the precontracted arteries, in the absence or presence of either indomethacin (50 μmol/l) or the NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) (10 μmol/l). L-NAME at 10 μmol/l has been shown to inhibit endothelium-dependent hydroxystilbene-induced vasorelaxation [13,17].

In all sets of experiments, arteries precontracted with NA were challenged with either acetylcholine (ACh; 100 μmol/l), to determine muscarinic-receptor-activated endothelium-dependent vasorelaxation, or the calcium ionophore A23187 (100 μmol/l), which is a non-receptor-mediated endothelium-dependent vasorelaxant [18].

Reagents
NA, indomethacin, L-NAME, A23187, resveratrol and ACh were all obtained from Sigma Chemical Co. (Poole, Dorset, U.K.). Resveratrol was dissolved in ethanol. Working concentrations of ethanol in the bath were < 0.01% (v/v). To exclude possible vascular effects of ethanol, two arteries from each animal in each experiment were studied in the presence of the same volume of ethanol as the test arteries. As reported previously, there was no significant effect of this low concentration of ethanol on contractility induced by either KPSS or NA [19,20].

Statistical analyses
All data are expressed as means ± S.E.M. for the group. An average response for all the vessels from a given animal was determined before group analysis. Statistical significance was tested using repeated-measures analysis of variance or Student’s t-test, as appropriate. n represents number of animals unless otherwise stated.

RESULTS

Diameters of vessels from day-7 animals were 324 ± 11 μm for MAs and 330 ± 9 μm for UAs (n = 15 animals; 30 vessels). Vessels obtained from day-15 guinea-pigs were 325 ± 10 and 315 ± 15 μm in diameter for MAs and UAs respectively. There were no significant differences in vessel diameter between any groups of arteries used in this study.

Effects of NA and KCl
In day-7 guinea-pigs, NA- and KCl-induced increases in tension in UAs were up to 2.5-fold greater than those
Table 1  Effects of KCl (125 mmol/l) and NA (10 μmol/l) on the rise in tension in MAs and UAs from female guinea-pigs

<table>
<thead>
<tr>
<th>Stage of oestrous cycle</th>
<th>Tension (mN/mm artery)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KCl MA</td>
</tr>
<tr>
<td>Day 7</td>
<td>3.2 ± 1.1</td>
</tr>
<tr>
<td>Day 15</td>
<td>4.7 ± 1.1</td>
</tr>
</tbody>
</table>

Data represent means ± S.E.M. (n = 15 animals in each group; 30 vessels in each group). Significance of differences: *P < 0.01 compared with MA.

in MAs (n = 15 animals; 30 vessels) (Table 1). In day-15 guinea-pigs, NA-induced increases in force for MAs and UAs were almost identical. There was also no difference in KCl-induced tension between UAs and MAs (Table 1).

Effects of ACh and A23187 on arteries contracted with NA

When arteries were contracted with NA, ACh (100 μmol/l) caused relaxation of MAs, but not of UAs, from both day-7 and day-15 guinea-pigs (Figure 1). The magnitude of the ACh-induced vasorelaxation was 86 ± 10% and 92 ± 11% of the maximum NA-induced contraction for day-7 and day-15 MAs respectively. ACh up to 500 μmol/l also failed to induce any significant relaxation of UAs from animals at day 7 or day 15 of the oestrous cycle (5 ± 3%). However, A23187 (31.6 μmol/l) caused rapid relaxation of UAs from both day-7 and day-15 guinea-pigs (Figure 1).

Effects of resveratrol on preconstricted arteries

In arteries precontracted with either NA or KCl from day-7 guinea-pigs, resveratrol (5–50 μmol/l) caused concentration-dependent relaxation of both MAs and UAs, with EC50 values of 16.6 ± 4.3 μmol/l (MA) and 32.1 ± 5.0 μmol/l (UA; P < 0.001 compared with MA) for NA, and of 17.4 ± 5.0 μmol/l (MA) and 27.7 ± 3.8 μmol/l (UA; P < 0.001 compared with MA) for KCl (Figure 2, upper panel).

In arteries precontracted with either NA or KCl from day-15 guinea-pigs, resveratrol (5–50 μmol/l) caused concentration-dependent relaxation of both MAs and UAs, with EC50 values of 10.4 ± 3.7 μmol/l (MA) and 25.7 ± 4.2 μmol/l (UA; P < 0.001 compared with MA) for NA, and of 14.8 ± 0.9 μmol/l (MA) and 27.5 ± 1.3 μmol/l (UA; P < 0.001 compared with MA) for KCl (Figure 2, lower panel).

Effects of indomethacin and L-NAME on resveratrol-induced vasorelaxation

With vessels from animals at both day 7 and day 15 of the oestrous cycle, resveratrol (50 μmol/l) induced time-dependent relaxation of MAs and UAs contracted with either NA or KCl. Resveratrol-induced vasorelaxation was significantly (P < 0.01, n = 4–6) faster and greater in

![Figure 1](https://example.com/figure1.png)

Figure 1  Effects of ACh and A23187 on MAs (upper panel) and UAs (lower panel) obtained from day-7 and day-15 female guinea-pigs

Arteries were contracted with NA (10 μmol/l). When contractions reached a plateau, ACh (a; 100 μmol/l) was added. A23187 (31.6 μmol/l) was further added to UAs when ACh (b; 500 μmol/l) did not produce significant vasorelaxation.
MAs than in UAs from both day-7 and day-15 guinea-pigs (Table 2; Figures 3 and 4).

When arteries from day-7 guinea-pigs were precontracted with NA, l-NAME had no effect on the time course of resveratrol-induced vasorelaxation in either MAs or UAs. However, indomethacin significantly ($P < 0.05$, $n = 5$) accelerated and enhanced resveratrol-induced vasorelaxation in UAs, but had no such effect in MAs (Figure 3, upper panel). In KCl-contracted arteries, indomethacin had no effect on the vasorelaxant property of resveratrol, but l-NAME significantly ($P < 0.05$, $n = 5$) caused right- and down-ward shifts of resveratrol-induced vasorelaxation of UAs, but not of MAs (Figure 3, lower panel).

In vessels from day-15 guinea-pigs, indomethacin had no effect on the time course of resveratrol-induced vasorelaxation in MAs or UAs, regardless of the constrictor used; however, l-NAME significantly ($P < 0.01$, $n = 4$) reduced resveratrol-induced vasorelaxation in MAs contracted with NA (Figure 4).

**DISCUSSION**

Several studies have suggested vasorelaxant properties of polyphenolic compounds [11,13,17,21]. It is thought that resveratrol is the major compound contributing to the vascular smooth muscle relaxant action of polyphenols. Resveratrol has been shown to inhibit NA- and phenylephrine-induced contraction of rat aorta [10] and guinea-pig trachea [8] *in vitro*. In the present study, we have shown that resveratrol induces vasorelaxation of both the MA (a resistance vessel) and the main UA (a conductance vessel) obtained from guinea-pigs at day-7 or day-15 of the oestrous cycle. Resveratrol reversed the tension generated by either depolarization (KCl) or membrane receptor stimulation (NA). The vasorelaxant responses to resveratrol in day-7 arteries (both MA and UA) and day-15 UA were similar regardless of the constrictor used, indicating that in these arteries the vascular effect of resveratrol does not depend on the mechanism of tension induction. However, its effect on day-15 MAs was more pronounced for arteries contracted with NA than with KCl, suggesting that, in these blood vessels, the mechanism of action of resveratrol may be dependent upon the specific mechanisms by which the arteries were contracted. Therefore it appears that both the blood vessel type and the mechanism of force induction may influence the vascular effects of resveratrol.

The EC$_{50}$ for the effect of resveratrol on the NA-induced contraction of day-7 UAs in the present study was almost identical with that reported for rat aorta (32 and 31 μmol/l respectively) [10]. However, the EC$_{50}$ for the effect of resveratrol on the MA was only 50% of that for the UA, indicating a more potent relaxant effect in resistance arteries than in conductive arteries.

There were no significant effects of the day of the oestrous cycle on resveratrol-induced vasorelaxation in either the MA or the UA, regardless of the type of constrictor used, suggesting that variations in general
Vasorelaxation effects of resveratrol

Figure 3 Effects of indomethacin and L-NAME on resveratrol-induced vasorelaxation of MAs and UAs from day-7 female guinea-pigs

Blood vessels were contracted with either NA (10 μmol/l; upper panel) or KCl (125 mmol/l; lower panel). When contractions reached a plateau, 50 μmol/l resveratrol was added to the arteries in the presence or absence of either indomethacin (50 μmol/l) or L-NAME (10 μmol/l). Significance of differences: **P < 0.01; NS, not significant.

Steroid levels do not modulate the vasorelaxant action of resveratrol.

The vascular effects of resveratrol [10] and other polyphenols [11] are thought to be both endothelium-dependent and endothelium-independent. The former effect is apparent at low resveratrol concentrations (10–30 μmol/l) and is blocked by inhibitors of NO synthase activity [10,21], whereas endothelium-independent effects appear at higher resveratrol concentrations (> 60 μmol/l) and are not blocked by endothelial denudation or NO synthase inhibitors [10,11]. In our study, pretreatment of arteries with L-NAME inhibited the vasorelaxant effect of resveratrol on day-7 UAs contracted with KCl (Figure 3) and on day-15 MAs contracted with NA (Figure 4). By contrast, L-NAME was ineffective in blocking resveratrol-induced relaxation of day-7 MAs and day-15 UAs, suggesting an endothelium-independent mechanism of action. Hence our findings confirm that resveratrol-induced relaxation may be either endothelium-dependent or -independent, and that the particular vascular bed and type of constrictor stimulus may both influence the action of resveratrol.

Several studies have shown that polyphenols inhibit cyclic nucleotide phosphodiesterases, which break down vasorelaxant cAMP and cGMP [22,23]. Other inhibitors of cyclic nucleotide phosphodiesterases induce relaxation of endothelium-denuded aortic rings [24,25]. Such a mechanism might therefore be involved in the endothelium-independent relaxation induced by resveratrol. Resveratrol could also become incorporated into the
Figure 4  Effects of indomethacin and l-NAME on resveratrol-induced vasorelaxation of MAs and UAs from day-15 female guinea-pigs

Arteries were contracted with either NA (10 μmol/l; upper panel) or KCl (125 mmol/l; lower panel). When contractions reached a plateau, 50 μmol/l resveratrol was added to the arteries in the presence or absence of either indomethacin (50 μmol/l) or l-NAME (10 μmol/l). Significance of differences: * P < 0.05; NS, not significant.

smooth muscle membrane, where it could either couple with a membrane receptor [26] or interact directly with membrane calcium channels [27], thus inducing vasorelaxation.

Reports have also implicated resveratrol in the inhibition of arachidonic acid metabolism in a variety of systems [12,28,29]. In the present study, indomethacin did not block resveratrol-induced vasorelaxation, regardless of the type of artery or the constrictor used. This suggests that the vascular effect of resveratrol is not mediated by the release of relaxant prostanoids. Indeed, indomethacin potentiated the action of resveratrol in day-7 UAs contracted with NA (Figure 3), reminiscent of the finding in guinea-pig trachea [8] that inhibition of arachidonic acid metabolism potentiated resveratrol-induced vasorelaxation.

Interestingly, we found that ACh did not induce vasorelaxation of UAs from either day-7 or day-15 guinea-pigs. By contrast, A23187, which causes the receptor-independent release of NO [18], and resveratrol both caused vasorelaxation in these arteries. This unexpected finding is at variance with the report of Weiner et al. [30], who described a dose-dependent vasorelaxation to ACh in UAs from both pregnant and non-pregnant guinea-pigs.

The lack of an effect of ACh on UAs is unexplained. Degradation of ACh activity is unlikely, as the same freshly prepared ACh induced marked relaxation of MAs. Endothelial damage is also unlikely, because this was a consistent finding in all 60 UAs studied. Thus we conclude either that the guinea-pig UA lacks muscarinic ACh receptors, or that the receptors are rendered inactive in some way.

In conclusion, we have shown that resveratrol can induce relaxation of UAs and MAs from guinea-pigs, and that this effect may be either endothelium-dependent (attenuated by l-NAME) or endothelium-independent. Resveratrol was more potent in relaxing resistance arteres.
arteries than conductance arteries. Vasodilator prostanoids were apparently not involved, and we found no effects of different stages of the oestrous cycle on the potency of resveratrol. This vasorelaxant effect of resveratrol (a polyphenolic compound) may result in lowered blood pressure [31] and, by reducing myocardial work, may contribute to the beneficial cardiovascular effects of resveratrol.

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