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(-)Epicatechin induces and modulates endothelium-dependent relaxation in isolated rat mesenteric artery rings

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KEY WORDS (-)epicatechin; vascular endothelium; nitric oxide; muscle relaxation; mesenteric artery; rats

ABSTRACT

AIM: The present study was aimed to examine the role of endothelial nitric oxide in the relaxant response to green tea (-)epicatechin and its modulation of endothelium-mediated relaxation in the isolated rat mesenteric artery rings. **METHODS:** Changes in the isometric tension were measured with Grass force-displacement transducers. **RESULTS:** The (-)epicatechin-induced relaxation was largely dependent on the presence of intact endothelium and was reversed by N^G-nitro-*L*-arginine methyl ester 10 μmol/L or methylene blue 10 μmol/L, the inhibitors of nitric oxide-mediated relaxation. *L*-Arginine at 1 mmol/L antagonized the effect of *L*-NAME or methylene blue. Pretreatment of endothelium-intact rings with (-)epicatechin 10 μmol/L enhanced the relaxation induced by endothelium-dependent vasodilator, acetylcholine, while this concentration did not influence the endothelium-independent relaxation induced by sodium nitroprusside in the endothelium-denuded artery rings. **CONCLUSION:** The results indicate that the endothelium-dependent vasodilation by (-)epicatechin is mainly mediated through nitric oxide and low concentration of (-)epicatechin augments endothelium-dependent vasorelaxation in the rat mesenteric arteries.

INTRODUCTION

Jasmine green tea is one of the most popular beverages consumed in China. It was reported that decaffeinated green tea could lower blood pressure and heart rate in mice[1]. Another benefit of drinking green tea has been related to hypocholestrolemic action of the bioflavonoids. The antioxidant activity may play a role in reduction of plasma cholesterol levels by certain flavonoids. Increased consumption of green tea (>10 cups per day) was related to a decreased serum concentration of total cholesterol, triglyceride, and the ratio of low density lipoprotein to high density lipoprotein cholesterol in human population[2], suggesting that green tea may act preventively against the cardiovascular disease. However, there is little investigation *in vitro* on the vascular effect of green tea except we reported vasodilator effects of four epicatechin derivatives[3,4]. In this study, we further examined the role of endothelial nitric oxide in the relaxant response to (-)epicatechin in the isolated rat mesenteric artery rings and the possible modulation by (-)epicatechin on the endothelium/nitric oxide-mediated relaxation. (-)Epicatechin was previously found to be present in aortic blood after oral administration[5].

MATERIALS AND METHODS

Procedure for extraction of (-)epicatechins from

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jasmine green tea was described previously by us[3,6]. The purity of (-)epicatechin was greater than 99 %. The pure (-)epicatechin was freeze-dried overnight and stored in dark at –20 °C.

Male Sprague-Dawley rats (250-300 g) were killed by cervical dislocation and bled. The main branch of the superior mesenteric artery was excised and cut into three 3 mm-wide ring segments. Each ring was placed between two stainless wire hooks in a 10-mL organ bath. The organ bath contained Krebs's solution of the following composition (in mmol/L): 119 NaCl, 4.7 KCl, 25 $NaHCO_3$, 2.5 $CaCl_2$, 1 $MgCl_2$, 1.2 KH_2PO_4, 11 *D*-glucose and 0.3 ascorbic acid. The bath solution was continuously gassed with a mixture of 95 % O_2 and 5 % CO_2, and maintained at 37 °C to give a pH of approximately 7.4. Changes in isometric tension were measured with force transducers. All rings were set to equilibrate for 90 min. The resting tension was readjusted to 5 mN when necessary. In some arterial rings the endothelial layer was mechanically removed by gently rubbing the luminal surface of a ring back and forth several times with plastic tubing. Endothelium integrity or functional removal was confirmed by the presence or absence, respectively, of the relaxant response to acetylcholine 1 μmol/L. The removal of endothelium was also evaluated by light microscopy of the histological section of the artery. Each experiment was performed on the rings prepared from different rats. This study was approved by the Animal Research Ethics Committee of the Chinese University of Hong Kong.

Thirty minutes after being set up in organ baths, the arterial rings were first contracted with 1 μmol/L phenylephrine to test their contractility after which they were rinsed with pre-warmed and oxygenated Krebs' solution several times until muscle tension returned to the basal level. In the first set of experiments, following the control concentration-response curve for phenylephrine, the ring was rinsed several times and thereafter exposed to 300 μmol/L (-)epicatechin for 30 min before the second concentration-response curve for phenylephrine was repeated. These experiments were performed in both endothelium-intact and -denuded rings. In the second group of experiments, the sustained contraction was first evoked by 30 nmol/L U46619, 300 μmol/L (-)epicatechin was added to induce relaxation, followed by addition of 10 μmol/L N^G-nitro-*L*-arginine methyl ester (*L*-NAME) or 10 μmol/L methylene blue (MB) and finally by addition of 1 mmol/L *L*-arginine in the endothelium-intact rings. The effects of (-)epicatechin or *L*-NAME were tested in endothelium-denuded rings. Besides, the possible effect of (-)epicatechin was also examined on the endothelium-dependent relaxation induced by acetylcholine (0.3-300 nmol/L) or on endothelium-independent relaxation induced by sodium nitroprusside (0.1-300 nmol/L).

The following chemicals were used: phenylephrine hydrochloride, acetylcholine hydrochloride, N^G-nitro-*L*-arginine methyl ester, methylene blue, *L*-arginine, sodium nitroprusside, U46619 (Sigma, St Louis, MO, USA). All agents except for U46619 were prepared in double-distilled water and further dilution was made in fresh Krebs' solution. U46619 was dissolved in dimethyl sulfoxide.

To study the effect of (-)epicatechin on phenylephrine-induced contraction or on dilator-induced relaxation, the negative logarithm of the drug contraction that caused 50 % of the maximal contraction (pEC_{50}) or maximal relaxation (pD_2) and the maximal responses (E_{max}) were calculated and compared in the absence and presence of (-)epicatechin. Data are expressed as Mean±SD of *n* experiments. Two-tailed Students' *t*-test was used to evaluate statistical difference.

RESULTS

Effect of (-)epicatechin on phenylephrine-induced contraction Phenylephrine produced greater contractile responses in the endothelium-denuded rings as compared with the endothelium-intact rings with the same maximal contraction. The pEC_{50} values were 5.70±0.14 (n=6) and 6.86±0.21 (n=6) in the presence and absence of endothelium, respectively (P<0.05). Fig 1 showed that pretreatment of the endothelium-intact rings with 300 μmol/L (-)epicatechin significantly attenuated the concentration-response to phenylephrine (E_{max}: 40.3 %±6.0 % as compared with 106.2 %± 1.8 % of control, P<0.05) even though pEC_{50} value (5.49±0.22) was not different from the control (5.70± 0.14). (-)Epicatechin started to reduce the contraction when the concentration of phenylephrine was higher than 0.3 μmol/L (Fig 1). In contrast, in endothelium-denuded rings, pretreatment with 300 μmol/L (-)epicatechin had no effect on the contractile response to phenylephrine (pEC_{50}: 6.86±0.21, n=6 for the control and 6.69±0.15, n=5 for epicatechin, P>0.05).

Effect of nitric oxide inhibitors on (-)epicatechin-induced relaxation Traces in Fig 2 showed that in endothelium-intact rings, (-)epicatechin at 300

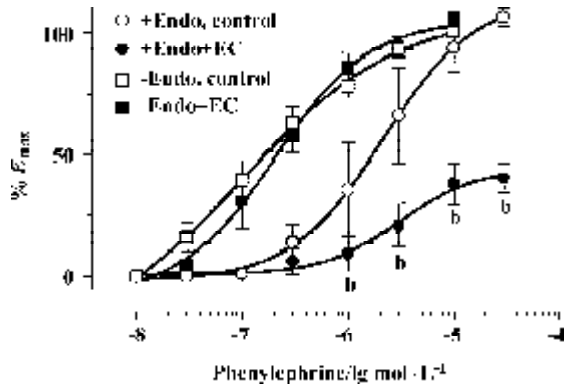

Fig 1. Concentration-response curves for the contractile response to phenylephrine in the isolated rat mesenteric artery rings (with endothelium: ○ *n*=6 for the control; ●, *n*=5 for 300 μmol/L (-)epicatechin; without endothelium: □, *n*=5 for the control; ■, *n*=5 for 300 μmol/L (-)epicatechin). Data are expressed as percentages of the maximum contraction induced by phenylephrine in the control concentration-response curve. $^bP<0.05$ *vs* control+Endo.

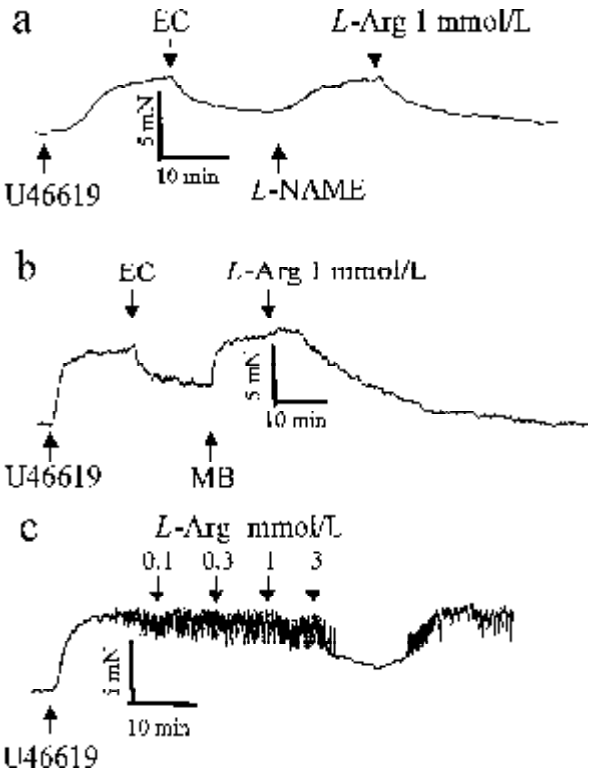

Fig 2. Traces showing the relaxant effect of 300 μmol/L (-) epicatechin (EC) in U46619-preconstricted endothelium-intact rings and reversed by 10 μmol/L *L*-NAME (a) or by 10 μmol/L methylene blue (b), followed by the addition of 1 mmol/L *L*-arginine. The concentration-dependent effect of *L*-arginine on U46619-preconstricted endothelium-intact rings (c).

μmol/L reduced the U46619-induced contraction and this relaxant effect was reversed by 10 μmol/L *L*-NAME (Fig 2a) or by 10 μmol/L methylene blue (Fig 2b). Subsequent addition of 1 mmol/L *L*-arginine relaxed the rings (Fig 2a&b). *L*-Arginine 1 mmol/L had little effect on the U46619 response but further increase of the L-arginine concentration (up to 3 mmol/L) started to reduce the evoked tone (Fig 2c). These results are summarized in Fig 3a&b. Pretreatment with 10 μmol/L *L*-NAME significant inhibited the relaxant effect of (-)epicatechin (Fig 4a) while (-)epicatechin had little effect in endothelium-denuded rings (Fig 4b).

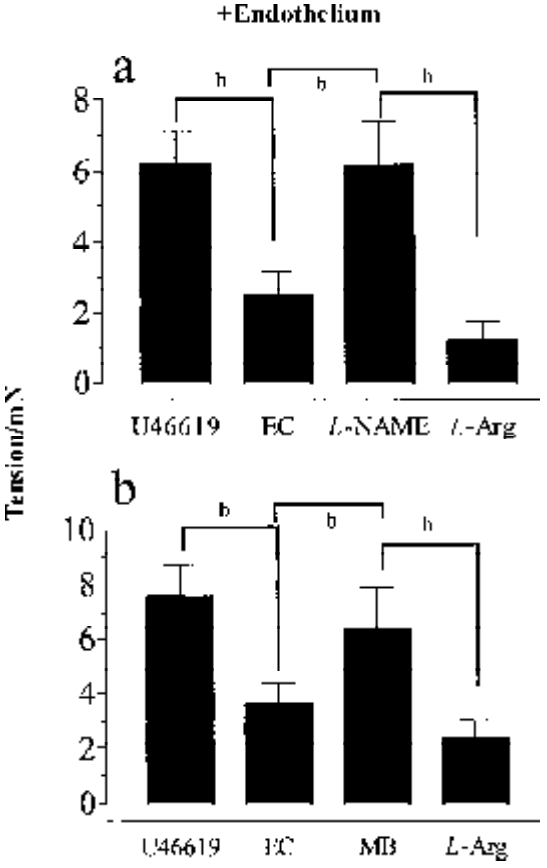

Fig 3. The effect of 10 μmol/L *L*-NAME (a) or 10 μmol/L methylene blue (b) on 300 μmol/L (-)epicatechin-induced relaxation in 30 nmol/L U46619-preconstricted endothelium-intact rings. Each later treatment was placed in the presence of the previous treatment(s). $^bP<0.05$ as compared with the immediate early treatment. *n*=5-6 experiments.

Effect of (-)epicatechin on endothelium-dependent and -independent relaxation Fig 5a showed that cumulative addition of acetylcholine induced concentration-dependent relaxations in the endothelium-intact rings with an pD_{50} of 7.66±0.06 (*n*=5). Pretreatment with 10 μmol/L (-)epicatechin slightly but signifi-

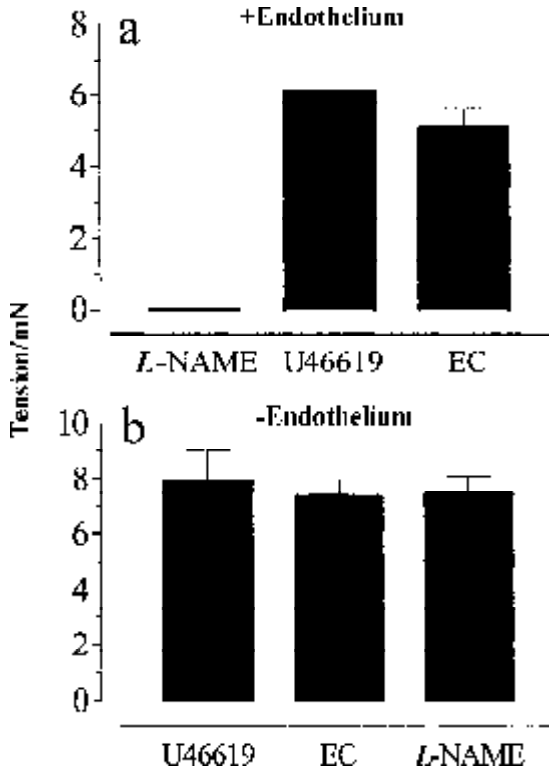

Fig 4. The effect of pretreatment with 10 μmol/L *L*-NAME on (-)epicatechin-induced relaxation in endothelium-intact rings (a) and the effect of 300 μmol/L (-)epicatechin in endothelium-denuded rings (b). *n*= 4-5 experiments.

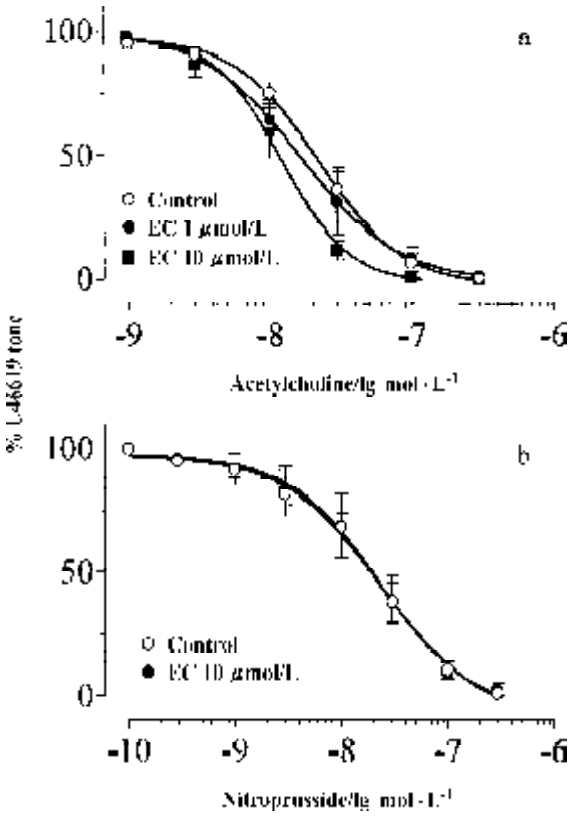

Fig 5. The effect of (-)epicatechin on relaxation induced by (a) acetylcholine in endothelium-intact rings (○ *n*=5 for the control; ● *n*=6 for 1 μmol/L EC and ■ *n*=5 for 10 μmol/L EC) or by (b) sodium nitroprusside in endothelium-denuded rings (○ *n*=7 for the control and ● *n*=5 for 10 μmol/L EC). The rings were exposed to (-)epicatechin for 30 min before the addition of phenylephrine.

cantly enhanced the acetylcholine-induced relaxation (pD_{50}: 7.92±0.05, *n*=5, *P*<0.05 compared with the control), while (-)epicatechin at 1 μmol/L had no effect (pD_{50}: 7.79 ±0.09, *n*=6). However, pretreatment with 10 μmol/L (-)epicatechin did not modify the relaxant effect of sodium nitroprusside in the endothelium-denuded rings (Fig 5b).

DISCUSSION

We isolated and purified four epicatechin derivatives from jasmine green tea and found that these four polyphenols reduced the concentration-response to phenylephrine in the isolated rat arteries[3]. The present results clearly demonstrated that the primary role of the functional endothelium in (-)epicatechin-induced relaxation in the U46619-preconstricted rat mesenteric artery rings since removal of the endothelium markedly reduced the relaxant effect of (-)epicatechin. Further experiments indicated the important role of endothelial nitric oxide in (-)epicatechin-induced relaxation. The (-)epicatechin-induced relaxation was completely reversed by *L*-NAME, an inhibitor of nitric oxide synthase, or by methylene blue, an inhibitor of soluble guanylate cyclase. While *L*-arginine (1 mmol/L), the precursor of nitric oxide formation, relaxed the arterial rings in the presence of *L*-NAME or methylene blue. This concentration of *L*-arginine did not significantly affect the arterial tone. Furthermore, pretreatment with *L*-NAME markedly inhibited the relaxant effect of (-)epicatechin or (-)epicatechin had little effect in endothelium-denuded rings. These findings strongly suggest that (-)epicatechin could act on endothelium to release nitric oxide which then diffused into the underlying smooth muscle to activate guanylate cyclase leading to muscle relaxation.

Since epicatechin derivatives (2.5-40 μmol/L) were found to be strong antioxidants[5] and oxidation of nitric oxide is one of the major pathways for its inactivation. Therefore, it is possible that epicatechin may enhance the nitric oxide-mediated vasorelaxation. Pretreatment for 30 min with 10 μmol/L (-)epicatechin

slightly but significantly potentiated the relaxation induced by acetylcholine, a well-defined endothelium-dependent dilator. In contrast, (-)epicatechin at 10 μmol/L had no effect on the endothelium-unrelated relaxation induced by nitroprusside, an exogenous nitric oxide donor. These new results indicate that (-)epicatechin could delay degradation of nitric oxide while this agent does not affect the activity of guanylate cyclase.

The present study has provided some new evidence for the involvement of endothelial nitric oxide in (-)epicatechin-induced relaxation in isolated rat mesenteric arteries and for the enhancement by (-)epicatechin of acetylcholine-induced relaxation. These effects may be linked to the health benefit of drinking green tea on the vascular system.

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