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Baicalin, a flavonoid from *Scutellaria baicalensis* Georgi, activates large-conductance Ca^{2+}-activated K^+ channels via cyclic nucleotide-dependent protein kinases in mesenteric artery

Yi-Ling Lin [a,b], Zen-Kong Dai [c], Rong-Jyh Lin [d], Koung-Shing Chu [e], Ing-Jun Chen [a], Jiunn-Ren Wu [c,*], Bin-Nan Wu [a,b,*]

[a] Department of Pharmacology, College of Medicine, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan
[b] Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung 807, Taiwan
[c] Department of Pediatrics, Division of Pediatric Pulmonology and Cardiology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[d] Department of Parasitology, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[e] Department of Anesthesiology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan

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ABSTRACT

Baicalin isolated from *Scutellaria baicalensis* is a traditional Chinese herbal medicine used for cardiovascular dysfunction. The ionic mechanism of the vasorelaxant effects of baicalin remains unclear. We investigated whether baicalin relaxes mesenteric arteries (MAs) via large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel activation and voltage-dependent Ca^{2+} channel (VDCC) inhibition. The contractility of MA was determined by dual wire myograph. BK_{Ca} channels and VDCCs were measured using whole-cell recordings in single myocytes, enzymatically dispersed from rat MAs. Baicalin (10-100 μM) attenuated 80 mM KCl-contracted MA in a concentration-related manner. L-NAME (30 μM) and indomethacin (10 μM) little affected baicalin (100 μM)-induced vasorelaxations. Contractions induced by iberiotoxin (IbTX, 0.1 μM), Bay K8644 (0.1 μM) or PMA (10 μM) were abolished by baicalin 100 μM. In MA myocytes, baicalin (0.3-30 μM) enhanced BK_{Ca} channel activity in a concentration-dependent manner. Increased BK_{Ca} currents were abolished by IbTX (0.1 μM). Baicalin-mediated (30 μM) BK_{Ca} current activation was significantly attenuated by an adenylate cyclase inhibitor (SQ 22536, 10 μM), a soluble guanylate cyclase inhibitor (ODQ, 10 μM), competitive antagonists of cAMP and cGMP (Rp-cAMP, 100 μM and Rp-cGMP, 100 μM), and cAMP- and cGMP-dependent protein kinase inhibitors (KT5720, 0.3 μM and KT5823, 0.3 μM). Perfusate with PMA (0.1 μM) abolished baicalin-enhanced BK_{Ca} currents. Additionally, baicalin (0.3-30 μM) reduced the amplitude of VDCC currents in a concentration-dependent manner and abolished VDCC activator Bay K8644-enhanced (0.1 μM) currents. Baicalin produced MA relaxation by activating BK_{Ca} and inhibiting VDCC channels by endothelium-independent mechanisms and by stimulating the cGMP/PKG and cAMP/PKA pathways.

Introduction

The root of *Scutellaria baicalensis* Georgi (common name, Huang-qin) has been extensively used as a herbal therapy to treat cardiovascular dysfunction (Huang et al. 2005). However the exact mechanisms by which Huang-qin alters vascular reactivity remain unclear. Given the recent increases in the use of herbal medicines by all societies, understanding the possible mechanisms of action of Hung-qin is an urgent demand and may prove future clinic impact. Huang-qin has a multitude of pharmacological activities including antihypertensive (Tang and Zhou 1958), anti-inflammatory (Huang et al. 2006), antithrombotic (Kubo et al. 1985), antioxidant (Su et al. 2000), antihyperlipidemic (Huang et al. 2005), and anticarcinogenic effects (Chan et al. 2000). Huang-qin is also known to contain numerous flavone derivatives, including baicalin, baicalein and wogonin (Sekiya and Okuda 1982). Among them, baicalin (5,6-Dihydroxy-flavone-7-O-glucuronide, Fig. 1) is known to have excellent anti-inflammatory effects (Lin and Shieh 1996; Lo et al. 2005) and provides potent free radical scavenging and xanthine oxidase inhibition (Shieh et al. 2000), thus improving endothelial function and conferring cardiovascular protective effects (Woo et al. 2005;

* Corresponding author at: Department of Pharmacology and Department of Pediatrics, College of Medicine, Kaohsiung Medical University, 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan. Fax: +886 7 3234686
E-mail addresses: binnan@kmu.edu.tw (B.-N. Wu), jirewu@kmu.edu.tw (J.-R. Wu).

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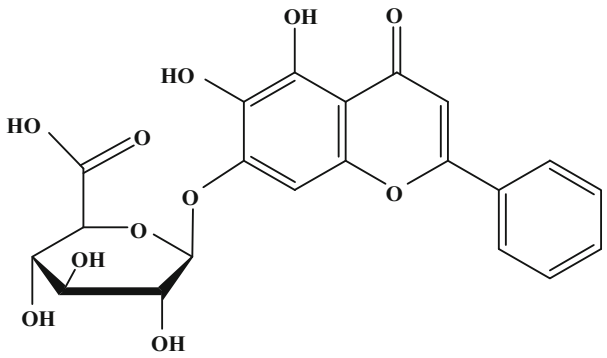

Fig. 1. Chemical structure of baicalin (5,6-Dihydroxyflavone-7-O-glucuronide).

Chang et al. 2007). One of previous reports showed that baicalin (1-100 μM) and its aglycone baicalein (1-50 μM) potentiated the MA contractile response to phenylephrine through inhibition of nitric oxide formation and/or release from the endothelium (Tsang et al. 2000). By contrast, high concentrations of baicalein (100-300 μM) reduced the phenylephrine-induced tone in endothelium-intact vessels (Tsang et al. 2000). Baicalein (30-300 μM) also reduced the protein kinase C (PKC)–mediated MA contractions in endothelium-denuded vessels (Chen et al. 1999). In this study, we found for the first time that baicalin (10-100 μM) produced direct relaxation in KCl-contracted MA and we further investigated the ionic mechanism by which it produces relaxation.

Large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels play a key role in regulating smooth muscle contractility and controlling the diameter of resistance arteries (Nelson and Quayle 1995). Previous reports showed that the functional role of BK_{Ca} channels is enhanced in arterial smooth muscle during chronic hypertension. A similar phenomenon occurs throughout the vasculature, including the mesenteric artery (Asano et al. 1993), cerebral vascular beds (Paterno et al. 1997), and the aorta (Rusch et al. 1992). Therefore, increased BK_{Ca} channel function in arterial smooth muscle cells may provide a protective mechanism against progressive increases in blood pressure.

The cAMP and the cGMP pathways are major regulators of smooth muscle contractility. It is widely accepted that activation of BK_{Ca} channels via cAMP-dependent protein kinase (PKA) and cGMP-dependent protein kinase (PKG) contributes to relaxation (Zhou et al. 2001). Likewise, agents that elevate cAMP and cGMP have also been shown to modulate BK_{Ca} channels and initiate vascular relaxation (Bayguinov et al. 2001; Zhou et al. 2001). Increases in cAMP and cGMP simultaneously activate PKA and PKG pathways resulting in the opening of BK_{Ca} channels. In contrast, since PKC is known to inhibit BK_{Ca} channel activity (Jaggar et al. 2000; Bayguinov et al. 2001), any activation of PKA or PKG would be likely to reduce PKC activation and increase BK_{Ca} activity (Jaggar et al. 2000). These protein kinases interact with each other in modifying channel activity. Physiologically, increasing the activity of the BK_{Ca} channel would result in the closure of voltage-dependent Ca^{2+} channels (VDCCs) by membrane hyperpolarization (Nelson and Quayle 1995). Thus, BK_{Ca} channels appear not only to integrate the output from several signaling cascades but also arrest VDCC activation to relax SMCs.

The main objective of this study was to investigate whether, and by what signaling and ionic mechanisms, baicalin relaxes resistance MA. To the best of our knowledge, this study provides the first evidence that the underlying mechanisms of baicalin-induced MA relaxation could be due to BK_{Ca} channel activation and VDCC inhibition.

Materials and Methods

Chemicals

Baicalin (95% purity), Bay K8644, collagenase type Ia, dithioerythritol, hyaluronidase, iberiotoxin (IbTX), KT5720, KT5823, N^{ω}-nitro-L-arginine methyl ester (L-NAME), 1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one (NS1619), 1H-[1,2,4]oxadiazolo[4,3-a]-quinoxalin-1-one (ODQ), papain, phorbol 12-myristate 13-acetate (PMA), Rp-cAMP, Rp-cGMP, 9-(terahydro-2-furanyl)-9H-purin-6-amine (SQ 22536) and tetraethylammonium chloride (TEA) were obtained from the Sigma-Aldrich Chemical Co. (St Louis, MO). All drugs and reagents were dissolved in distilled water unless otherwise noted. Baicalin, KT 5823, NS1619, ODQ and PMA (10 mM) were dissolved in dimethylsulfoxide; indomethacin (10 mM) was dissolved in ethanol. Serial dilutions were made in phosphate-buffered solution.

Animal procedures and tissue preparations

All procedures and protocols were approved by the Animal Care and Use Committee at the Kaohsiung Medical University. Female Sprague–Dawley rats (200-250 g) were sacrificed with an overdose of urethane (2 g kg^{-1}) by the intraperitoneal route. MAs were carefully removed and placed in cold oxygenated Krebs solution (in mM) 137 NaCl, 5.6 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 4.17 $NaHCO_3$, 0.44 NaH_2PO_4, 0.42 Na_2HPO_4, 10 HEPES and 5 Glucose (pH 7.4). A segment of MA was dissected free of fat and connective tissue, and then cut into 2–3 mm long rings for isometric tension measurement or preparation of isolated arterial smooth muscle cells.

Contractile tension recordings

Resistance MA rings (~250 μm internal diameter) were fitted with two stainless steel wires (40 μm internal diameter) and mounted in a dual-channel Mulvany-Halpern myograph (DMT A/S, Model 410A, Aarhus, Denmark) for measurement of isometric tension. The rings were equilibrated with a resting tension of 5 mN for 90 min. After equilibrium, MA rings were contracted with a depolarizing concentration of KCl (80 mM), which was considered 100% of contraction. All experiments were performed in endothelium-intact arteries. Endothelium function was verified by the presence of relaxation response (> 70%) to acetylcholine (1 μM) contracted by phenylephrine (10 μM) as described previously (Wu et al. 2001). To determine whether baicalin-induced vasorelaxation involved nitric oxide synthase (NOS) or prostanoid related mechanisms, some rings were co-treated with baicalin (100 μM) and L-NAME (30 μM) or baicalin and indomethacin (10 μM). Next, to evaluate whether baicalin (100 μM)-induced vasorelaxation modulated the channel activity, it was added after BK_{Ca} channel inhibitor iberiotoxin (IbTX, 0.1 μM), VDCC activator Bay K8644 (0.1 μM) or PKC activator PMA (10 μM) induced MA contractions.

Preparation of mesenteric artery smooth muscle cells

SMCs from rat MAs were enzymatically isolated. In brief, arterial segments were placed in a warm (37 °C) cell isolation medium containing (in mM) 137 NaCl, 5.6 KCl, 0.1 $CaCl_2$, 1 $MgCl_2$, 4.17 $NaHCO_3$, 0.44 NaH_2PO_4, 0.42 Na_2HPO_4, 10 HEPES and 5 glucose with 5 mg/ml albumin (pH 7.4) for 10 min. After this equilibration step, arterial segments were initially incubated (37 °C) in Ca^{2+}-Free isolation medium, papain (0.3 mg ml^{-1}) and dithioerythritol (1 mg ml^{-1}) for 30 min. This was followed by a