Activation of Large-Conductance Calcium-Activated Potassium Channels by Puerarin: The Underlying Mechanism of Puerarin-Mediated Vasodilation

Xiao-Hui Sun, Jiu-Ping Ding, Hui Li, Na Pan, Lu Gan, Xiang-Liang Yang, and Hui-Bi Xu

Key Laboratory of Molecular Biophysics of the Ministry of Education, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, People’s Republic of China

Received May 9, 2007; accepted July 24, 2007

ABSTRACT

Puerarin is the main isoflavone found in Pueraria lobata (Wildl) Ohwi, which has been used in therapy for various cardiovascular diseases. The present study examined the effects of puerarin on the large-conductance voltage- and Ca$^{2+}$-activated potassium (BK$_{Ca}$) channel and on rat thoracic aortas. BK$_{Ca}$ channels encoded with either α (BK-α) or α/β subunits (BK-α+β1) were heterologously expressed in Xenopus oocytes or human embryonic kidney 293 cells. The activities of BK$_{Ca}$ channels were measured using excised patch-clamp recordings. Puerarin activated BK-α+β1 currents with a half-maximal concentration (EC$_{50}$) of 0.8 nM and a Hill coefficient of 1.11 at 10 μM Ca$^{2+}$ and with an EC$_{50}$ of 12.6 nM and a Hill coefficient of 1.08 at 0 μM Ca$^{2+}$. Puerarin (1 nM) induced a 16-mV leftward shift in the conductance-voltage curve for BK-α+β1 currents at 10 μM Ca$^{2+}$ and at 100 nM induced a 26-mV leftward shift at 0 μM Ca$^{2+}$. Puerarin mainly increased the BK-α+β1 channel open probability without changing the unitary conductance. Activation was also detected in the absence of the β1 subunit. A deglycosylated analog of puerarin, daidzein, also activated BK$_{Ca}$ channels with weaker potency. In addition, puerarin (0.1 to 1000 μM) caused concentration-dependent relaxations of rat thoracic aortic rings contracted with 1 μM noradrenaline bitartrate (EC$_{50}$ = 1.1 μM). These were significantly inhibited by 50 nM ibotxin, a specific blocker of BK$_{Ca}$ channels. This is the first study demonstrating that puerarin activates BK$_{Ca}$ channels, especially BK-α+β1 channels. The activation of the BK$_{Ca}$ channel probably contributes to the puerarin-mediated vasodilation action.

Puerarin [7-hydroxy-3-(4-hydroxyphenyl)-1-benzopyran-4-one 8-(β-D-glucopyranoside), Fig. 1] is the main isoflavone isolated from the Chinese medicinal herb Ge-gen (also known as “Kudzu” in the West), the root of the wild leguminous creeper Pueraria lobata (Wildl) Ohwi. Puerarin has multiple pharmacological activities (Gao, 2003) and has been used to treat many cardiovascular diseases such as hypertension (Song et al., 1988; Wu et al., 2006), angina (Chen, 2004; Wang et al., 2006), and myocardial infarction (Xiao et al., 2005; Zhang et al., 2006). The vascular relaxing action of puerarin is one of the focuses of research on its pharmacology. It involves a variety of molecular mechanisms such as activation of endothelial nitric-oxide synthase (Ma et al., 2003) and the cAMP pathway (Yeung et al., 2006). Some researchers (Sun and Li, 2002; Dong et al., 2004) have shown that the potassium channel blocker tetraethylammonium can significantly block puerarin-induced vasodilation. Other studies have shown that puerarin blocks the L-type Ca$^{2+}$ channel and the K$^{+}$ channel in isolated guinea pig ventricular myocytes (Miao et al., 1998; Qian et al., 1999; Zhang et al., 2001) and inhibits the Na$^{+}$ current in rat ventricular myocytes (Zhang et al., 2003). However, there are no reports of effects of puerarin on K$^{+}$ channels in smooth muscle cells.

The BK$_{Ca}$ channel, the large-conductance voltage- and Ca$^{2+}$-activated potassium channel abundantly expressed in vascular smooth muscle cells, plays a critical role in controlling vascular tone. Activation of BK$_{Ca}$ channels leads to hyperpolarization of the cell membrane, which causes deactivation of voltage-dependent calcium channels and vasodi-
lulation (Brenner et al., 2000). Deletion of the BKCa channel leads to high blood pressure in small arteries of mice (Sausbier et al., 2005). The smooth muscle BKCa channels are typically composed of four pore-forming α subunits and an unknown number of regulatory β subunits. The accessory β1 subunits of BKCa channels play a vital role in coupling Ca2+ sparks to BKCa channel activation in vascular smooth muscles (Chang et al., 2006). Its down-regulation has been shown to play an important role in hypertension (Plüger et al., 2000; Amberg et al., 2003).

In this study, we investigated the effects of puerarin on cloned BKCa channels and examined the vasodilation effects of puerarin in the presence or absence of the specific BKCa channel blocker iberiotoxin (IbTX). These results may help to understand the underlying mechanisms of puerarin-mediated vasodilation.

**Materials and Methods**

**Materials.** Puerarin and daidzein (7,4′-dihydroxyisoflavone) were purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (purity ≥99%; Beijing, China). Daidzein was dissolved with dimethyl sulfoxide. The final concentration of dimethyl sulfoxide did not exceed 0.1%, which did not alter the kinetic properties of BKCa channels (data not shown). All of the other chemicals were obtained from Sigma-Aldrich (St. Louis, MO) except where indicated.

**Expression of mslo and hβ1 Channels in Xenopus Oocytes.** Methods of expression of mslo and hβ1 channels in stage V to VI Xenopus oocytes were described previously (Xia et al., 1999; Yao et al., 2005). In brief, oocytes were digested by treatment with 2 mg/ml collagenase I in zero calcium ND-96 solution. Between 24 and 24 h after digestion, 1 to 2 ng (mslo and hβ1) of cRNA (a gift of Dr. Christopher Lingle, Washington University, St. Louis, MO) was injected into Xenopus oocytes using a Drummond Nanoinject II (Drummond Scientific Co., Broomall, PA). After injection, oocytes were then incubated in ND-96 solution at 18°C. Currents were recorded 2 to 7 days after RNA injection. ND-96 solution (pH 7.5) contained the following concentrations: 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 2.5 mM sodium pyruvate, and 10 mM HEPES. It was supplemented with 100 IU/ml penicillin and 100 μg/ml streptomycin for incubation.

**Expression of dslo Channels in HEK293 Cells.** Human embryonic kidney 293 (HEK293) cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (McCay et al., 2005). HEK293 cells grew on 24-well plates at a density of 1 × 10^5/well and were transfected with 0.6 μg of green fluorescent protein/pcDNA3.1 and 0.6 μg of dslo/pcDNA3.1 (a gift of Dr. Christopher Lingle) using Lipofectamine 2000 (Invitrogen). Cells were used for electrophysiological recordings in 1 to 2 days after transfection.

**Electrophysiology.** Patch pipettes were pulled from borosilicate glass capillaries with a resistance between 2 and 5 megohms. All experiments in excised patch configurations were performed and recorded using a PC202 patch-clamp amplifier (Inhibio Life Science Instrument Co., Ltd., Wuhan City, China) and PClamp software (Molecular Devices, Sunnyvale, CA). Currents were typically digitized at 20 kHz. Macroscopic records were filtered at 10 kHz during digitization. Single-channel records were filtered at 5 kHz and digitized at 10 kHz. For an inside-out patch experiment, the intracellular solution contained 160 mM MeSO4-K, 10 mM HEPES, and 2 mM MgCl2, adjusted to pH 7.0 with methanesulfonic acid (MeSO4-H). The bath solution contained 160 mM MeSO4-K, 10 mM HEPES, and 5 mM N-hydroxymethlendiaminetricarboxylic acid with Ca2+ added to make 10 μM free Ca2+, as defined by the EGTAC program (E. McCleskey, Vollum Institute, Portland, OR), with the pH adjusted to 7.0.

During recording, drugs and control/wash solutions were puffed locally onto the cell via a puffer pipette containing seven solution channels. The tip (~300 μm diameter) of the puffer pipette was located approximately 120 μm from the cell. As determined by conductance tests, the solution around a cell under study was fully controlled by the application solution with a flow rate of 100 μl/min or greater. All experiments were done at room temperature (22–25°C).

**Vasoreactivity Measurements.** Male Wistar rats (weighing 200–250 g; obtained from the Animal Center, Institute of Health and Epidemic Prevention, Hubei, China) were sacrificed by decapitation under ether anesthesia. The thoracic aortas were excised and cleaned of adherent connective tissue and were cut into 3-mm ring segments. The endothelial layer was removed in some experiments by gently rubbing the internal surface of the vessels with a hypodermic needle. Conversely, in other cases, the endothelium was maintained. Arterial rings were mounted on two stainless steel hooks in 5-ml organ baths filled with Krebs-Ringer buffer (pH 7.4) of the
following composition: 119 mM NaCl, 25 mM NaHCO₃, 11.1 mM glucose, 1.6 mM CaCl₂, 4.7 mM KCl, 1.2 mM KH₂PO₄, and 1.2 mM MgSO₄ and gassed with a mixture of 95% O₂/5% CO₂. The rings were equilibrated for 45 min at +37°C with a resting tension of 1.0 g. After the equilibration, the endothelial removal/integrity was confirmed by the administration of acetylcholine (10 μM) to noradrenaline bitartrate (NA; 1 μM)-precontracted vascular rings. A relaxation <10% of the NA-induced contraction was considered the representative of an acceptable lack of the endothelial layer, whereas a relaxation ≥70% of the NA-induced contraction was considered the representative of an acceptable integrity of the endothelium. Rings were then washed in prewarmed Krebs-Ringer solution until the baseline tone was regained. The rings were then contracted with 1 μM NA to the maximal contraction. Following washout of NA, cumulative concentration-response curves to stepwise cumulative addition of puerarin were measured with an isometric force-displacement transducer and recorded on a polygraph (RM6240 transducer, RM6240B/C Polygraph; Chengdu Instrument Co., Chengdu, China). Relaxation responses were expressed as a percentage of NA-induced contraction. All animal experiments were approved by the Institutional Animal Care and Use Committee and were in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (publication 85-23, revised 1996).

Data Analysis. Data were analyzed with Clampfit (Molecular Devices), Sigmaplot (SPSS Inc., Chicago, IL), and QUB (State University of New York, Buffalo, NY) software. Unless indicated, the data are presented as means ± S.E.M.; statistical significance between two groups and among multiple groups was tested using the t test and one-way analysis of variance, respectively. Differences in the mean values were considered significant at a probability of <0.05. Dose-response relationships for puerarin activating BKCa channels and relaxing rat thoracic aortic rings were fitted to a Hill equation of the following form: \( f = f_{\text{max}} / (1 + (EC_{50}/|\text{puerarin}|)^n) \), where \( f \) is activation percentage of BKCa currents or relaxation percentage of rat thoracic aortic rings, \( f_{\text{max}} \) is maximum value of \( f \), and [puerarin] is the concentration of puerarin. EC₅₀ and \( n \) denote the puerarin concentration of half-maximal effect and the Hill coefficient, respectively. G-V curves were fitted to a Boltzmann equation of following form: \( G/G_{\max} = 1/(1+exp(V-V_{50}))/k \), where \( G \) is the conductance of the channel, \( G_{\max} \) is the maximal G, \( V \) is the holding potential, \( V_{50} \) is the voltage for half-maximal activation, and \( k \) represents the slope factor. G-V curves were generated from steady-state currents. Single-channel analysis was performed using QUB. Single-channel amplitudes were measured by using an all-points histogram of current records. NPO values (the open probability for a multichannel patch) was determined over at least 10 s of recording. NPO values were calculated from the area under the curve of the Gaussian fit of all-points amplitude histograms. Assuming a Poisson distribution, \( \text{NPO} = \sum X_i \), with \( i = 1 \ldots n \), where \( n \) is the maximum number of simultaneous conducting channels during the observation period, and \( X_i \) is the relative area under the curve corresponding to each opening.

Results

Puerarin Activating Macroscopic BK-α+β1 Channel Currents. Macroscopic BKCa currents in Xenopus oocytes coexpressing mslo and hβ1 (mslo+hβ1) were obtained at different potentials in excised patches. Puerarin does not activate BKCa channel currents when applied to the outside of excised cell membrane patches, even at 100 μM concentration (data not shown). However, puerarin potently activated channels when applied to the cytoplasmic side of excised cell membrane in the presence of 0 and 10 μM intracellular Ca²⁺ (Fig. 1). Figure 1A shows the voltage dependence of puerarin-induced enhancement of BKCa currents at 10 μM Ca²⁺. Application of 1 nM puerarin significantly increased the currents at negative potentials, and the activation was partially reversible. The middle panel summarizes the relationships between conductance and voltage. Puerarin (1 nM) resulted in a 16-mV leftward shift of the \( V_{50} \) of G-V curves. The bottom panel shows the enhancement percentage of BKCa currents by puerarin at different potentials. The activation of BKCa channel currents by puerarin was markedly inverse voltage-dependent; i.e., there was a larger increase at lower potentials (76.6 ± 13.5 and 27.5 ± 6.1% at −40 and −20 mV, respectively, \( p < 0.05 \), versus control, \( n = 6 \)). Figure 1B summarizes the activation by 100 nM puerarin at 0 μM Ca²⁺. Puerarin (100 nM) also stimulated BKCa currents and induced a 26-mV leftward shift in the \( V_{50} \) of G-V curves.

Currents of mslo+hβ1 in Xenopus oocytes shown in Fig. 2 were recorded in inside-out patches at −20 and +100 mV with 10 and 0 μM Ca²⁺, respectively. In Fig. 2A, 1 nM puerarin increased BKCa currents, and the time courses of the currents, which were activated at various concentrations of puerarin, indicate that the whole process goes in a rapid and partially reversible manner. The dose-response curve is fitted to a Hill equation with an EC₅₀ of 0.8 nM and a Hill coefficient of 1.11. Likewise, puerarin increased the BKCa currents at +100 mV and zero Ca²⁺ concentration (Fig. 2B). However, the EC₅₀ and the Hill coefficient derived from the dose-response curve in this case are 12.6 nM and 1.08, respectively, indicating that Ca²⁺ facilitates activation by puerarin.

The Effect of Puerarin on Single BK-α+β1 Channel Currents. Single-channel currents of mslo+hβ1 were recorded in inside-out patches at 10 μM free Ca²⁺ concentration. Figure 3 and Supplemental Fig. 1 show the effect of puerarin on the single BK-α+β1 channel currents at −20 and +50 mV, respectively. In Fig. 3A, the initial NPO was 0.51, and after the application of 1 nM puerarin the NPO was increased to 0.70. The single-channel amplitude was almost unchanged after the application of 1 nM puerarin. A summary of the effects of 1 nM puerarin on NPO, and the unitary amplitude of single BKCa channels at −20 mV is shown in Fig. 3B. Puerarin induced a 37 ± 7% enhancement in NPO and slight changes in unitary amplitude at −20 mV (\( n = 6 \)). To further confirm any possible change in single BKCa channel conductance caused by puerarin, a series of experiments was performed at several holding potentials ranging from −60 to +60 mV with 10 μM Ca²⁺. In Fig. 3C, the current-voltage plots show that there is no significant change in conductance before and after the application of 1 nM puerarin (242.8 ± 12.4 and 248.5 ± 16.0 pS, control and plus puerarin, respectively, \( n = 6 \)).

Puerarin Activating Currents of BK-α (mslo) Channel. One often asks whether the auxiliary β1 subunit of BKCa channels plays a critical role in the activation of BKCa currents by puerarin A (Giangiacomo et al., 1998; Pérez, 2005; Zakharov et al., 2005). Consequently, we have further explored the effect of puerarin in the absence of the β1 subunit. A series of experiments shown in Fig. 4 were conducted at zero Ca²⁺ concentration. Under those conditions, puerarin
also shows an activation effect on mslo currents but at higher concentrations. Figure 4A shows that 1 μM puerarin significantly activates mslo currents with an 18-mV leftward shift in the V_{50}. The dose-response curve was fitted to a Hill equation with an EC_{50} of 166.6 nM and a Hill coefficient of 0.72 (Fig. 4B).

**Puerarin Has No Effect on dslo Currents.** To estimate the domain of BK_{Ca} channels that puerarin may bind to, the BK_{Ca} encoded with dslo subunit expressed in HEK293 cells was used. Puerarin has no effect on the dslo currents at 0 μM Ca^{2+} (data not shown) and 10 μM Ca^{2+} (Fig. 5). Therefore, we infer that the possible interaction domain may locate at the S0-S1 and S8-S9 linkers, because the BK_{Ca} dslo channel contains the above regions significantly different from that of the BK_{Ca} mslo channel.

**Daidzein, an Analog to Puerarin, Activating mslo Currents.** Daidzein is an aglycone of puerarin, in other words, it is a hydrolysate of puerarin. To investigate the role of β-D-glycosyl residue at 8-position of the puerarin isoflavone core in the activation process of BK_{Ca} channels, the effect of daidzein on BK_{Ca} mslo channels was examined at zero Ca^{2+} concentration. In Fig. 6, 1 μM daidzein also increases BK_{Ca} currents in inside-out patches with an 11-mV leftward shift in the V_{50}, which is smaller than that of puerarin of 18-mV shift as shown in Fig. 4A. That means that the β-D-glycosyl residue also plays a role in enhancing channels.

**Puerarin Relaxing Rat Thoracic Aortic Rings.** Puerarin (0.1 to 1000 μM) caused concentration-dependent relaxations in endothelium-intact and endothelium-denuded rat thoracic aortic rings contracted with NA (1 μM) (Fig. 7). The EC_{50} was 1.1 and 2.2 μM, and the maximal relaxation was 45.9 ± 5.1% (n = 5) and 33.5 ± 4.6% (n = 6) to endothelium-intact and endothelium-denuded aortic rings, respectively, indicating that endothelium was involved in puerarin-induced vasodilation. The specific BK_{Ca} channel blocker IbTX (50 nM) significantly inhibited puerarin-induced relaxations on both endothelium-intact and endothelium-denuded aortic rings, the fractional inhibition at a puerarin concentration of 1 μM on endothelium-intact and endothelium-denuded aortic rings was 34.0 and 48.0%, respectively, suggesting that the activation of BK_{Ca} channels also contributes to the puerarin-mediated vasodilation action.
The major findings of this study are as follows. Puerarin potently activated BK-α+β1 currents in the nanomolar concentration range. It increased the activity of BK-α+β1 channels with no change in single-channel conductance. Puerarin also stimulated BK-α currents with weaker potency. A puerarin analog, daidzein, activated BK-α currents with weaker potency than puerarin. Puerarin dilated rat thoracic aortic rings in a concentration-dependent manner, and this relaxation response could be inhibited by IbTX.

**Mechanisms of the Effect of Puerarin on BKCa Channels.** Puerarin potently activated cloned BK-α+β1 channels, shifting the G-V relationship to the left in cell-free patches. Compared with many other natural BKCa channel openers, including BMS-204352 (EC50 ≥ 300 nM) (Gribkoff et al., 2001), dehydrosoyasaponin-I (EC50 ≥ 100 nM) (Giangiacomo et al., 1998), and mallotoxin (effective concentration ≥500 nM) (Zakharov et al., 2005), the effective concentration range for puerarin is considerably lower (EC50 values, 0.8 nM at 10 μM Ca2+ and 12.6 nM at 0 μM Ca2+). The effect of puerarin on BKCa channels is in a voltage- and Ca2+-dependent manner. It is very notable that the effect of puerarin is inverse voltage-dependent; i.e., the activation of BKCa channels by puerarin is significantly larger at negative potentials, which is similar to another BKCa channel opener 12,14-dichlorodehydroabietic acid (Sakamoto et al., 2006). The EC50 of puerarin on BKCa channels is a 16-fold augmentation at zero Ca2+ compared with at 10 μM Ca2+, which may imply that Ca2+ facilitates the activation of puerarin. On the other side, Ca2+ produced a more leftward shift in V50 of BKCa channels in the presence of puerarin than that in the absence of puerarin; e.g., the V50 of BK-α+β1 was −27 ± 3 mV in the absence of 1 nM puerarin but −43 ± 4 mV in the presence of 1 nM.
puerarin at 10 μM Ca^{2+}, indicating that puerarin was able to increase the Ca^{2+} sensitivity of BK_{Ca} channel gating. Single-channel recordings showed that puerarin mainly increased the BK_{Ca} channel NO without changing the unitary conductance. In addition, the Hill coefficients of dose-response curves were approximately unity both at 10 and 0 μM Ca^{2+}, suggesting only one site for interaction between puerarin and the BK_{Ca} channel protein.

The Role of the β1 Subunit. Puerarin activating BK_{Ca} channels was also observed in the absence of the β1 subunit, which indicates that β1 subunit is not required for the activation. However, puerarin has a stronger effect on the BK-α+β1 channels than the BK-α channel alone. In the absence of the β1 subunit, the EC_{50} of puerarin is greater by 13 times, suggesting that the role of the β1 subunit is probably to facilitate puerarin binding. The β1 subunit is composed of two transmembrane domains, a long extracellular loop, and two short intracellular segments (Knaus et al., 1994), leaving little to construct an intracellular binding site for puerarin. Because puerarin can activate the BK_{Ca} channel alone when applied to the cytoplasmic side of cell membrane, we infer that puerarin-binding sites are located on the extracellular side of the α subunits. The N terminus and S0 of the BK-α subunit have been supposed to be the possible regions to regulate the gating of BK_{Ca} channels via interacting with β1 subunits (Wallner et al., 1996). We examined the effect of puerarin on dslo; interestingly, no action was observed. Aligning the sequences of mslo and dslo, we find that there is a main difference in two cytoplasmic motifs, i.e., the S0-S1 linker and S8-S9 linker. The sites for puerarin binding to BK_{Ca} channels may be located in one of these domains. More experiments are needed to determine the precise locations.

As we know, both dslo and mslo channels contain the same calcium binding sites in RCK1 (regulator of conductance for K^+) domain and calcium bowl regions but have very different Ca^{2+} sensitivity (Xia et al., 2002). Furthermore, considering that the S0-S1 and S8-S9 linkers are the major differences in the sequences between dslo and mslo channels, we speculate that they could be the candidates of locations affecting Ca^{2+} sensitivity of BK_{Ca} channels.

**The Function of the Glycosyl Residue of Puerarin.** Puerarin is an isoflavone glycoside with a β-d-glucopyranoside at 8-position and two hydroxyl groups at 4’, 7-positions. The isoflavone nucleus is a rigid and hydrophobic structure, whereas the glycosyl residue and hydroxyl group are hydrophilic. Daidzein, an analog of puerarin, has no glycosyl residue and activates BK_{Ca} channels with weaker potency than puerarin. These results indicate that the β-D-glucopyranoside at 8-position plays an important role in puerarin activating BK_{Ca} channels. Thus, engineering in new BK_{Ca} channel openers should focus on the modification of the number or location of glycosyl group in the isoflavone nucleus.

**Vasodilation and the Activation of BK_{Ca} Channels Induced by Puerarin.** Puerarin caused concentration-dependent relaxations in isolated rat thoracic aortic rings contracted with NA. IbTX, a specific BK_{Ca} channel blocker, markedly inhibited puerarin-induced relaxations. It is likely, therefore, that the mechanism of this response to puerarin involves opening of BK_{Ca} channels. In the absence of endothelium, puerarin produced less relaxation than that in the presence of endothelium, indicating that endothelium was also involved in puerarin-mediated vasodilation. However, the inhibition of relaxation by IbTX was not decreased in the absence of endothelium, implying that the contribution of BK_{Ca} channels to puerarin-mediated vasodilation is independent to the endothelium. It is worth noting that the EC_{50} of puerarin required to relax aortic rings (1.1 μM) was ~1000-fold higher than that required to directly activate BK_{Ca} channels. This difference is attributed to the fact that puerarin hardly penetrates the cell membrane as mentioned above. However, puerarin could be taken into the cytoplasm through endocytosis. The average diameter of a vesicle is about 140 nm (Zhang et al., 1995), so each vesicle could carry drugs in 3 × 10^{-6} of the extracellular concentration taking a 10 μm cell in diameter. There are about 200 vesicles taking in one secretion event (Gillis et al., 1996), and from this we can calculate that the concentration of puerarin in the cytoplasm of vascular smooth muscle cells would be in 6 × 10^{-4} of the extracellular concentration, which would be the concentration activating the BK_{Ca} channels. The plasma concentration of puerarin is in the range of 1 to 1000 μM in humans and animals (Jin et al., 1991; Deng et al., 2004; Wu et al., 2004), so the concentration used in this study is of therapeutic relevance.

In summary, we have provided direct evidences for the activation of cloned BK_{Ca} channels by puerarin, and shown that BK_{Ca} channel activation contributes to puerarin-induced vasodilation, which is likely to be a mechanism by which puerarin exerts its action on rat thoracic aortas, in addition to its activation of endothelial nitric-oxide synthase and cAMP pathway, as demonstrated by other investigators (Ma et al., 2003; Yeung et al., 2006). The present findings are of interest for understanding the contribution of BK_{Ca} openers to lowering blood pressure and improving other cardiovascular symptoms.
Acknowledgments

We thank Dr. Geng Hui, Dr. Tao Yunhai, and Dr. Gao Shangbang for helpful discussions. We thank Prof. Chen Jianguo and Dr. Wang Fang for guidance in vasoreactivity experiments. We thank Prof. John Cram and Prof. He Guangyuan for critical comments on the manuscript.

References


Hsing AC, Chien SR, and Chien C (1999) Role of inactivation in the regulation of large-conductance 


Address correspondence to: Xiang-Liang Yang, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, PR China. E-mail: yangx3@mail.hust.edu.cn