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Role of endothelium/nitric oxide in vascular response to flavonoids and epicatechin

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KEY WORDS flavones; (-) epicatechin; endothelium; relaxation; nitric oxide; artery; rats

ABSTRACT

AIM: To examine the role of endothelium in the vascular responses to flavonoids, baicalein, baicalin, cardamonin, alpinetin, and to purified jasmine green tea (-) epicatechin in the isolated rat mesenteric artery rings. **METHODS**: The isometric contraction was measured by Grass force-displacement transducers. **RESULTS**: Both baicalein and baicalin enhanced the phenylephrine-induced contractile response in the endothelium-intact rings. This enhancement was abolished by pretreatment with the nitric oxide inhibitor N^G-nitro-*L*-arginine or in the absence of the endothelium. Both flavonoids also inhibited the acetylcholine-induced endothelial nitric oxide-dependent relaxation. In contrast, cardamonin, alpinetin or (-)epicatechin induced both endothelium-dependent and -independent relaxation. N^G-nitro-*L*-arginine meyhyl ester or endothelium denudation attenuated the endothelium-dependent relaxation to the same extent. **CONCLUSION**: Baicalein and baicalin enhanced the phenylephrine-induced contraction most likely through inhibiting production or/and release of endothelial nitric oxide. *Whilst, cardamonin-, alpinetin- or (-)epicatechin-induced* endothelium-dependent relaxation is primarily mediated through endothelial nitric oxide.

INTRODUCTION

Both polyphenols and flavonoids are richly contained in many herbal plants traditionally used to produce vascular protection in oriental population. However, the mechanisms of their vascular action are incompletely understood. Recently, we isolated four epicatechin derivatives (polyphenols) from jasmine green tea and showed that catechins possess antioxidant and hypolipodemic activity[1]. We also demonstrated the antioxidative action of baicalein, baicalin, and other flavonoids isolated from the dry roots of *Scutellaria baicalensis* Georgi (Huangqin)[2] or from *Scutellaria rehderiana*[3]. Some commercially available flavonoids such as kempferol, morin, and myricetin, inhibit oxidation of low-density lipoprotein[4]. The aforementioned effects may represent some of the major mechanisms underlying the cardiovascular protective action of these bioactive compounds.

Vascular tone is the most important regulator of blood pressure. The herbal plants used to treat hypertension normally contain polyphenols or/and flavonoids. For example, the wine polyphenols were shown to relax isolated rat aorta[5]. In our previous studies, green tea epicatechins inhibited the constrictor-induced arterial tone[6,7] and suppressed the serum-stimulated proliferation of aortic smooth muscle[8]. Besides, the endothelial nitric oxide accounted for over 80 % of relaxation induced by Crataegus extract[9]. The vasorelaxing effects may contribute largely to the hypotensive action of these medicinal plants. However, regulation of smooth muscle contractility involves multiple factors. Both endothelium and the underlying vascular smooth fibers may be targets for the vascular effects of many polyphenols and flavonoids.

In this report, we describe the different roles of endothelium/nitric oxide in the vascular response to some flavonoids isolated from three Chinese herbal plants in the rat mesenteric arteries *in vitro*.

MATERIALS AND METHODS

Artery preparation After approval of animal use was obtained from the Animal Ethics Committee, Chinese University of Hong Kong, male Sprague-Dawley rats weighing 250-300 g were sacrificed by cervical dislocation and bled. The main branch of the superior mesen-

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teric artery was dissected out and the surrounding connective tissue was carefully trimmed off. The artery from each rat was cut into three ring segments, 3 mm in length; each ring was mounted between two stainless steel hooks in Krebs solution-filled organ baths (10 mL). The Krebs-Henseleit solution contained (mmol/L): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1, $NaHCO_3$ 25, KH_2PO_4 1.2, *D*-glucose 11.1. The bath solution was continuously bubbled with 95 % O_2/5 % CO_2, and kept at 37 ℃ and pH ~ 7.4. During an equilibrium period of about 90 min, the basal tone of the vessel was maintained at 5 mN. The isometric contraction was measured with a Grass FT03 force-displacement transducer. In some experiments, the endothelial layer was disrupted mechanically by rubbing the lumen with fine plastic tubing. Functional removal of the endothelium was verified by the absence of relaxant response (over 80 %) to 1 μmol/L acetylcholine at the beginning of each experiment. Each experiment was performed on the ring prepared from different rats.

Experimental protocol Some thirty minutes after setting up in the organ baths, the contraction of each ring was initially induced by 1 μmol/L phenylephrine to evaluate the contractile capacity. The ring was then washed several times in Krebs solution to restore tension to the preconstricted level. In the first group of experiments, the arterial ring was contracted with phenylephrine applied cumulatively (ranging from 1 nmol/L to 30 μmol/L) to obtain the first concentration-contraction curve. Once the maximum response to phenylephrine had been obtained, the ring was rinsed with Krebs solution every 20 min until the tension returned to the basal level, then exposed for 30 min to vehicle (0.2 % Me_2SO) or to baicalein or baicalin. Another concentration-contraction curve to phenylephrine was repeated. The effects of both flavonoids were also examined in the endothelium-denuded rings. In another set of experiments, after the first concentration-response curve for phenylephrine-induced contraction, the endothelium-intact rings were incubated for 30 min with 100 μmol/L N^G-nitro-*L*-arginine (*L*-NNA) or with 100 μmol/L *L*-NNA plus 10 μmol/L baicalin or 10 μmol/L baicalein before repeating the second concentration-response curve to phenylephrine.

In some cases, the effects of baicalein, baicalin or N^G-nitro-*L*-arginine methyl ester (*L*-NAME) were examined on the endothelium-dependent relaxation induced by acetylcholine in the endothelium-intact rings.

In the final set of experiments, the concentration-dependent relaxant response to (-)epicatechin, cardamonin or alpinetin was determined in the absence and presence of *L*-NAME, or in the endothelium-denuded arterial rings.

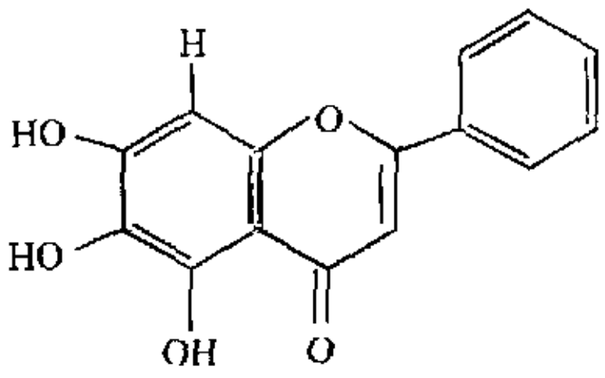
Chemicals Phenylephrine hydrochloride, acetylcholine hydrochloride, N^G-nitro-*L*-arginine, N^G-nitro-*L*-arginine methyl ester (Sigma, St. Louis, MO, USA). Baicalein and baicalin were isolated and purified from the ground roots of Huangqin that was purchased from a local store of traditional Chinese medicine in Hong Kong (Chen *et al*, 2000). (-)Epicatechin was purified by us[7]. Cardamonin and alpinetin were gifts from Prof ZT WANG, China Pharmaceutical University. The chemical structures of four flavonoids are shown in Fig 1. Four flavonoids were dissolved in dimethyl sulphoxide (Me_2SO) and further dilution was made in fresh Krebs solution. Me_2SO at 0.2 % in organ baths did not affect the basal tone or the phenylephrine-induced contractions.

Statistical analysis All data were presented as $\bar{x} \pm s$ of n experiments. To study the effects of the individual flavonoid on the phenylephrine-induced contractile response, the values of pEC_{50} (negative log of the phenylephrine molar concentration that caused 50 % of the maximal increase of muscle tension) were compared. For relaxation studies, pD_2 was calculated as the negative log of the relaxant molar concentration that caused 50 % of the maximal relaxation. These values were compared in the absence and presence of baicalin or baicalein. Statistical significance was analyzed by Student's t test (two-tailed). $P < 0.05$ was considered significant.

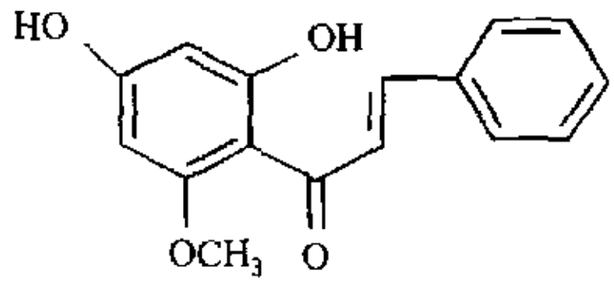
RESULTS

Flavonoid-induced vascular response Pretreatment with 10 μmol/L baicalein (Fig 2a) or 10 μmol/L baicalin (Fig 2b) significantly potentiated the phenylephrine-induced concentration-dependent contractions in the isolated rat endothelium-intact mesenteric artery rings (Tab 1). Pretreatment with 100 μmol/L *L*-NNA induced greater enhancement of the phenylephrine-induced contraction (Fig 2a, b). In the presence of *L*-NNA, neither 10 μmol/L baicalein nor 10 μmol/L baicalin affect the concentration-response curve for phenylephrine (Fig 2a, b, Tab 1). In the endothelium-denuded rings, the enhancing effect of baicalein or baicalin was abolished (Fig 2c).

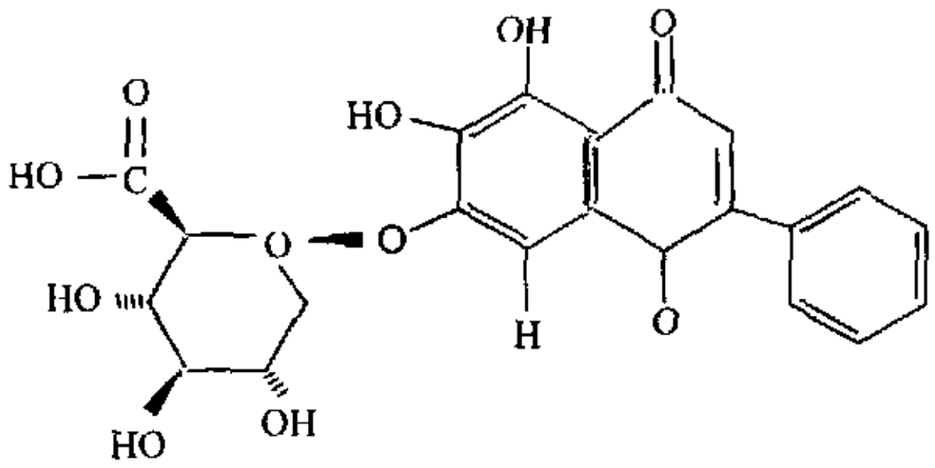
In the phenylephrine-preconstricted endothelium-



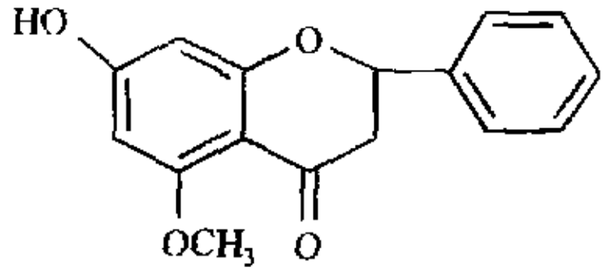
Baicalein



Cardamonin



Baicalin



Alpinetin

Fig 1. Chemical structures of baicalein, baicalin, cardamonin and alpinetin.

Tab 1. pEC_{50} values for the phenylephrine-induced contraction. $n = 5$ experiments. $\bar{x} \pm s$. $^bP < 0.05$ *vs* control group.

Drug	pEC_{50}
Control (+ endothelium)	6.4 ± 0.1
Baicalein 10 μmol/L	6.8 ± 0.2^b
Baicalin 10 μmol/L	7.1 ± 0.2^b
L-NNA 100 μmol/L	7.0 ± 0.2^b
L-NNA + Baicalein	7.1 ± 0.2^b
L-NNA + Baicalin	7.2 ± 0.3^b
Control (- endothelium)	7.2 ± 0.1
Baicalein 10 μmol/L	7.3 ± 0.2
Baicalin 10 μmol/L	7.4 ± 0.3

Drug at indicated concentrations was incubated for 30 min prior to repeating the second concentration-response curve for phenylephrine in rat isolated mesenteric artery rings.

intact rings, acetylcholine induced relaxation with a pD_2 of 7.7 ± 0.2 ($n = 6$). This relaxation was significantly reduced by pretreatment with 10 μmol/L baicalein (pD_2 of 6.5 ± 0.2, $n = 5$), 10 μmol/L baicalin (pD_2 of 7.2 ± 0.1, $n = 5$) or 30 μmol/L *L*-NAME (pD_2 of 6.7 ± 0.3, $n = 5$) ($P < 0.05$ compared with control, Fig 3). The acetylcholine-induced relaxation was absent in the endothelium-denuded rings ($n = 4$, Fig 3).

Cardamonin and alpinetin reduced the phenylephrine-induced tone in the endothelium-intact rings with respective pD_2 values of 5.0 ± 0.2 ($n = 5$) and 4.6 ± 0.2 ($n = 5$). Pretreatment with 100 μmol/L *L*-NAME or removal of the endothelium attenuated the relaxant response to cardamonin (pD_2: 3.2 ± 0.1, $n = 5$ in *L*-NAME, Fig 4a) or to alpinetin (pD_2: 4.0 ± 0.2, $n = 5$ in *L*-NAME, Fig 4b).

Green tea epicatechin-induced vascular response Purified green tea (-)epicatechin induced concentration-dependent relaxations of the rat mesenteric artery rings with endothelium with a pD_2 of 3.7 ± 0.2 ($n = 6$). Pretreatment with 100 μmol/L *L*-NAME reduced the epicatechin-induced relaxation to the similar extent as that in the endothelium-denuded rings (pD_2: 3.2 ± 0.1, $n = 6$ for *L*-NAME and 3.4 ± 0.1, $n = 5$ for endothelium-denuded rings, Fig 5).

DISCUSSION

The results of the present investigation show that endothelial nitric oxide plays different roles in the arterial response to four flavonoids isolated from two widely used medicinal plants in China. Baicalein from the roots of *Scutellaria baicalensis* Georgi has been reported to lower blood pressure[10] and to exert an antiproliferative effect on arterial smooth muscle[11]. Indeed, baicalein at higher concentrations (>30 μmol/L) relaxed the isolated rat arteries contracted by phenylephrine or active phorbol

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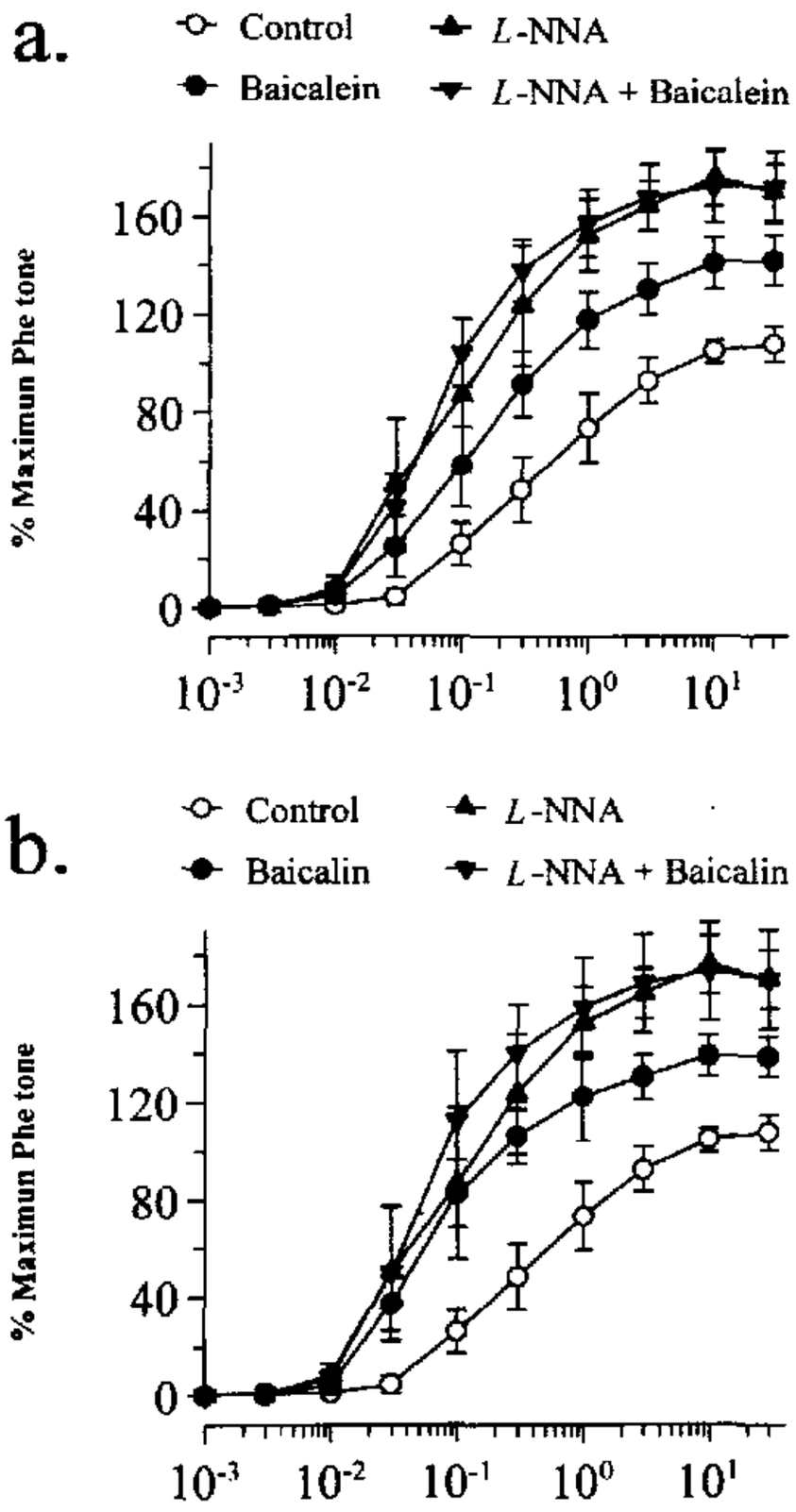

Fig 2. Concentration-response curves for the phenylephrine-induced contraction in the isolated rat mesenteric arteries in the control (○), (●) 10 μmol/L baicalein (a) or 10 μmol/L baicalin (b), 100 μmol/L *L*-NNA (▲), 100 μmol/L *L*-NNA + 10 μmol/L baicalein or baicalin (▼). (c) Concentration-response curves for phenylephrine in the endothelium-denuded rings (○, control; ●, 10 μmol/L baicalein, and ■, 10 μmol/L baicalin). $n = 5$ rings (or 5 rats). $\bar{x} \pm s$.

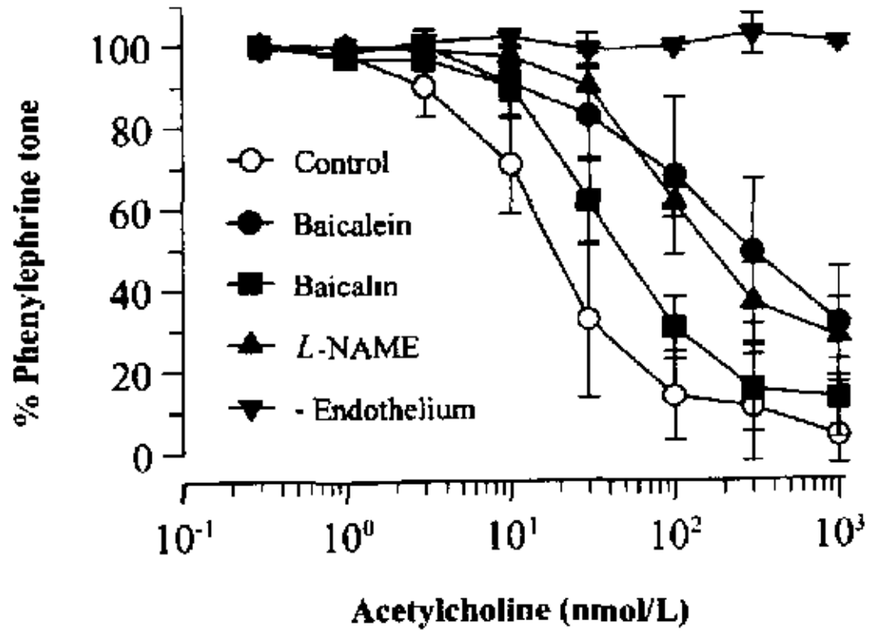

Fig 3. Inhibitory effects of flavonoids on the acetylcholine-induced endothelium-dependent relaxation. (○) $n = 6$ for acetylcholine control; (●) $n = 5$ for 10 μmol/L baicalein; (■) $n = 5$ for 10 μmol/L baicalin; (▲) $n = 4$ in 30 μmol/L *L*-NAME; (▼) $n = 4$ in endothelium-denuded rings). The results are $\bar{x} \pm s$ of n rings.

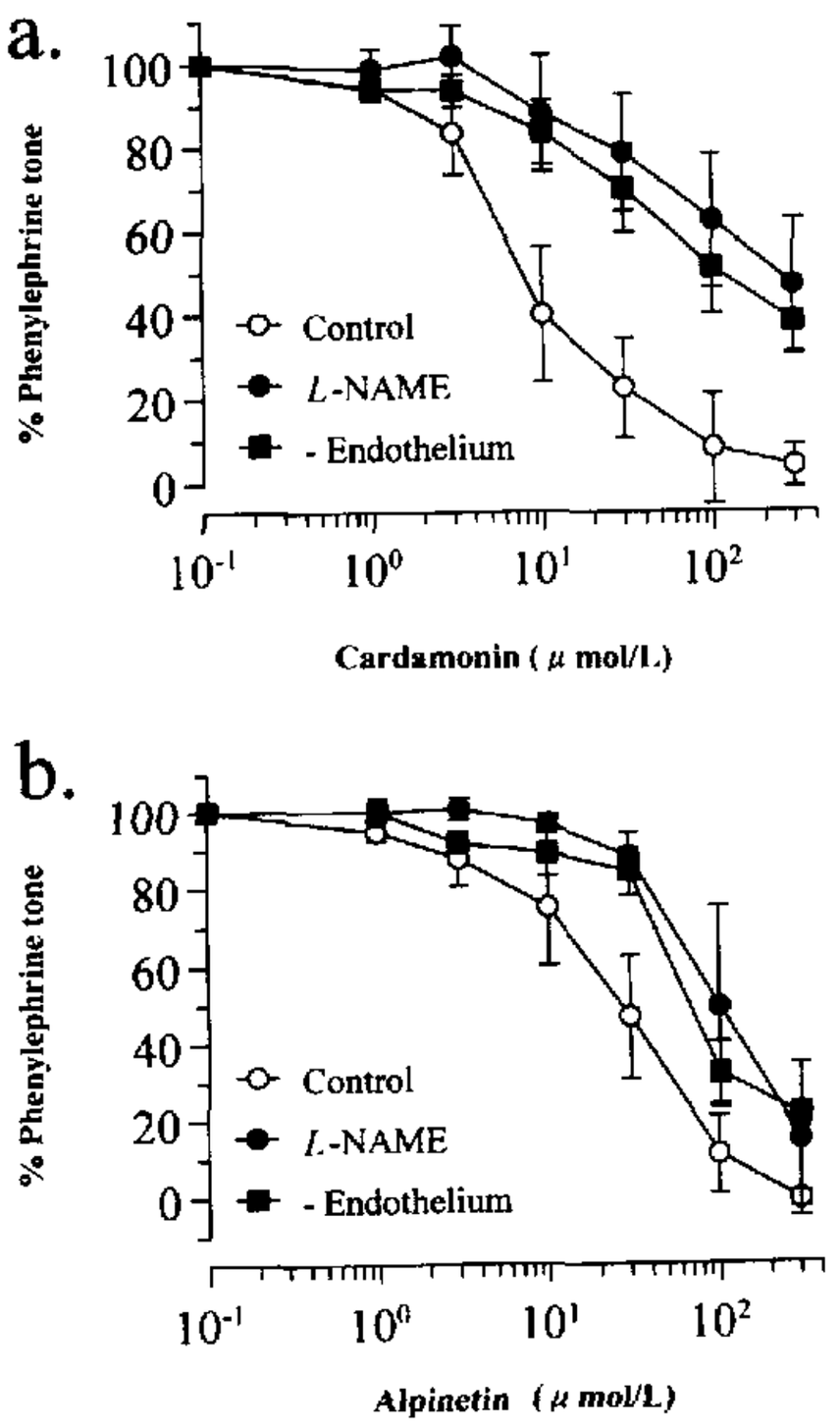

Fig 4. Concentration-response curves for the relaxant response to cardamonin (a) or to alpinetin (b) in the isolated rat mesenteric artery rings. (○) Control; (●) 100 μmol/L *L*-NAME; (■) the endothelium-denuded rings). $n = 5$ rings. $\bar{x} \pm s$.

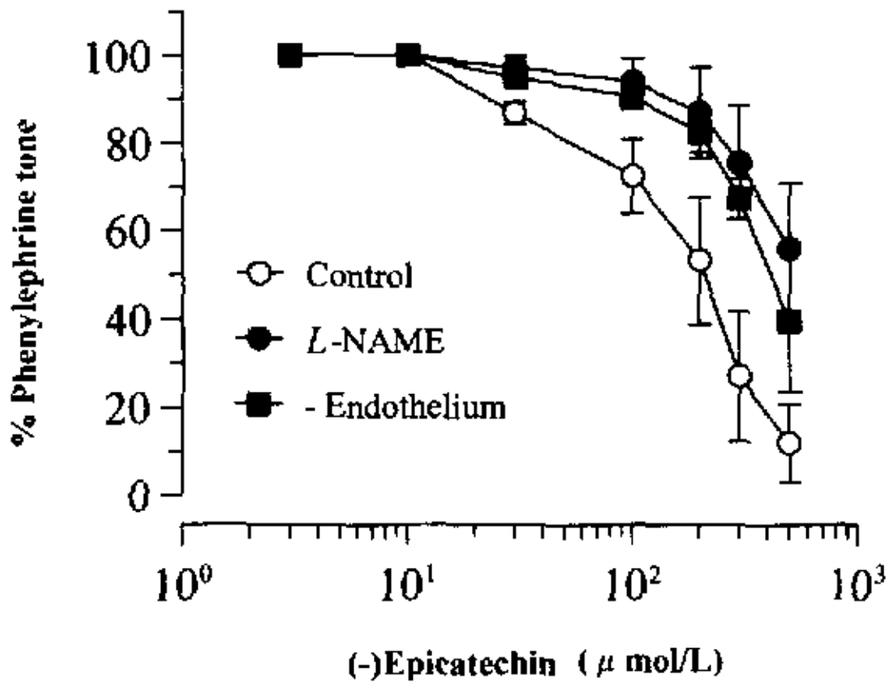

Fig 5. Concentration-response curves for the relaxant response to purified green tea (-)epicatechin in the isolated rat mesenteric artery rings. (○) $n = 6$ for the control; (●) $n = 6$ for 100 μmol/L *L*-NAME; (■) $n = 5$ in endothelium-denuded rings). The results are $\bar{x} \pm s$ of n rings.

ester[12]. However, baicalein and baicalin at 10 μmol/L enhanced the contractile response to phenylephrine, observed in the present study; this enhancement was completely abolished in the endothelium-intact rings pretreated with the nitric oxide synthase inhibitor *L*-NNA or in the endothelium-denuded rings. Furthermore, following exposure to either baicalein or baicalin in the endothelium-intact rings, the acetylcholine-induced relaxation was significantly attenuated. *L*-NAME partially inhibited the acetylcholine response. Acetylcholine failed to affect the muscle tone in the endothelium-denuded rings. These new findings clearly point to the important contribution of the endothelium/nitric oxide to the vascular action of baicalein and baicalin. However, these effects would counteract with the reported hypotensive effect of baicalein. It is therefore possible that the observed hypotensive action of baicalein may occur only at its high plasma concentrations together with the reported centrally sedative effect[13].

Cardamonin and alpinetin, both isolated from the seeds of *Alpinia blepharocalyx* K Schum concentration-dependently relaxed the phenylephrine-preconstricted artery rings. The relaxant potency was significantly impaired in the absence of the functional endothelium. Pretreatment of the endothelium-intact rings with another nitric oxide synthase inhibitor *L*-NAME inhibited the cardamonin- or alpinetin-induced relaxation to the same extent as that seen in the endothelium-denuded rings. Baicalein did not affect the endothelium-independent relaxation induced by exogenous nitric oxide donor nitroprusside[12]. The present results indicate a primary role of endothelial nitric oxide in the endothelium-dependent relaxant response to cardamonin or to alpinetin. Elevated Ca^{2+} concentration is essential for stimulation of endothelial nitric oxide synthase, however, the exact mechanisms remain to be elucidated, by which cardamonin or alpinetin activates endothelial nitric oxide-mediated pathways. Similarly, it is unknown whether or not baicalein or baicalin could inhibit the activity of nitric oxide synthase by reducing the endothelial Ca^{2+} levels.

In conclusion, both baicalein and baicalin act on the endothelium to inhibit production or/and liberation of nitric oxide; this effect appears to solely account for their potentiating effect on the phenylephrine-induced tone. Cardamonin and alpinetin produced both endothelium-dependent and -independent relaxation; both flavonoids also act on the endothelium, instead, to release nitric oxide which is the key contributor to the flavonoid-induced endothelium-dependent relaxation. Besides, the endothelial nitric oxide plays a significant role in the vasorelaxation induced by jasmine green tea (-)epicatechin. Our results suggest that the endothelium is an important target for action of many biologically active ingredients contained in Chinese traditionally used medicinal plants. The endothelium-derived factors must be taken into consideration when one searches for the natural products as potential therapeutic agents in treatment of the vascular disease.

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