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Inhibition of nitric oxide/cyclic GMP-mediated relaxation by purified flavonoids, baicalin and baicalein, in rat aortic rings

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Abstract

The dried roots of *Scutellaria baicalensis* Georgi (Huangqin) are widely used in traditional Chinese medicine. We purified two flavonoids, baicalin and baicalein from *S. baicalensis* Georgi and examined their effects on isolated rat aortic rings. Baicalin (3–50 μM) inhibited endothelium/nitric oxide (NO)-dependent relaxation induced by acetylcholine (Ach) or cyclopiazonic acid (CPA). Baicalein at 50 μM abolished Ach-induced relaxation and markedly reduced CPA-induced relaxation. Treatment with 1 mM L-arginine partially but significantly reversed the effects of baicalin (50 μM) or baicalein (50 μM) on Ach-induced relaxation. In endothelium-denuded rings, treatment with baicalin, baicalein or methylene blue partially inhibited relaxations induced by the NO donors, sodium nitroprusside (SNP) and hydroxylamine. Both flavonoids markedly reduced the increase in cyclic GMP levels stimulated by Ach in endothelium-intact rings and by SNP in endothelium-denuded rings. In contrast, exposure of endothelium-denuded rings to baicalin or baicalein did not affect relaxations induced by pinacidil or NS 1619, putative K^+ channel activators. Neither flavonoids affected agonist-induced increase in the endothelial $[Ca^{2+}]_i$. Our results indicate that baicalin and baicalein attenuated NO-mediated aortic relaxation and cyclic GMP increases, likely through inhibition of NO-dependent guanylate cyclase activity.

Keywords: Flavonoids; *Scutellaria baicalensis*; Nitric oxide; Cyclic GMP; Endothelium; Artery

1. Introduction

The dried root of *Scutellaria baicalensis* Georgi (Huangqin) is an important medicinal plant widely used in China and Japan due to its therapeutic effectiveness in the treatment of chest discomfort, nausea, acute dysentery, jaundice and carbuncles [1]. The root possesses a multitude of pharmacological activities, having anti-inflammatory [2], antiviral [3], sedative [4], antithrombotic [5], hypocholesterol [4], antioxidant [6,7], and anticancer [8] effects.

The roots of *S. baicalensis* Georgi that are rich in baicalein and baicalin, exert an antihypertensive effects [9,10]. Baicalein is a potent free radical scavenger and xanthine oxidase inhibitor [11,12], thus improving endothelial function and conferring cardiovascular protective actions against oxidative stress-induced cell injury [13]. Baicalein lowers blood pressure in renin-dependent hypertension. The *in vivo* hypotensive effect and *in vitro* vasorelaxnt effect may be partly attributed to its inhibition of lipoxygenase, resulting in reduced biosynthesis and release of arachidonic acid-derived vasoconstrictor products [14,15]. The anti-thrombotic, anti-proliferative and anti-mitogenic effects of the roots or baicalein are also reported [16–18]. These pharmacological findings highlight the therapeutic potential of plant-derived baicalein and its analogues for the treatment of arteriosclerosis and hypertension even though the mechanisms by which baicalin and baicalein exert other vascular effects are unclear.

In contrast, lower concentrations of baicalein enhance sensitivity to a number of receptor-dependent vasoconstrictor agonists in isolated rat arteries [19,20]. Inhibition of endothelium-dependent regulation may likely be a

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Abbreviations: Ach, acetylcholine; CPA, cyclopiazonic acid; L-NNA, N^G-nitro-L-arginine; NO, nitric oxide; ODQ, 1*H*-[1,2,4]oxadiazolo[4,2-α]quinoxalin-1-one; SNP, sodium nitroprusside.

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contributing mechanism [21,22]. Baicalin and baicalein inhibit thrombin-induced elevation of $[Ca^{2+}]_i$ in cultured human umbilical vein endothelial cells [18]. The endothelium plays a critical role in the regulation of vascular tone by liberating endothelium-derived relaxing factors. If these flavonoids lower endothelial $[Ca^{2+}]_i$ that triggers the release of endothelial relaxing factors, endothelium-dependent relaxation would be attenuated. This possibility could limit the potential usefulness of these compounds as hypotensive agents. On the other hand, if the flavonoids inhibit NO/cyclic GMP-mediated vascular regulation, they could act as anti-inflammatory agents in acute inflammation where NO regulates edema through increases in vascular permeability.

We purified baicalin and baicalein from the dried roots of *S. baicalensis* Georgi, and found that both flavonoids enhanced agonist-induced contraction only in endothelium-intact rat arteries [21,23]. However, it is unclear how both agents affect endothelial function. We hypothesize that both flavonoids reduce endothelium-dependent relaxation through inhibition of the NO-cyclic GMP pathway. To this end, we examined the effects of both flavonoids on endothelium-dependent and -independent relaxation of rat aortic rings and measured changes in tissue cyclic GMP, and endothelial cell $[Ca^{2+}]_i$.

2. Materials and methods

2.1. Artery preparation

Male Sprague–Dawley rats (~250–300 g) were sacrificed by cervical dislocation and bled. The thoracic aorta was excised. After surrounding connective tissue had been carefully cleaned off, four 3 mm-wide ring segments were prepared from each aorta. Each was dispensed between two stainless wire hooks in a 10-mL organ bath. The upper wire was connected to a force–displacement transducer (Grass Instruments Co.) and the lower one was fixed at the bottom of the organ bath. The organ bath was filled with Krebs solution of the following composition (in mM): 119 NaCl, 4.7 KCl, 25 $NaHCO_3$, 2.5 $CaCl_2$, 1 $MgCl_2$, 1.2 KH_2PO_4, and 11 D-glucose. The bathing solution was gassed with 95% O_2–5% CO_2 at 37° (pH ≈ 7.4). The rings were placed under an optimal basal tone of 15 mN, determined from previous length–tension experiments. Changes in isometric tension were measured with a Grass force transducer and stored on MacLab software (Version 3.0, AD Instruments) for later data analysis. Twenty minutes after mounting in organ baths, the rings were first contracted with 0.3 μM phenylephrine to test the contractility and then relaxed by 1 μM Ach. They were rinsed several times until baseline tone was restored. The rings were thereafter allowed to equilibrate for 60 min. Baseline tone was readjusted to 15 mN when necessary. In some rings the endothelial layer was mechanically disrupted by gently rubbing the luminal surface back and forth several times with plastic tubing. Functional removal of the endothelium was confirmed by the absence of a relaxant response to 1 μM Ach. Each set of experiments was performed on rings prepared from different rats. The use of laboratory animals was approved by the Animal Research Ethical Committee of the Chinese University of Hong Kong.

2.2. Primary culture of endothelial cells

Rat aortic endothelial cells were prepared as previously described [24]. Endothelial cells were isolated by gently scraping the intimal surface of rat aortas with a scalpel blade. The cells were rinsed off the scalpel into dishes containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum plus penicillin (100 units/mL) and streptomycin (100 μg/mL). The cells were allowed to grow in an incubator at 37° under a 5% CO_2 atmosphere until islands of endothelial cells appeared. Culture medium was changed everyday until a confluent layer of endothelial cells was obtained. Three days later, endothelial cell identity was confirmed by positive reactivity to an antibody against Von Willebrand factor (DAKO A/S); the results confirmed that more than 99% of cells were of endothelial origin.

2.3. Tension measurements

In the first set of experiments, relaxation of U46619-contracted endothelium-intact rings was induced by Ach (30 nM–10 μM) or CPA (0.3–10 μM) in the absence and presence of baicalin (3–50 μM), baicalein (50 μM) or L-NNA (10 μM). Rings were incubated with each drug for 30 min prior to application of U46619. In some experiments, L-arginine (1 mM) was added 10 min before application of baicalin (50 μM) or baicalein (50 μM). In the second series of experiments, the effects of baicalin (3 μM), baicalein (3 μM), methylene blue (3 μM) or L-NNA (10 μM) were examined on the relaxant responses to SNP (0.1 nM–1 μM) or hydroxylamine (10 nM–100 μM) in endothelium-denuded rings. SNP is an exogenous NO donor while hydroxylamine is suggested to be an intermediate product of the pathway for the oxidative conversion of L-arginine to NO and thus may serve as an endogenous NO donor [25]. Higher concentrations (>10 μM) of baicalin or baicalein were not tested since the U46619-induced tone was not maintained in endothelium-denuded rings after exposure to such concentrations [19]. In the last group of experiments, the effects of baicalin and baicalein, each at 50 μM were tested on relaxations induced by pinacidil (0.3–100 μM) or NS 1619 (1–300 μM) in endothelium-denuded rings. U46619 (30 nM) induced a larger contraction in endothelium-denuded rings; we therefore reduced the concentration of U46619 to 10–20 nM so that we obtained a similar level of contraction in endothelium-intact and -denuded rings.

2.4. Cyclic GMP measurements

After 60-min equilibration in oxygenated Krebs solution at 37°, the aortic rings were first incubated with phenylephrine (0.3 μM) for 10 min, then with 0.3 μM Ach or with 0.1 μM SNP for 3 min in the absence and presence of baicalin (1–30 μM) or baicalein (1–30 μM). At the end of the reaction, rings were rapidly frozen in liquid nitrogen and stored at −70° until homogenization in 0.5 mL of ice-cold 6% trichloroacetic acid using a glass homogenizer. The homogenate was centrifuged at 2000 *g* for 10 min at 4°. The supernatant was extracted three times with 3 vols. of diethyl ether before lyophilization. The tissue content of cyclic GMP was measured by radioimmunoassay using a [^{125}I] cyclic GMP RIA kit (DuPont). The tissue residue was dissolved in 2 M NaOH and the protein content was determined using a protein assay kit (Sigma Chemical Co.) with bovine serum albumin as the standard. The tissue content of cyclic GMP was presented as pmol/mg protein.

2.5. Ca^{2+} fluorescence measurements

Rat aortic endothelial cells were cultured on 25 mm glass coverslips in DMEM supplemented with 10% FBS and penicillin–streptomycin in a humidified atmosphere (5% CO_2) at 37°. Cells were grown for 4 days, and starved of serum (0.4% FBS) for 24 hr before the experiment. Cells were incubated at room temperature (∼24°) for 60 min in a modified Tyrode solution that contained (in mM) 150 NaCl, 2.7 KCl, 1.2 KH_2PO_4, 1.2 $MgCl_2$, 1.0 $CaCl_2$, and 10 HEPES (pH 7.4) with 10% FBS, 3 μM Fura-2/acetoxymethyl ester (Fura-2/AM; Molecular Probes) and 1.6 μM pluornic F127. The cells were then rinsed several times with Tyrode solution to remove the serum and fluorescent Ca^{2+} indicator from the extracellular fluid and left for 30 min in a fresh, serum-free Tyrode solution to allow intracellular hydrolysis of Fura-2/AM into Fura-2 by esterases. Changes in $[Ca^{2+}]_i$ were monitored by Fura-2 ratiometric fluorescence using a PTI Fluorescence Spectroscopy and analyzed by a Felix software (Photon Technology International Inc.). The cells were excited at 340 and 380 nm, and emission was monitored at 510 nm. The fluorescence ratio was obtained by dividing, after background subtraction, the 340 nm × 380 nm images as an index of changes in $[Ca^{2+}]_i$. All experiments were performed at 24°. Since Ach did not always induce an increase in $[Ca^{2+}]_i$ in cultured endothelial cells probably due to loss of the Ach receptors, bradykinin was instead used as a stimulus.

2.6. Chemicals

Phenylephrine, Ach, U46619, SNP, hydroxylamine, pinacidil, L-NNA, CPA, methylene blue, L-arginine, bradykinin, NS 1619 (Sigma). ODQ (Tocris). Baicalin and baicalein were isolated and purified from the dried roots of *S. baicalensis* Georgi as previously described [12]. The chemical structure of baicalein and baicalin were verified on the basis of their spectra data of UV, LC–MS, ^{1}H and ^{13}C NMR [12]. All chemicals were dissolved in Krebs solution except for U46619, pinacidil, baicalin and baicalein which were dissolved in dimethylsulfoxide. Dimethylsulfoxide at 0.2% did not affect U46619-induced tone.

2.7. Data analysis

The relaxant effects of the vasodilators were expressed as 100 minus percentage reduction from the U46619-induced contractile response. Non-linear regression curve fitting was performed on individual cumulative concentration–response curves (GraphPad software, Version 3.0). pD_2 values were calculated as negative log molar of dilator that induced 50% of the maximal relaxation (E_{max}). All data were shown as means ± SEM. Statistical significance was determined by two-tailed Student's *t*-test or one-way ANOVA followed by the Newman–Keuls test when more than two treatments were compared. A *P* value of less than 0.05 was regarded to be significant.

3. Results

3.1. Effects on endothelium-dependent relaxation

Traces in Fig. 1 show that Ach concentration dependently relaxed an U46619-contracted rat aortic ring with

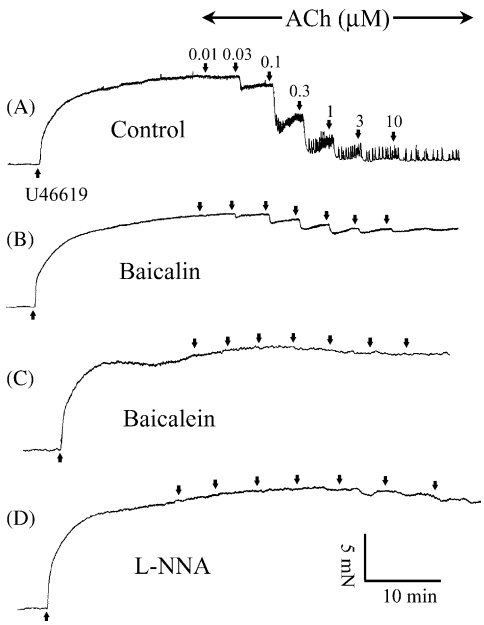

Fig. 1. The representative traces showing the aortic relaxation induced by Ach in control (A) and in the presence of 50 μM baicalin (B), 50 μM baicalein (C), or 10 μM L-NNA (D) in endothelium-intact rings. Scale bars apply to all records.

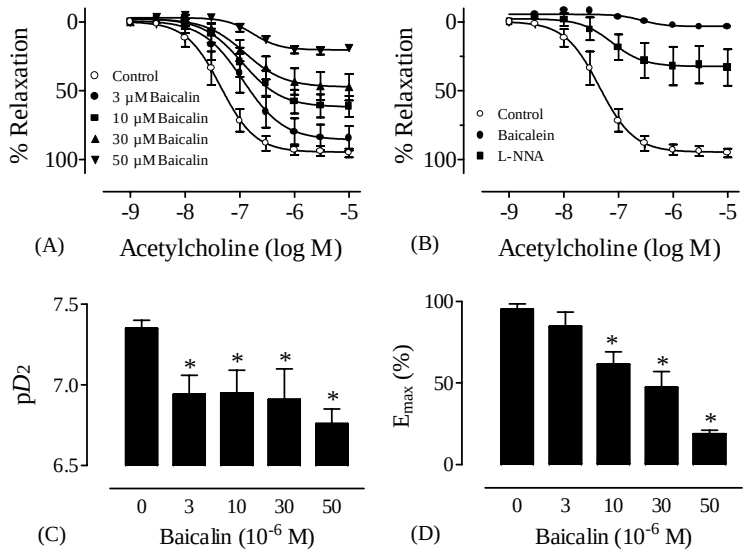

Fig. 2. (A) Concentration–response curves for the relaxant effect of Ach in control (○, N = 7) and in the presence of baicalin (●, N = 5; ■, N = 5; ▲, N = 5; ▼, N = 5). (B) Concentration–response curves for the relaxant effect of Ach (○, N = 7 for control; ●, N = 5 for 50 μM baicalein; ■, N = 5, for 10 μM L-NNA). The pD_2 (C) and E_{max} (%) values (D) for Ach-induced relaxation in the presence of baicalin. Significant difference is indicated by * ($P < 0.05$) from controls. The results are means ± SEM of N experiments.

endothelium (Fig. 1A); treatment with 50 μM baicalin (Fig. 1B) or 50 μM baicalein (Fig. 1C) reduced or abolished Ach-induced relaxation. L-NNA (10 μM) abolished relaxation to Ach (Fig. 1D). Figure 2A shows that baicalin (3–50 μM) produced concentration-dependent inhibition of the Ach response. Baicalin reduced both sensitivity (Fig. 2C) and the maximal relaxation (Fig. 2D), while baicalein at 50 μM abolished and L-NNA at 10 μM reduced the relaxant response to Ach (Fig. 2B). Similarly, both baicalin (3–50 μM, Fig. 3A) and baicalein (50 μM, Fig. 3B) attenuated the relaxation to CPA, another endothelial NO-dependent dilator. The pD_2 values and E_{max} for the CPA-induced relaxation were collectively presented in Fig. 3C and D. Neither flavonoid affected baseline tone.

Treatment of endothelium-intact rings with 1 mM L-arginine caused a small but significant reversal effect on baicalin- or baicalein-induced inhibition of the Ach response (Fig. 4A and B). L-Arginine produced a greater effect on rings treated with baicalin than with baicalein (E_{max}: 18.9 ± 0.8% in baicalin; 47.7 ± 4.4% in baicalin + L-arginine; 6.5 ± 3.7% in baicalein; 17.3 ± 1.3% in baicalein +

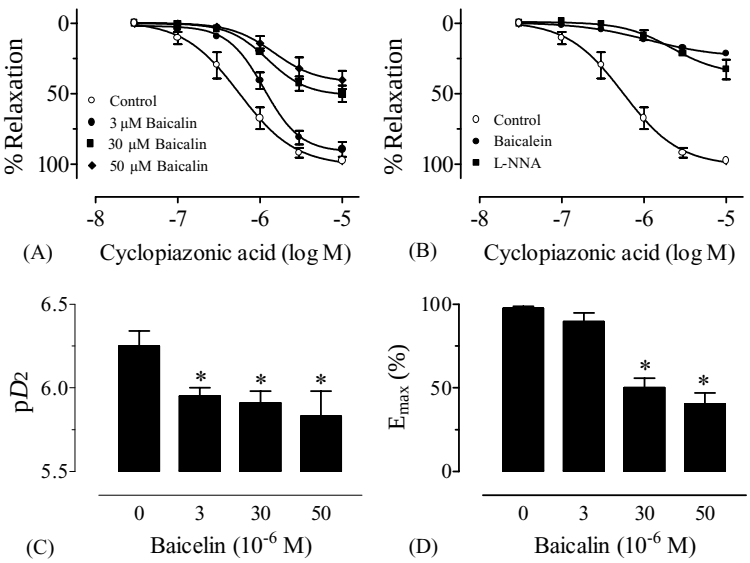

Fig. 3. (A) Concentration–response curves for the relaxant effect of CPA in control (○, N = 6) and in the presence of baicalin (●, N = 5; ■, N = 5; ◆, N = 5). (B) Concentration–response curves for the relaxant effect of CPA (○, N = 6 for control; ●, N = 5 for 50 μM baicalein; ■, N = 5 for 10 μM L-NNA). The pD_2 (C) and E_{max} (%) values (D) for CPA-induced relaxation in the presence of baicalin. Significant difference is indicated by * ($P < 0.05$–0.01) from controls. The results are means ± SEM of N experiments.

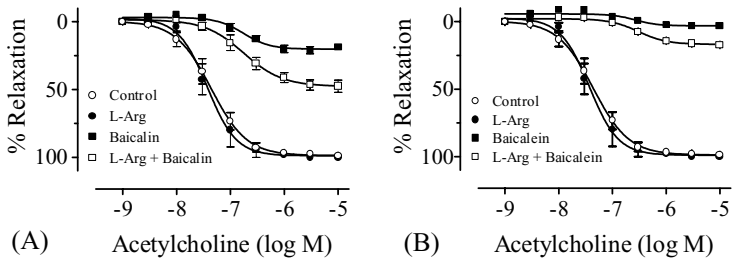

Fig. 4. (A) Concentration–response curves for Ach in control (○, N = 7) and in the presence of 1 mM L-arginine (L-Arg) (●, N = 5), 50 μM baicalin (■, N = 5), or L-Arg + baicalin (□, N = 5). (B) Concentration–response curves for Ach in control (○, N = 7) and in the presence of 1 mM L-Arg (●, N = 5), of 50 μM baicalein (■, N = 5), or of L-Arg + baicalein (□, N = 5). L-Arginine was added 10 min prior to application of the flavonoid. The results are means ± SEM of N experiments.

L-arginine; Fig. 4). L-Arginine did not modify the relaxant response to Ach (pD_2: 7.36 ± 0.06 in control and 7.43 ± 0.06 in L-arginine, $P > 0.05$; Fig. 4).

3.2. Effects on NO donor-mediated relaxation

Traces in Fig. 5 show an inhibitory effect of baicalin, baicalein, or methylene blue, each at 3 μM on the relaxant response to hydroxylamine in endothelium-denuded aortic rings. Hydroxylamine-induced relaxations with a pD_2 of 6.98 ± 0.12. Treatment with baicalin, baicalein or methylene blue caused rightward shift of the concentration-relaxation curve for hydroxylamine with little effect on the maximal relaxation (Fig. 6A and B). Similarly, SNP-induced relaxation of U46619-preconstricted rings with a pD_2 of 8.73 ± 0.06 (Fig. 6C). Treatment with baicalin, baicalein or methylene blue each at 3 μM also attenuated SNP-induced relaxation (Fig. 6C and D). In contrast, L-NNA at 10 μM did not alter SNP-induced relaxation (pD_2: 8.62 ± 0.11, $P > 0.05$ compared with control).

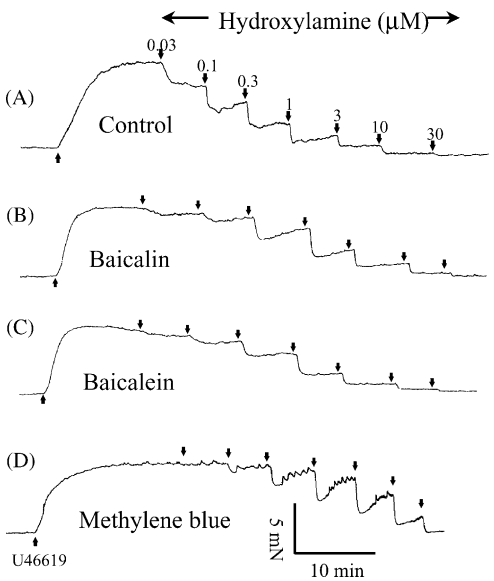

Fig. 5. The traces showing endothelium-independent relaxation induced by hydroxylamine in control (A) and in the presence of 3 μM baicalin (B), 3 μM baicalein (C), or 3 μM methylene blue (D) in endothelium-denuded aortic rings. Calibration bars apply to all traces.

3.3. Effects on relaxation induced by K^+ channel activators

Pinacidil or NS 1619 induced concentration-dependent relaxations in endothelium-denuded rings. Treatment with baicalin (50 μM) or baicalein (50 μM) did not influence relaxation induced by pinacidil (pD_2: 5.86 ± 0.14 in control; 5.83 ± 0.12 in baicalin and 5.72 ± 0.09 in baicalein, N = 5, $P > 0.05$ as compared with control) or by NS 1619 (pD_2: 4.44 ± 0.03 in control; 4.53 ± 0.02 in baicalin and 4.56 ± 0.03 in baicalein, N = 5, $P > 0.05$ as compared with control).

3.4. Effects on cyclic GMP formation

Figure 7A shows that Ach at 0.3 μM caused approximately 21-fold increase in tissue content of cyclic GMP in endothelium-intact rings and this effect was abolished by 10 μM LNNA. Treatment of endothelium-intact rings with baicalein (10–30 μM) or baicalin (10–30 μM) markedly reduced Ach-induced increase in cyclic GMP levels. Both flavonoids at 30 μM also inhibited the production of cyclic GMP stimulated by 0.1 μM SNP, and ODQ (3 μM) abolished the effect of SNP (Fig. 7B).

3.5. Effects on the endothelial $[Ca^{2+}]_i$ response

Bradykinin (0.3 μM) increased the peak $[Ca^{2+}]_i$ by approximately 3-fold in cultured endothelial cells. The second exposure to the same concentration of bradykinin induced smaller effect (Fig. 8A) probably due to a slow development of desensitization for bradykinin receptor-mediated cellular response. Treatment with baicalin (30 μM, Fig. 8B) or baicalein (30 μM, Fig. 8C) showed little effect on bradykinin-induced increase in endothelial $[Ca^{2+}]_i$. Figure 8D shows ratio values (2nd/1st) of peak increase in $[Ca^{2+}]_i$ in response to two consecutive applications of bradykinin under three experimental conditions.

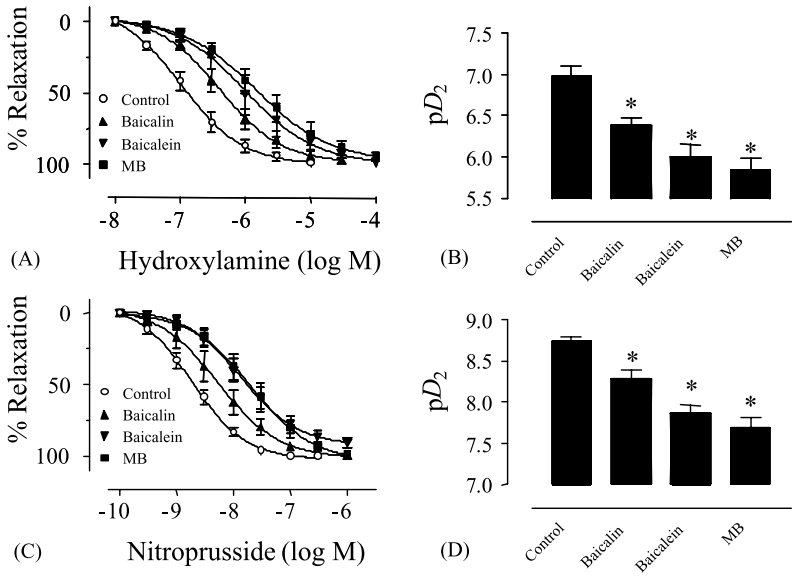

Fig. 6. Concentration–response curves for the relaxant effects of (A) hydroxylamine and (C) SNP (○, N = 8 for control; ▲, N = 6 for 3 μM baicalin; ▼, N = 5 for 3 μM baicalein; ■, N = 7 for 3 μM methylene blue (MB)). The pD_2 values for relaxation induced by hydroxylamine (B) or SNP (D) in the presence of baicalin, baicalein, or MB. Endothelium-denuded rings were incubated with each drug for 30 min before application of U46619. Significant difference is indicated by * ($P < 0.05$–0.01) from controls. The results are means ± SEM of N experiments.

4. Discussion

The present results show that baicalein and baicalin, purified flavonoids from a popular Chinese herbal plant, *S. baicalensis* Georgi impaired endothelial NO-mediated relaxation in isolated rat aortic rings. Both agents also inhibited endothelium-independent relaxation induced by NO donors. We have thus provided novel evidence suggesting that the aortic action of the two flavonoids is mediated through acting on both endothelium and vascular smooth muscle via inhibition of NO production/release and of a cyclic GMP-dependent mechanism.

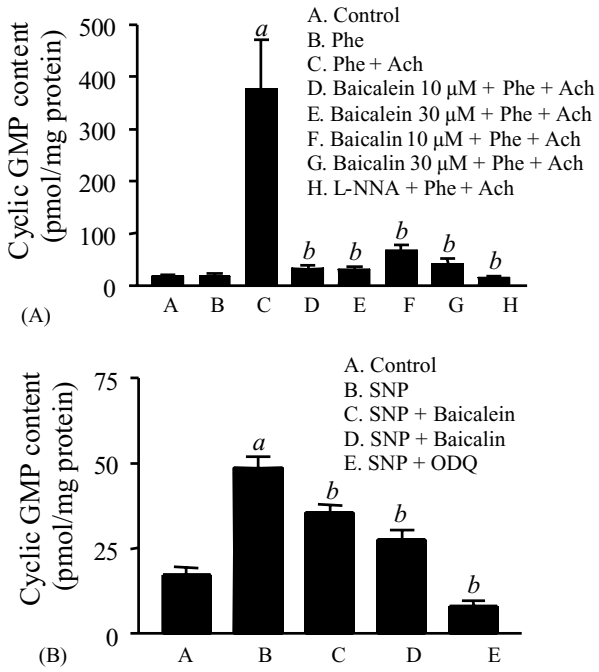

Fig. 7. Effects of baicalin and baicalein on the increase of cyclic GMP levels induced by 0.3 μM Ach (A) endothelium-intact rings or 0.1 μM SNP (B) in endothelium-denuded rings. Significant difference ($P < 0.05$–0.01) is indicated by 'a' between control group and Ach (or SNP) group and 'b' between Ach (or SNP) group and other treatment groups. The results are means ± SEM of six to seven experiments.

Baicalin and baicalein reduced endothelial NO-dependent relaxation to similar degrees in the aortic responses to Ach (a receptor-mediated dilator) and CPA (a receptor-independent dilator), suggesting that they did not act as Ach receptor antagonists. L-NNA abolished both Ach-induced cyclic GMP elevation and aortic relaxation, indicating that Ach-induced relaxation is predominantly attributable to NO-induced accumulation of cyclic GMP. Baicalein was slightly more effective than baicalin at 50 μM. In rat mesenteric artery baicalin and baicalein enhanced phenylephrine- or U46619-induced contractile responses and this effect was abolished in the presence of L-NNA, indicating that baicalein may inhibit NO release [21]. The present study shows that treatment with L-arginine, the precursor of NO biosynthesis antagonized the inhibitory effect of baicalin and baicalein. L-Arginine at 1 mM had greater reversal effect on baicalin- than baicalein-treated rings. Treatment with baicalin, baicalein or L-NNA attenuated the increase of cyclic GMP in response to Ach. These data indicate that like the NOS inhibitors, both flavonoids may act on endothelium to inhibition NO production and/or release in response to endothelial NO-dependent dilators. Like L-NNA, both flavonoids reduced the maximal relaxation induced by Ach or CPA. Though unproven in this study, it is conceivable that both flavonoids reduce substrate (L-arginine) availability and/or

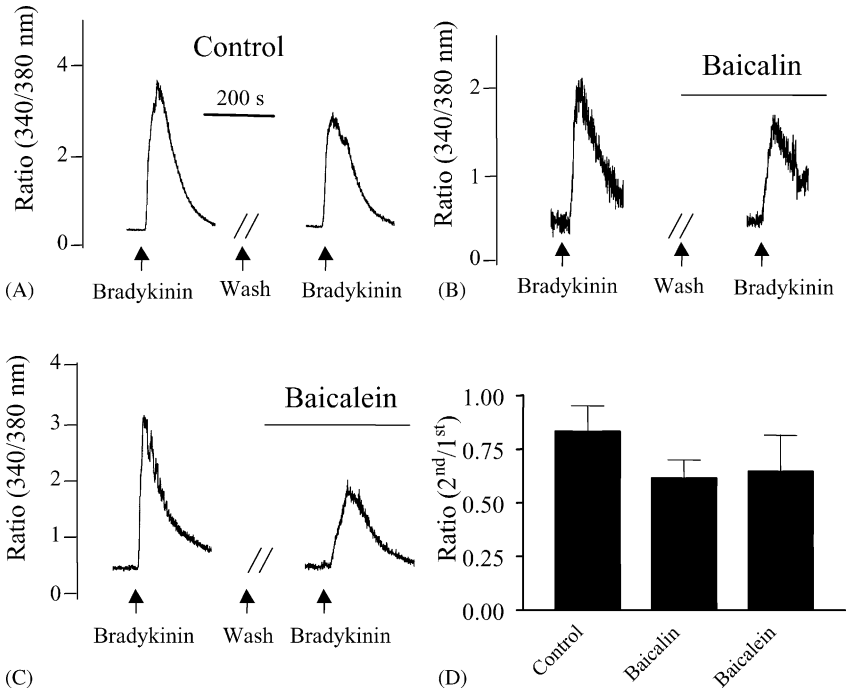

Fig. 8. Transient increase in $[Ca^{2+}]_i$ of cultured rat aortic endothelium in response to two consecutive applications with bradykinin (0.3 μM) in control (A) and in the presence of 30 μM baicalin (B) or 30 μM baicalein (C) applied 30 min before the second bradykinin stimulation. The Ca^{2+} signal ratio (2nd/1st) (D). The results are means ± SEM of five experiments.

cofactor levels. Such effects would inhibit endothelial NOS activity.

Acute activation of endothelial NOS in blood vessels in response to the application of a dilator agonist such as Ach or bradykinin results in the activation of guanylate cyclase in vascular smooth muscle cells and the formation of cyclic GMP. An elevation in intracellular cyclic GMP levels leads to vasorelaxation. In order to examine whether baicalin and baicalein inhibit endothelial NO-mediated response partly via inhibition of guanylate cyclase, their effects were tested on endothelium-denuded rings. Both flavonoids inhibited aortic relaxation induced by NO donors, SNP and hydroxylamine. This effect is similar to that of methylene blue, a known inhibitor of guanylate cyclase. Again, baicalein had a greater inhibitory effect than baicalin. Baicalin and baicalein produced marked reduction in SNP-stimulated cyclic GMP production. These findings clearly point to the flavonoid-induced inhibition of guanylate cyclase.

Baicalein was reported to inhibit thrombin-induced elevation in $[Ca^{2+}]_i$ in cultured human vascular endothelial cells [18]. If this effect were to occur in endothelium-intact aortic rings, the lowered endothelial $[Ca^{2+}]_i$ would inhibit the Ca^{2+}-dependent endothelial NOS activity and thus impairs NO formation and subsequent NO-dependent relaxation. To establish whether the flavonoid-induced inhibition of NO-mediated relaxation is associated with a reduced $[Ca^{2+}]_i$ in endothelium, we examined their effects on the endothelial $[Ca^{2+}]_i$. The results obtained in Ca^{2+} fluorescence measurements in the cultured rat aortic endothelial cells, however, do not indicate such an association. Agonist-stimulated increase in endothelial $[Ca^{2+}]_i$ was unaffected in the presence of baicalin or baicalein at a concentration (30 μM) that inhibited markedly NO-mediated aortic relaxation.

Neither baicalin nor baicalein affected endothelium-independent relaxation induced by putative K^+ channel activators, pinacidil or NS 1619, suggesting that neither flavonoids acts as a K^+ channel blocker and that their inhibitory effects on NO-mediated relaxation is not non-specific.

Taken together, the present study has provided evidence showing the novel vascular effects for purified baicalin and baicalein from the dried roots of *S. baicalensis* Georgi. Both flavonoids inhibited endothelial NO-mediated aortic relaxation via inhibition of NO-dependent cyclic GMP production. They reduced exogenous NO-mediated relaxation via inhibition of cyclic GMP accumulation in the vascular smooth muscle cells. If the inhibitory effect on NO-dependent relaxation were to occur in small blood vessels *in vivo*, vascular permeability would be reduced. This may contribute to the reported anti-inflammatory action of the flavonoids against acute edema [2], in additional to their inhibition of lipoxygenase [26], a key enzyme involved in the inflammatory response.

Oral administration of baicalein reduces the inflammatory response in a murine model of acute experimental colitis [27]. After oral administration, the absolute absorption is 40% for baicalein [28]. It is suggested that baicalein is directly absorbed through the small intestine while baicalin may be absorbed only after hydrolysis by colonic

bacterial enzymes to form baicalein [28,29]. The conjugated form of baicalein is present almost exclusively in circulating blood following oral administration of baicalin and baicalein [28], indicating that the *in vivo* effects of both flavonoids may be mediated by their conjugated metabolites. Nevertheless, further investigation is needed to determine whether oral baicalein or baicalin exert similar effects on endothelial or exogenous NO-mediated vasodilation *in vivo*.

Acknowledgments

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