The mechanism of resveratrol-induced vasorelaxation differs in the mesenteric resistance arteries of lean and obese rats

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ABSTRACT

Resveratrol has been shown to induce vasorelaxation. In this study, we investigated the mechanism(s) of resveratrol-induced vasorelaxation in resistance mesenteric arteries from male lean and dietary-induced obese rats. Compared with lean rats, arteries from dietary-obese rats showed significant ($P < 0.001$) endothelial dysfunction, as indicated by a decrease ($>20\%$) in maximal acetylcholine-induced vasorelaxation. Resveratrol (5–35 μmol/l) induced concentration-dependent relaxation of mesenteric arteries preconstricted with noradrenaline (8 μmol/l) or KCl (125 mmol/l) from both lean and dietary-obese rats. There were no significant differences between the two groups, achieving a maximum relaxation of $>95\%$ at a concentration of 35 μmol/l. In noradrenaline-preconstricted arteries from lean rats, Nω-nitro-L-arginine methyl ester (L-NAME; 100 and 300 μmol/l) caused a significant ($P < 0.01$) concentration-dependent rightward shift in resveratrol activity, with no effect on maximal responses. However, L-NAME (100 and 300 μmol/l) did not alter the effects of resveratrol on arteries from dietary-obese rats, giving superimposed concentration–responses curves. Indomethacin was also ineffective in altering resveratrol activity in arteries from both lean and dietary-obese rats. In noradrenaline-precontracted arteries from dietary-obese rats, responses to resveratrol were not attenuated by endothelial denudation, indicating an action independent of the endothelium.

This study indicates that: (a) the maximal effects of resveratrol on resistance arteries from lean and dietary-obese rats are not effected by endothelial dysfunction, and (b) the effects of resveratrol in lean animals (where endothelial function is not impaired), but not in dietary-obese rats, are mediated via NO.

INTRODUCTION

Resveratrol is a naturally occurring phenolic trihydroxystilbene, present in a variety of plants [1–4]. A protective effect against atherosclerosis and coronary heart disease through various mechanisms [5–7], that may include vasorelaxation [8–11] and an anti-platelet effect [7], has been attributed to resveratrol. The mechanisms by which resveratrol causes vasodilatation are uncertain, but may include inhibition of arachidonate metabolism [8,12] and induction of nitric oxide (NO) synthesis [11,13,14].

The endothelium is vital to various important physiological functions in the arterial wall, including the regulation of vascular tone [15–17]. Endothelial cells...
produce various vasoactive mediators, such as the vasodilator NO and the vasoconstrictor endothelin, both of which act on the underlying vascular smooth muscle to modulate arterial contractility [18]. NO production is regulated by NO synthase, which is stimulated by mediators and hormones that include acetylcholine (ACh), bradykinin and insulin [18,19]. A range of deficits in endothelial function have been identified in several conditions that are associated with abnormal vascular reactivity and/or atherogenesis [20,21]. In particular, impaired NO-mediated vasodilatation has been demonstrated in obesity, Type II diabetes, hypertension and hypercholesterolaemia [22–25], and in an animal model of dietary-induced obesity [26], with the most striking abnormality being blunted vasodilatation in response to ACh. Therefore in the present study we aimed to investigate the mechanism(s) of resveratrol-induced vasorelaxation in mesenteric resistance arteries from lean and dietary-obese rats.

**METHODS**

**Experimental procedures**

Male Wistar rats (*n* = 14) were randomly assigned to a control group (*n* = 7) or a test group (*n* = 7). All had free access to water, and were housed in groups of two to three under controlled environmental conditions (19–22 °C; 30–40% humidity) and a 12-h light/dark cycle (lights on at 07.00 hours). Control animals were fed a standard laboratory pelleted diet (CRM Biosure, Cambridge, U.K.), while the test group had free access to a highly palatable, high-energy diet consisting of 33% condensed milk, 7% sucrose and 27% water, as described previously [27]. The two groups were initially matched for body weight (Table 1). All animals in the study were maintained on their respective diet for 16 weeks before being killed by CO₂ inhalation.

**Assessment of metabolic changes**

On the day of the experiment, the gonadal and perirenal fat pads were dissected out and weighed. Blood was removed by cardiac puncture into cold heparinized tubes. The plasma was immediately separated by centrifugation before being frozen for later measurements of blood analytes [glucose, non-esterified (‘free’) fatty acids and triacylglycerols (triglycerides)]. The plasma glucose concentration was determined using a glucose oxidase method, and non-esterified fatty acid (Boehringer Mannheim, Milton Keynes, Bucks., U.K.) and triacylglycerol (Sigma Diagnostics, Poole, Dorset, U.K.) concentrations were measured using commercial diagnostic kits.

**Assessment of vascular function**

Eight third-order mesenteric arteries (180–250 μm diameter; 2 mm length) were carefully dissected from each animal. Each artery was freed of fat and connective tissue and mounted in an automated myograph (Cambustion, Cambridge, U.K.), based on the principle of the Mulvany myograph, which measures isometric tension generated in response to various stimuli [28]. The vessels (in duplicate) were incubated in a 5 ml organ bath containing physiological salt solution [PSS; composition (in mM): 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 period, the length–tension characteristics for each vessel were determined as described previously [14]. Arteries were allowed a further 30 min to equilibrate before being depolarized twice with high-potassium PSS (KPSS; 125 mM) in which NaCl in normal PSS was replaced by an equimolar concentration of KCl. Any vessel failing to reach its predetermined target tension in response to vasoconstriction with KCl (125 mM) was discarded.

Arteries were then contracted with noradrenaline (NA; 8 μmol/l). When contractions reached a plateau, relaxation response curves to either ACh alone or

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<th>Physiological and metabolic characteristics of chow-fed and dietary-obese rats</th>
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<td>Chow-fed</td>
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<tr>
<td>Body weight (g)</td>
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<tr>
<td>Initial</td>
<td>210 ± 4</td>
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<tr>
<td>Final</td>
<td>510.8 ± 10.7</td>
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<tr>
<td>Gain</td>
<td>300.7 ± 16.4</td>
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<tr>
<td>Epididymal fat pad mass (g)</td>
<td>7.9 ± 1.0</td>
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<td>Perirenal fat mass (g)</td>
<td>10.2 ± 1.8</td>
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<td>Plasma glucose (mM)</td>
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<td>Plasma triacylglycerols (mM)</td>
<td>0.45 ± 0.02</td>
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<td>Plasma non-esterified fatty acids (mM)</td>
<td>0.33 ± 0.04</td>
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resveratrol (5–35 μmol/l), in the presence or absence of N^\text{N-}\text{nitro-}L\text{-arginine methyl ester (L-NAME; 100 and 300 μmol/l) or indomethacin (10 μmol/l), were carried out. To evaluate the role of endothelium-derived NO in NA-induced vasocontraction, arteries were also contracted with NA (8 μmol/l) in the presence or absence of L-NAME (300 μmol/l). In another set of experiments, resveratrol relaxation response curves were carried out on arteries preconstricted with KCl (125 mmol/l). We have also examined the effects of resveratrol on arteries with intact or denuded endothelium precontracted with NA (8 μmol/l).

Endothelium denudation was achieved by gently rubbing the internal diameter of arteries around 40 μm stainless steel wires. Endothelial function was assessed by responses to ACh. Failure of arteries to relax to ACh (100 μmol/l) was considered to indicate a state of endothelial denudation.

Reagents
Noradrenaline, resveratrol, ACh and L-NAME were all obtained from Sigma. 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 [29,30].

Statistical analyses
All data are expressed as the mean ± 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 ANOVA or Student’s t-test, as appropriate. n represents the number of animals, unless otherwise stated.

RESULTS

Metabolic changes
At the end of the experiment, animals fed on the highly palatable diet weighed significantly (> 40%; P < 0.001) more than their lean counterparts. The epididymal and perirenal fat pads weighed significantly more (> 120%; P < 0.001) in dietary-induced obese rats than in the chow-fed controls (Table 1). Moreover, in dietary-induced obese rats, terminal plasma non-esterified fatty acid levels were significantly higher (> 45%; P < 0.05) than in their chow-fed counterparts, as were triacylglycerol levels (> 78%; P < 0.001) (Table 1), indicating the induction of obesity in animals fed on the highly palatable diet.

Vascular and contractile responses to NA
There were no significant differences in vessel diameter between the two groups of arteries used in this study. Diameters of vessels from lean and dietary-obese animals were 260 ± 10 μm and 250 ± 15 μm (n = 7 animals, 40 vessels) respectively.

There were no significant differences in the NA-induced rise in tension between vessels from chow-fed and dietary-induced obese rats (6.24 ± 0.61 and 6.66 ± 0.75 mN/mm of artery respectively; P > 0.5). L-NAME caused a slight increase in NA-induced force in arteries from both chow-fed (7.20 ± 0.49 mN/mm of artery) and dietary-induced obese (7.25 ± 0.79 mN/mm of artery) rats. However, these increases in tension development were not significant compared with those in the absence of L-NAME.

Effects of ACh on arteries contracted with NA
NA (8 μmol/l)-precontracted arteries from lean rats demonstrated progressive relaxation to cumulative additions of ACh (10 nmol/l–100 μmol/l), with a maximal relaxation of 93 ± 2% at 100 μmol/l ACh. Arteries from the dietary-obese group that were similarly exposed to ACh displayed a significant (20 ± 2%; P < 0.001) decrease in maximal relaxation. There was also a significant rightward shift of the concentration–response curves in arteries from dietary-obese rats compared with those from lean animals (EC_{50} values of 3.14 ± 0.04 and 0.40 ± 0.03 μmol/l respectively; P < 0.001) (Figure 1).

Effects of resveratrol on NA- and KCl-preconstricted arteries
Resveratrol caused a concentration-dependent relaxation of arteries precontracted with either NA or KCl,
from both lean control and dietary-obese rats. There was no significant difference between the vasoactivity of resveratrol on arteries from lean control and dietary-obese rats, with a maximal relaxation of up to 99% being achieved (Figure 2).

**Effects of L-NAME and indomethacin on resveratrol activity**

In arteries from lean animals, L-NAME caused a concentration-dependent rightward shift of resveratrol-induced vasorelaxation, with no change in maximal relaxation. The EC\textsubscript{50} values for resveratrol were 10.8 ± 0.2 μmol/l (control), 15.1 ± 0.3 μmol/l (100 μmol/l L-NAME; P < 0.05 compared with control) and 19.5 ± 0.5 μmol/l (300 μmol/l L-NAME; P < 0.01 compared with control) (Figure 3, upper panel). In arteries from dietary-obese rats, L-NAME had no effect on resveratrol-induced vasorelaxation, with EC\textsubscript{50} values of 11.2 ± 0.4 μmol/l (control), 11.0 ± 0.3 μmol/l (100 μmol/l L-NAME) and 11.3 ± 0.4 μmol/l (300 μmol/l L-NAME) (Figure 3, lower panel).

Indomethacin (10 μmol/l) had no significant effect on the vascular activity of resveratrol on arteries from lean or dietary-obese rats, giving EC\textsubscript{50} values of 11.1 ± 0.3 μmol/l (control) and 11.5 ± 0.5 μmol/l (indomethacin) for lean rats, and 10.8 ± 0.5 μmol/l (control) and 11.1 ± 0.5 μmol/l (indomethacin) for dietary-obese rats.

**Effects of endothelial denudation on resveratrol activity**

In dietary-obese rats, endothelial denudation failed to alter resveratrol activity. Resveratrol caused concentration-dependent vasorelaxation of arteries in the presence or absence of functional endothelium, giving EC\textsubscript{50} values of 11.2 ± 0.4 μmol/l (endothelium intact) and 11.8 ± 0.5 μmol/l (endothelium denuded) (Figure 4).
DISCUSSION

Several recent studies have indicated vasorelaxant properties for resveratrol [11–14]. Resveratrol has been shown to inhibit KCl-, noradrenaline- and phenylephrine-induced contraction of rat aorta [10], guinea-pig trachea [8] and guinea-pig mesenteric and uterine arteries [14] in vitro. The mechanism(s) of action of resveratrol [10,14] 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 [10,31], whereas endothelium-independent effects appear at high resveratrol concentrations (> 60 μmol/l) and are not blocked by endothelial denudation or NO synthase inhibitors [10,11]. In the present study, resveratrol (5–35 μmol/l) caused concentration-dependent relaxation of NA-precontracted arteries from both lean and dietary-obese rats, indicating the involvement of the endothelium-dependent component of resveratrol activity. Inhibitory effects of L-NAME on the vasoactivity of resveratrol would have further strengthened such a hypothesis. Indeed, L-NAME attenuated the responses to resveratrol of arteries from lean animals, indicating activation of endothelial NO synthesis by resveratrol. This finding is in agreement with previous reports in which resveratrol-induced vasodilatation was attenuated with inhibitors of NO synthase activity [10,31]. However, L-NAME failed to affect the vasorelaxant property of resveratrol on arteries from dietary-obese animals, indicating a lack of involvement of the endothelial NO system in the resveratrol-induced vasorelaxation of arteries from dietary-obese rats. This raises a number of questions as to the mechanism(s) of resveratrol-induced vasorelaxation in arteries from animals with dietary-induced obesity.

The attenuation of ACh-induced vasorelaxation seen in the present study is in agreement with our previous report [26] indicating the induction of endothelial dysfunction in dietary-obese animals. Therefore it appears that the mechanism of resveratrol-induced vasorelaxation in arteries from obese animals is influenced by the state of endothelial function. That is, if there is no apparent endothelial dysfunction, then the mechanism of resveratrol-induced vasorelaxation is via the activation and release of endothelial NO. If, however, the endothelium is damaged, another component of resveratrol’s mechanism of action becomes more important. This secondary endothelium-independent effect has been shown to occur at high concentrations (> 60 μmol/l) [10,11]; nonetheless, in the present study the endothelium-independent effect was apparent at all resveratrol concentrations used (Figures 2 and 3).

In agreement with previous reports [10,14], indo-methacin did not attenuate responses to resveratrol in the present study, indicating that endogenous prostanoids play little or no part in the vascular activity of resveratrol. In arteries from dietary-obese rats, resveratrol activity was similar in the presence or absence of functional endothelium, suggesting that the endothelium-independent component of the effect of resveratrol responses is not NO-mediated [10,11].

Inhibitors of cyclic nucleotide phosphodiesterases induce the relaxation of endothelium-denuded aortic rings [32,33]. Polyphenols have also been shown to inhibit cyclic nucleotide phosphodiesterases, which break down the vasorelaxants cAMP and cGMP [34,35]. Such a mechanism might therefore be involved in the endothelium-independent relaxation induced by resveratrol. Resveratrol might also become incorporated into the smooth muscle membrane, where it could either couple with a membrane receptor [36] or interact directly with membrane calcium channels [37], thus inducing endothelium-independent vasorelaxation.

In conclusion, we have shown that resveratrol can induce the relaxation of mesenteric resistance arteries from both lean and dietary-obese rats. Resveratrol-induced vasorelaxation may either be endothelium-dependent (attenuated by L-NAME) or endothelium-independent, in which case endothelial denudation and L-NAME treatment fail to alter the vasorelaxant effect of resveratrol. This vasorelaxant effect of resveratrol may contribute to reduce blood pressure [38] and, by lowering myocardial work, may contribute to the beneficial cardiovascular effects of resveratrol in conditions such as obesity, Type II diabetes, hypertension and hypercholesterolaemia [20–25] where endothelial function is blunted.

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REFERENCES


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