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Endothelium-dependent contraction and direct relaxation induced by baicalein in rat mesenteric artery

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Abstract

The vascular effect of purified baicalein from *Scutellaria baicalensis* Georgi (Huangqin) was examined in rat isolated mesenteric arteries. Baicalein exerts both contractile and relaxant effects on the U46619-, phenylephrine- or high K^+-contracted endothelium-intact arteries. In endothelium-denuded arteries, the contractile response to baicalein (0.3–10 μM) was absent while the relaxant response to baicalein (30–300 μM) remained. Pretreatment with 100 μM N^G-nitro-L-arginine (L-NNA) or 3 μM methylene blue abolished the baicalein-induced contraction while 10 μM indomethacin or 100 nM BQ610 had no effect. Pretreatment with baicalein (3–10 μM) significantly attenuated the relaxation induced by acetylcholine or by A23187. In contrast, baicalein did not affect the sodium nitroprusside-induced relaxation in endothelium-denuded arteries. Baicalein also concentration dependently inhibited the contractile response to 1 μM phorbol 12,13-diacetate (PDA) in Ca^{2+}-free solution. Baicalein had little effect on the contractile response to 60 mM K^+ or to 10 mM caffeine in endothelium-denuded arteries. The baicalein-induced relaxation was unaffected by 1 μM glibenclamide or by 3 mM tetraethylammonium ions in endothelium-denuded arteries. These results indicate that baicalein at low concentrations caused a contractile response and inhibited the endothelium-dependent relaxation, probably through inhibition of endothelial nitric oxide (NO) formation/release. At higher concentrations, baicalein relaxed the arterial smooth muscle partially through inhibition of the protein kinase C-mediated contractile mechanism.

Keywords: Baicalein; Endothelium; Nitric oxide (NO); Relaxation; Smooth muscle; Mesenteric artery; (Rat)

1. Introduction

The dry root of *Scutellaria baicalensis* Georgi (Huangqin) has been widely used in traditional Chinese herbal medicine. The roots have antiinflammatory (Lin and Shieh, 1996), antiviral (Nagai et al., 1995), sedative (Ying and Guo, 1994), antithrombotic (Kubo et al., 1985), hypocholesterol (Ying and Guo, 1994), and antioxidant (Gao et al., 1995; Gabrielska et al., 1997) effects. Baicalein, a flavonoid contained in the roots of *S. baicalensis* Georgi, was reported to have hypotensive effects (Lin et al., 1958; Tang and Zhou, 1958). Baicalein has been recently shown to exert an antiproliferative effect on vascular smooth muscle cells (Huang et al., 1994). Baicalein inhibited the production of plasminogen activator inhibitor-1 induced by thrombin in cultured human umbilical vein endothelial cells and this effect may be caused by a reduction of intracellular $[Ca^{2+}]_i$ in endothelial cells (Kimura et al., 1997). Thrombin plays an important role in blood coagulation (Furie and Furie, 1988) and platelet activation (Kimura et al., 1988). In addition, baicalein may act as an inhibitor of lipoxygenase in rat platelets (Sekiya and Okuda, 1982). These studies indicate that baicalein may have a potential use in the treatment of arteriosclerosis and thrombosis. However, the mechanisms by which baicalein exerts its hypotensive effect are unknown.

In this study, we have purified baicalein from the dry roots of *S. baicalensis* Georgi (Huangqin) and examined its effect on rat isolated mesenteric arteries. Our results indicated a complex effect of baicalein in blood vessels. In a low concentration range (0.3–10 μM), baicalein further increased muscle tension in the agonist-contracted arteries and reduced the endothelium/nitric oxide (NO)-mediated relaxation. These effects appeared to be caused by inhibition of NO production in the endothelium. In a high concentration range (30–300 μM), baicalein relaxed the

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endothelium-denuded vessels partially through inhibition of the protein kinase C-mediated contractile pathway in arterial smooth muscle cells.

2. Materials and methods

2.1. Extraction of S. baicalensis Georgi

The ground roots of *S. baicalensis* Georgi were extracted with hexane. Briefly, 5 g of the sample was placed into a flask attached to a water-cooled condenser through a ground glass joint and 100 ml hexane was then added. The flask was then heated to a temperature at which hexane evaporated. After refluxing for 2 h, the flask was cooled to room temperature and the contents were filtered. The residue was refluxed again with a fresh 100 ml of hexane for 2 h. The hexane extracts were then pooled and kept. An acetone extract was then obtained by refluxing the remaining residue twice after hexane extraction with 100 ml of acetone followed by filtration. Finally, a methanol extract was obtained by extracting the residue twice after acetone extraction with 100 ml of methanol in a similar way. The solvents from the three extracts were then evaporated under vacuum. The resulting three extracts were then weighed and kept at −20°C.

Two and a half grama of the acetone extract was dissolved in 150 ml methanol and then was placed overnight at −20°C in a freezer. The yellow precipitate was filtered, redissolved in methanol and then placed again overnight at −20°C. The final yellow crystals obtained (210 mg) after filtration had a melting point of 268–271°C.

2.2. Arterial ring preparation

Male Sprague–Dawley rats weighing ~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. The tissues were then mounted between two stainless wire hooks in a 10-ml organ bath. The upper wire was connected to a force–displacement transducer (Grass Instruments, USA) 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, 11 D-glucose. The bath solution was continuously gassed with a mixture of 95% O_2 and 5% CO_2, and kept at 37°C (pH ≈ 7.4). The rings were placed under an optimal resting tension of 0.5 g, which had been determined by length–tension relationship experiments. Changes in isometric tension were measured and stored on a Maclab software (version 3.0). Twenty minutes after being set up in organ baths, the tissues were first contracted with a single concentration of phenylephrine (1 μM) to test their contractility, after which time they were rinsed with prewarmed and oxygenated Krebs solution several times until the basal level of tension was restored. The tissues were then allowed to equilibrate further for 60 min. The resting tension was readjusted to 0.5 g when necessary. In some arterial rings the endothelial layer was mechanically disrupted 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 1 μM acetylcholine. Each set of experiments was performed with rings prepared from different rats.

2.3. Experimental protocol 1

Baicalein increased muscle tension only in the agonist-contracted endothelium-intact arteries. An attempt was therefore made to identify the endothelium-derived components involved in the contractile response to baicalein. U46619 (30 nM) or phenylephrine (3 μM) was used to induce a sustained contraction, and baicalein (0.3–300 μM) was then added cumulatively. In order to examine whether baicalein inhibits the NO- or prostacyclin-mediated response, the tissues were incubated with 100 μM N^G-nitro-L-arginine (L-NNA), 3 μM methylene blue or 10 μM indomethacin for 30 min prior to application of U46619, then baicalein was cumulatively added to the bath solution. In addition, the possible effect of 100 nM BQ610 was tested on the baicalein-induced potentiation of muscle tension. In some experiments, the effect of baicalein was examined on the contraction induced by 60 mM extracellular K^+ in both endothelium-intact and -denuded arteries. In these experiments, Na^+ was replaced by an equimolar amount of K^+ in order to maintain the same ionic strength.

2.4. Experimental protocol 2

To examine the possible inhibitory effect of baicalein on the NO-induced relaxation, acetylcholine, A23187 or sodium nitroprusside was used. The tissues were incubated with baicalein (3–10 μM) or L-NNA (10 μM) for 30 min before addition of U46619. Once a sustained tension was obtained, acetylcholine (0.1–10 000 nM) or A23187 (0.3–1000 nM) was added cumulatively to the bath. In addition, the effects of these agents on the sodium nitroprusside (0.03–300 nM)-induced relaxation were tested in endothelium-denuded arteries. U46619 at 30 nM induced a larger contraction in endothelium-denuded tissues, therefore, the concentration of U46619 was lowered to 10–20 nM and the amplitude of the evoked contraction was similar to that in the endothelium-intact arteries.

2.5. Experimental protocol 3

The endothelium-denuded arteries were incubated with putative K^+ channel blockers (3 mM tetraethylammonium ions, 1 μM glibenclamide) for 30 min prior to application

of U46619, baicalein (3–300 μM) was then added cumulatively. In some experiments with endothelium-denuded arteries, the tissues were first contracted with 60 mM K^+ and then washed three times with normal Krebs solution until the basal tension returned. The tissues were exposed to Ca^{2+}-free solution containing 0.3 mM Na_2–EGTA and washed with this solution twice and left for 15 min before the application of 10 mM caffeine. The tissues were thereafter washed twice with normal Krebs solution (30-min contact time for Ca^{2+} refilling of the intracellular stores) and then twice with Ca^{2+}-free solution (15-min contact time). The second contractile response to caffeine was tested in the absence and presence of 100 μM baicalein. In another set of experiments, sustained contractions of endothelium-denuded arteries to 1 μM phorbol 12,13-diacetate (PDA) were obtained in Ca^{2+}-free solution to which 0.5 mM Na_2–EGTA had been included, and then baicalein was added cumulatively. The effect of staurosporine (1–300 nM), the protein kinase C inhibitor, on the agonist-induced contraction was also examined in endothelium-denuded preparations.

2.6. Chemicals

The following chemicals were used: phenylephrine hydrochloride, acetylcholine hydrochloride, U46619, sodium nitroprusside, indomethacin, L-NNA, methylene blue, A23187, caffeine, endothelin I, BQ610, nifedipine, glibenclamide, tetraethylammonium chloride, PDA, staurosporine (Sigma, St. Louis, MO, USA). Baicalein was isolated and purified from the dry roots of *S. baicalensis* Georgi (Huangqin). All chemicals were dissolved in Krebs solution except for U46619, A23187, indomethacin, glibenclamide, nifedipine, PDA and staurosporine which were dissolved in dimethyl sulfoxide (0.2% final concentration). Dimethyl sulfoxide at 0.2% did not affect the U46619-induced contraction.

2.7. Data analysis

The contractile effect of baicalein was expressed as percentage increase of the agonist-contracted artery. The relaxant effects of baicalein or other dilators were expressed as percentage reduction from the agonist-induced contractile response. IC_{50} values were calculated as the drug concentration inducing 50% of the maximum inhibition. All data are shown as means ± S.E.M. Statistical significance was estimated by Student's *t*-test for unpaired observations. A *P*-value of less than 0.05 was regarded to be significant.

3. Results

3.1. Purification of baicalein

To prove the purity of extracted baicalein, thin-layer chromatographic analysis was performed using two developing solvent systems (chloroform:methanol:acetic acid, 20:2:1, v/v/v; butanol:water:acetic acid; 4:1:1; v/v/v) and only one single dot was visualized, indicating that it was a pure compound. It also had the same Rf value as pure baicalein (Sigma). The UV spectrum further demonstrated that this compound had λ_{max} at 325, 275 and 251 nm, similar to those of baicalein reported by Liu et al. (1984). ^1H nuclear magnetic resonance spectrum in ^2H $HOCH_3$, had the following characteristics (δ): 7.79 (d, 2H, C2′ and C6′–H), 7.40–7.37 (m, 3H, C3′, C4′ and C5′–H), 6.54 (s, 1H, C3–H), and 6.43 (s, 1H, C8–H), confirming that this compound is identical to baicalein (Fig. 1).

3.2. Vascular effects of baicalein

In the U46619-contracted arteries with a functional endothelium, baicalein induced two distinct effects. In a lower concentration range (0.3–10 μM), baicalein caused further contraction; the contracted artery started to relax when the concentration of baicalein was increased over 30 μM (Fig. 2a). Baicalein induced a 46.7 ± 7% maximal increase of the U46619-induced contraction ($n = 13$). Similarly, baicalein at 10 μM caused an approximately 39% increase in arterial tone of the phenylephrine (3 μM)-contracted arteries ($n = 4$). The contractile response to baicalein was totally abolished in endothelium-denuded arteries (Fig. 2b, Fig. 3a). Pretreatment of endothelium-intact tissues with 100 μM L-NNA ($n = 7$, Fig. 2c) or with 3 μM methylene blue ($n = 6$) also abolished the contractile effect of baicalein (Fig. 3a). In contrast, pretreatment with 10 μM indomethacin or with 100 nM BQ610 had no effect ($n = 5$ for each case, Fig. 3d). BQ610 at 100 nM caused an about 48% reduction of the endothelin I (10 nM)-induced contraction ($n = 4$). Baicalein alone (0.3–300 μM) did not affect the basal tension ($n = 4$, data not shown). Similarly, L-NNA (100 μM) failed to influence the basal tone in endothelium-intact tissues ($n = 5$, data not shown). These results indicate that baicalein may inhibit the NO-mediated response in the preconstricted arteries.

3.3. The effect of baicalein on endothelium-dependent relaxation

Acetylcholine induced relaxation of the U46619-contracted endothelium-intact arteries with an IC_{50} of 18.6 ±

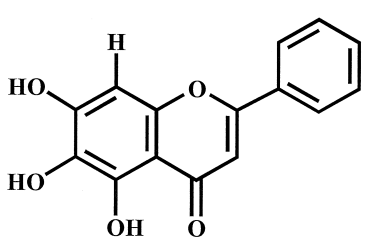

Fig. 1. Chemical structure of baicalein.

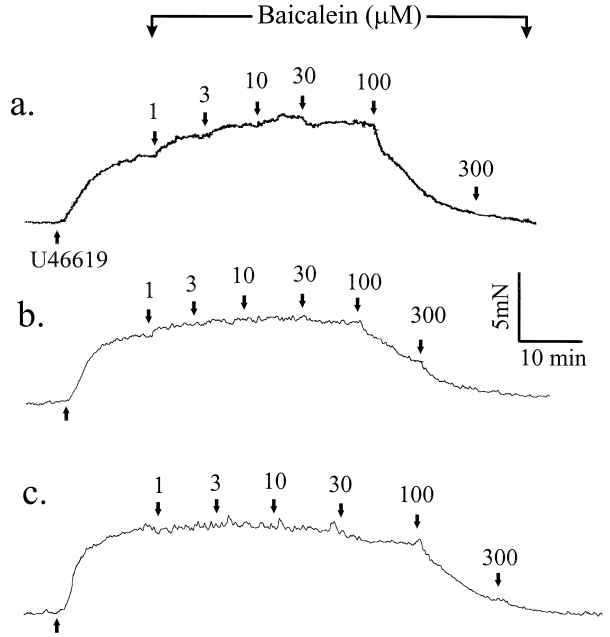

Fig. 2. Representative tracings showing the concentration-dependent effect of baicalein on the U46619-contracted rat mesenteric artery with endothelium (a), without endothelium (b), and in the presence of 100 μM L-NNA (c) in the endothelium-intact artery.

1.4 nM ($n = 6$). Fig. 4a shows that pretreatment of the arterial tissues with baicalein significantly inhibited the acetylcholine-induced relaxation (IC_{50}: 40.8 ± 3.9 nM, $n = 5$ and 184.1 ± 1.29 nM, $n = 5$, respectively, for 3 and 10 μM baicalein, $P < 0.05$ compared with control). Similarly, 10 μM baicalein inhibited the relaxation induced by A23187, another endothelium-dependent dilator (IC_{50}: 26.6 ± 3.8 nM, $n = 5$ for control and 189.1 ± 12.7 nM, $n = 5$ for baicalein, $P < 0.05$ compared with control, Fig. 4b). L-NNA at 100 μM markedly attenuated the maximum relaxation induced by both dilators ($n = 4$ for each case, Fig. 4a and b).

3.4. The effect of baicalein on endothelium-independent relaxation

Sodium nitroprusside (0.03–300 nM) evoked a concentration-dependent relaxation of the U46619-preconstricted endothelium-denuded arteries with an IC_{50} of 2.98 ± 0.16 nM ($n = 6$). Fig. 4c shows that pretreatment of tissues with 10 μM baicalein did not affect the nitroprusside-induced relaxation (IC_{50}: 3.18 ± 0.13 nM, $n = 6$, $P > 0.05$ compared with control).

3.5. Effect of baicalein on the high K^+- and caffeine-induced contraction

Baicalein induced further contraction in a 60 mM K^+-contracted artery with endothelium; removal of the endothelium abolished this effect (Fig. 5a). Within the same concentration range (1–300 μM), baicalein had only a slight inhibitory effect on the high-K^+ response (10.5 ± 3.3% reduction at 300 μM). In contrast, the voltage-sensitive Ca^{2+} channel blocker, nifedipine (10 nM), completely abolished the high-K^+-induced contraction ($n = 4$, data not shown).

In the endothelium-denuded tissues, caffeine (10 mM) induced a transient contraction (1.8 ± 0.2 mN, $n = 4$) in Ca^{2+}-free solution containing 0.3 mM Na_2–EGTA. Incubation of tissues with 10 μM baicalein for 10 min did not affect the caffeine-induced response (1.7 ± 0.2 mN, $n = 4$). In control experiments, the ratio of the second contraction to the first contraction in Ca^{2+}-free solution was 110 ± 6% ($n = 4$) for 10 mM caffeine.

3.6. Effect of K^+ channel blockers on baicalein-induced relaxation

In endothelium-denuded arteries, baicalein induced a concentration-dependent relaxation with an IC_{50} of 50.9 ±

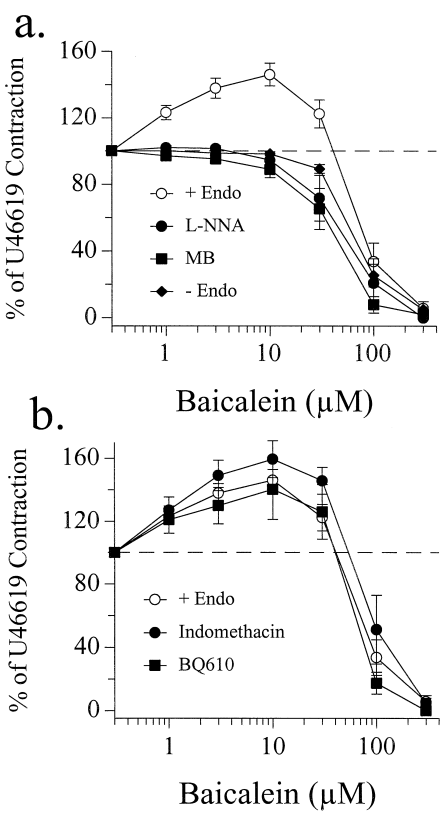

Fig. 3. (a) Concentration–response curves for the effect of baicalein on the U46619-contracted rat mesenteric arteries (with endothelium: ○ $n = 13$ for control; ● $n = 7$ in the presence of 100 μM L-NNA; ■ $n = 6$ in the presence of 3 μM methylene blue; without endothelium: ♦ $n = 10$). (b) Concentration–response curves for the effect of baicalein on the U46619-contracted endothelium-intact arteries (○ $n = 10$ for control; ● $n = 5$ in the presence of 10 μM indomethacin; ■ $n = 5$ in the presence of 100 nM BQ 610). The tissues were exposed to each inhibitor for 30 min prior to addition of U46619 to the bath. Curves were drawn by joining the adjacent data points and the results are means ± S.E.M. from n experiments.

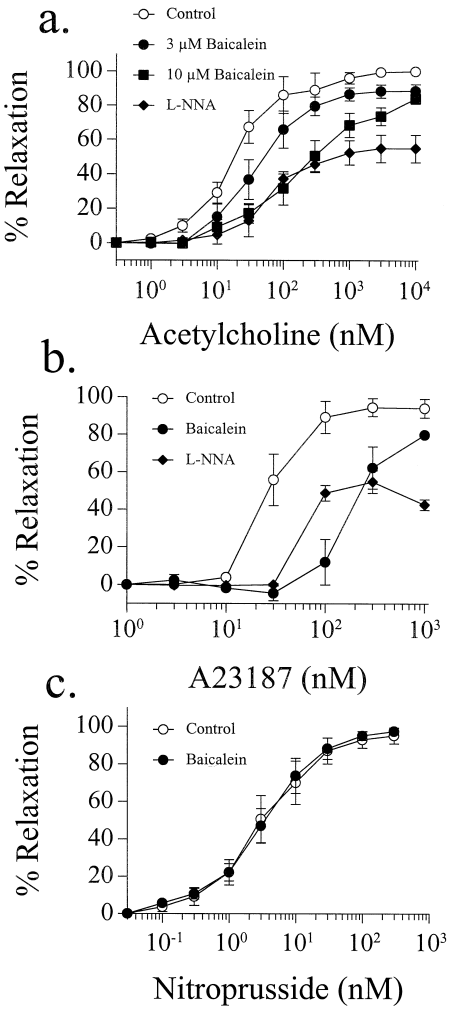

Fig. 4. (a) Concentration–response curves for the relaxant effect of acetylcholine in the U46619-contracted endothelium-intact tissues (○ $n=6$ for control; ● $n=5$ for 3 μM baicalein; ■ $n=5$ for 10 μM baicalein; and ♦ $n=4$ for 10 μM L-NNA). (b) Concentration–response curves for the relaxant effect of A23187 in endothelium-intact arteries (○ $n=5$ for control; ● $n=5$ for 10 μM baicalein; ♦ $n=4$ for 10 μM L-NNA). (c) Concentration–response curves for the relaxant effect of sodium nitroprusside in the endothelium-denuded arteries (○ $n=6$ for control; ● $n=6$ for 10 μM baicalein). The tissues were exposed to each drug for 30 min prior to addition of U46619 to the bath. Curves were drawn by joining the adjacent data points and the results are means ± S.E.M. from n experiments.

5.2 μM, $n=7$) and this relaxation was unaffected by 3 mM tetraethylammonium ion (IC_{50}: 45.2 ± 6.8 μM, $n=6$) or by 1 μM glibenclamide (IC_{50}: 39.6 ± 4.9 μM, $n=5$, $P>0.05$ compared with control).

3.7. Effect of baicalein on the phorbol ester-induced contraction

In order to test the possibility that baicalein also relaxed the arteries through a Ca^{2+}-independent mechanism. PDA, an exogenous protein kinase C activator, was used to evoke contractions in the absence of extracellular Ca^{2+} (zero Ca^{2+} plus 0.5 mM Na_2–EGTA). PDA (1 μM) induced a slowly developing contraction and the maximum sustained tension was 8.7 ± 1.2 mN ($n=6$). The cumulative addition of baicalein at concentrations above 30 μM caused relaxation of the PDA-precontracted tissues with an IC_{50} value of 153.6 ± 10.4 μM ($n=6$, Fig. 5b). Staurosporine, a protein kinase C inhibitor, inhibited the PDA-induced contraction in Ca^{2+}-free solution (IC_{50}: 48.5 ± 5.4 nM, $n=4$) and it also inhibited the U46619- or 60 mM K^+-induced contraction in the normal 2.5 mM Ca^{2+} solution with respective IC_{50} values of 106.5 ± 17.1 nM and 122.7 ± 16.9 nM ($n=4$ in each case, Fig. 5c). Staurosporine (100–300 nM) induced complete relaxation of arteries precontracted with these three agonists (Fig. 5c).

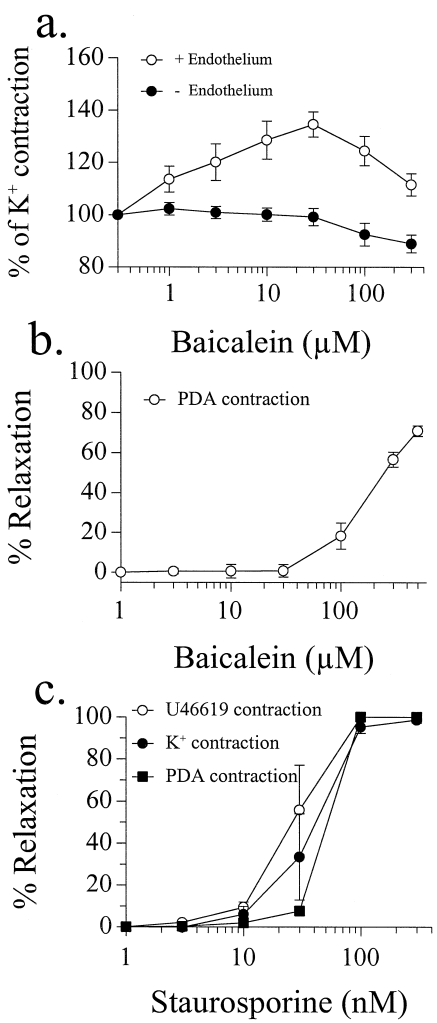

Fig. 5. (a) Concentration–response curves for the effect of baicalein on 60 mM K^+-contracted arteries with endothelium (○ $n=6$) and without endothelium (● $n=6$). (b) The relaxant effect of baicalein on contractile response to 1 μM PDA (○ $n=6$) in the absence of extracellular Ca^{2+}. (c) Concentration–response curves for the effect of staurosporine on contractions induced by 10 nM U46619 (○ $n=4$) or by 60 mM K^+ (● $n=4$) in normal Krebs solution, and by 1 μM PDA (■ $n=4$) in Ca^{2+}-free solution. Curves were drawn by joining the adjacent data points and the results are means ± S.E.M. from n experiments.

Besides, staurosporine at 100 nM significantly reduced the caffeine (10 mM)-induced transient contraction in Ca^{2+}-free solution (2.1 ± 0.4 mN and 0.7 ± 0.2 mN, $n = 4$, before and after staurosporine, $P < 0.01$ for paired data).

4. Discussion

The present study showed that baicalein, isolated and purified from the dry roots of *S. baicalensis* Georgi, exerted a complex effect on the agonist-contracted rat isolated mesenteric arteries. Within a low concentration range (0.3–10 μM) baicalein potentiated the sustained tension induced by phenylephrine, U46619 or high-K^+ in endothelium-intact tissues, whilst it induced concentration-dependent relaxation at concentrations greater than 10 μM. Therefore, it is unlikely that baicalein serves as a receptor antagonist against the vascular response to various constrictors. Removal of the functional endothelium abolished this contractile response without affecting the relaxant effect of baicalein. Pretreatment of endothelium-intact arteries with inhibitors of NO activity, such as L-NNA or methylene blue, also abolished the contractile response to baicalein. In contrast, the cyclooxygenase inhibitor, indomethacin, had no effect, suggesting that inhibition of prostacyclin was not involved. These results indicate that baicalein may inhibit the NO-mediated cellular response. The lack of the constrictive effect of baicalein or L-NNA on the basal tone suggests that the basal NO release may not be involved in maintenance of the resting tension of 0.5 g applied to our preparations.

To further examine the inhibitory effect of baicalein on the NO-mediated pathway, the effect of baicalein was investigated on both endothelium/NO-dependent and endothelium-independent relaxation. It was found that baicalein significantly attenuated the relaxation induced by either acetylcholine or A23187 with a slight reduction of the maximum response. The NO synthase inhibitor, L-NNA, significantly reduced the maximum relaxation induced by these two NO-dependent vasodilators. In contrast, baicalein did not inhibit the relaxation induced by sodium nitroprusside, an exogenous NO donor. These new findings clearly indicate that, like the NO synthase inhibitors, baicalein may inhibit NO production or/and release in rat mesenteric arteries. The lack of effect of baicalein on the nitroprusside-induced relaxation suggests that baicalein may not inhibit the activity of guanylate cyclase as does methylene blue in vascular smooth muscle.

In addition to the endothelium-derived relaxing factors, the endothelial cells also produce and release endothelins which are potent vasoconstrictor peptides (Yanagisawa et al., 1988). It is possible that baicalein may promote endothelin release from the endothelium to enhance the contractile response to U46619. However, our results did not show any effect of the endothelin ET_A receptor antagonist, BQ610, on the contractile response to baicalein, while the same concentration of BQ610 induced an approximately 50% relaxation of endothelin I-contracted arteries. These experiments ruled out the possible involvement of endothelins in the baicalein-induced vascular response.

At higher concentrations baicalein produced a relaxant response in both endothelium-intact and -denuded preparations. It is possible that baicalein may interfere with both Ca^{2+}-dependent and -independent contractile mechanisms. Activation of smooth muscle K^+ channels and subsequent membrane hyperpolarization normally inhibits Ca^{2+} influx through voltage-gated Ca^{2+} channels and thus causes vasorelaxation (Nelson and Quayle, 1995). The baicalein-induced relaxation in endothelium-denuded arteries was affected by neither 1 μM glibenclamide nor 3 mM tetraethylammonium ions. Glibenclamide and tetraethylammonium ions at concentrations used in the present study were found to cause a substantial inhibition of the ATP-sensitive and Ca^{2+}-activated K^+ channels, respectively, in myocytes isolated from rat mesenteric arteries (Standen et al., 1989; Langton et al., 1991). This suggests that baicalein did not affect the K^+ channel activity in vascular smooth muscle. Baicalein only slightly reduced the high-K^+-induced contraction (approximately 11%) at 300 μM, while this concentration caused nearly a full relaxation in the arteries contracted by U46619 or by phenylephrine. In contrast, 10 nM nifedipine abolished the high-K^+ response. Besides, baicalein at 100 μM did not reduce the transient contractile response to caffeine in the absence of extracellular Ca^{2+}. Caffeine was demonstrated to mobilize internal Ca^{2+} stores in vascular smooth muscle (Saida and Van Breemen, 1984; Kwok et al., 1998). Therefore, the results suggest that baicalein does not inhibit either Ca^{2+} influx or intracellular Ca^{2+} release as a main mechanism underlying its vasorelaxant effect.

Protein kinase C activators induce a slowly developing tone in vascular smooth muscle, which is not associated with Ca^{2+}-calmodulin-dependent phosphorylation of myosin light chain, but is related to phosphorylation of intermediate filaments (Rasmussen et al., 1984). The present study showed that PDA produced a slow tonic contraction in the absence of extracellular Ca^{2+}, indicating that activation of protein kinase C can interact with contractile filaments at the resting $[Ca^{2+}]_i$ (Huang, 1996). Baicalein reduced the PDA-induced tension in a concentration-dependent manner. These results suggest that the protein kinase C-mediated steps in excitation–contraction coupling may be the site of action for baicalein when it is used at higher concentrations. Baicalein was shown to suppress vascular smooth muscle cell proliferation in the presence of platelet-derived growth factor (Huang et al., 1994). It is at present unclear whether the antiproliferative effect of baicalein is partly mediated through its inhibition of protein kinase C activity. Both protein kinase C and platelet-derived growth factor (which can stimulate protein kinase C in vascular smooth muscle) are potent stimulators of vascular smooth muscle proliferation (Lee and Sever-

son, 1994; Touyz and Schiffrin, 1997). However, the present study indicates that baicalein and the protein kinase C inhibitor do not act exactly in the same manner. Staurosporine, an inhibitor of protein kinase C, inhibited the high-K^+- or caffeine-induced contractile response while baicalein was without effect.

Taken together, the present results provide novel information about the vascular response to baicalein purified from the dry roots of *S. baicalensis* Georgi. Baicalein at low concentrations could act on the endothelium to inhibit the NO-mediated relaxation. At high concentrations, baicalein relaxed the arteries probably in part through inhibition of the protein kinase C-mediated cellular pathway in vascular smooth muscle. Activation of glibenclamide- or tetraethylammonium-sensitive K^+ channels was not involved in baicalein-induced relaxation. It appeared that baicalein had little influence on the nifedipine-sensitive Ca^{2+} channels or caffeine-sensitive intracellular Ca^{2+} release.

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