Calcium Channel Blockade in Vascular Smooth Muscle Cells:
Major Hypotensive Mechanism of S-Petasin, a Hypotensive
Sesquiterpene from Petasites formosanus

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Received November 6, 2000; accepted January 3, 2001
This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

In vivo and in vitro studies were carried out to examine the putative hypotensive actions of S-petasin, a sesquiterpene extracted from the medicinal plant Petasites formosanus. Intravenous S-petasin (0.1–1.5 mg/kg) in anesthetized rats produced a dose-dependent hypotensive effect. In isolated aortic ring, isometric contraction elicited by KCl or the L-type Ca2+-channel agonist Bay K 8644 was reduced by S-petasin (0.1–100 μM), an action not affected by the cyclooxygenase inhibitor indomethacin, nitric-oxide synthase inhibitor N“-nitro-L-arginine, guanylyl cyclase inhibitor methylene blue, or removal of vascular endothelium. Pretreatment with S-petasin for 10 min shifted the concentration-response curve for KCl (15–90 mM)-induced contraction to the right and reduced the maximal response. In Ca2+-depleted and high K+-depolarized aortic rings preincubation with S-petasin attenuated the Ca2+-induced contraction in a concentration-dependent manner, suggesting that S-petasin reduced Ca2+ influx into vascular smooth muscle cells (VSMCs). Moreover, in cultured VSMCs, whole-cell patch-clamp recording indicated that S-petasin (1–50 μM) inhibited the L-type voltage-dependent Ca2+-channel (VDCC) activities. Intracellular Ca2+ concentration ([Ca2+]i) estimation using the fluorescent probe 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2’-amino-5’-methylphenoxo)-ethane-N,N,N,N,tetraacetic acid pentaaetoxymethyl ester indicated that S-petasin (10, 100 μM) suppressed the KCl-stimulated increase in [Ca2+]i. Taken together, the results suggested that a direct Ca2+-antagonism of L-type VDCC in vascular smooth muscle may account, at least in part, for the hypotensive action of S-petasin.

Extracts from Petasites plants (Compositae) have been used for thousands of years for therapeutic purposes in folk medicine. They have been claimed to improve conditions in respiratory diseases such as asthma and cough (Ziolo and Samochowiec, 1998), gastrointestinal pain, as well as spasms of the urogenital tract (Brune et al., 1993). P. formosanus is an indigenous species of Petasites in Taiwan and has been used as a folk medicine to treat hypertension. However, its active ingredients are unknown and the mechanisms of action obscure. In attempting to assess its potential as an antihypertensive agent, a series of in vivo and in vitro experiments were conducted to systematically verify its hypotensive properties, identify the active ingredients, and define its hypotensive mechanisms. A series of compounds, mainly sesquiterpenes, were isolated from the aerial part of P. formosanus (Lin et al., 1998). Of these the major one was S-petasin (Fig. 1). Intravenous administration of S-petasin in anesthetized rat caused a dose-dependent fall in blood pressure. In defining its mechanism of action, first of all the isolated aortic ring was used. It was found that S-petasin concentration-dependently relaxed the contraction induced by various vasoconstricting agents, thus providing a basis for hypotensive action. Endothelium dependence was then examined using endothelium-intact and -denuded preparations as well as inhibitors of endothelium-related mediators such as prostacyclin, NO, and guanylyl cyclase (Marczin et al., 1992; Ribeiro et al., 1992; Schrör, 1993). Further mechanistic studies were carried out in cultured VSMCs with the focus on the role of Ca2+ because it plays a central role in the regulation

ABBREVIATIONS: NO, nitric oxide; VSMC, vascular smooth muscle cell; [Ca2+]i, intracellular Ca2+ concentration; Fura-2/AM, 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2’-amino-5’-methylphenoxo)-ethane-N,N,N,N-tetraacetic acid pentaaetoxymethyl ester; VDCC, voltage-dependent Ca2+ channel; MAP, mean arterial blood pressure; L-NNA, N“-nitro-L-arginine; I-V, current-voltage.
of vascular tension. Subtle alterations in the activity of Ca\(^{2+}\) regulatory mechanism can have profound effects on \([\text{Ca}^{2+}]_{i}\), which in turn can affect muscle tone and vascular resistance. Possible changes in \([\text{Ca}^{2+}]_{i}\), associated with S-petasin-induced vasorelaxation were monitored using the fluorescent dye Fura-2/AM. Finally, the L-type VCDD, which is electrically activated by membrane depolarization, represents the principal route by which \(\text{Ca}^{2+}\) enters vascular smooth muscle (Bolton, 1979) and plays an essential role in excitation-contraction coupling (van Bremeen and Saida, 1989). Whole-cell patch-clamp technique was applied to single cultured rat VSMCs to study the \(\text{Ca}^{2+}\) fluxes.

The results indicated that S-petasin relaxed precontracted isolated rat aortic rings. Such vasorelaxation was independent of the endothelium or the associated mediators such as prostacyclin, NO, and guanylyl cyclase. On the other hand, reduction in \([\text{Ca}^{2+}]_{i}\), and \(\text{Ca}^{2+}\) influx in VSMCs suggested that prevention of \(\text{Ca}^{2+}\) entry from the extracellular fluid through L-type VCDD, leading to the reduction in \([\text{Ca}^{2+}]_{i}\), in VSMCs, could account, at least in part, for the hypotensive action of S-petasin.

**Materials and Methods**

**Rats**

Male Sprague-Dawley rats, weighing 250 to 350 g (Laboratory Animal Science Center of the National Yang-Ming University, Taipei, Taiwan), were used. The rats were allowed to acclimate in environmentally controlled quarters with temperature maintained at 20–22°C and lighting at 12-h light/dark cycles for at least a week before being used in experiments. Standard laboratory chow (Laboratory rodent diet no. 5P14; PMI Feeds Inc., Richmond, IN) and drinking water were provided ad libitum.

**Assessment of Effects on Blood Pressure**

The rats were anesthetized by i.p. administration of sodium pentobarbital (50 mg/kg) and kept on a heating pad for the maintenance of body temperature at 37 ± 1°C. The right femoral artery and vein were cannulated using PE-50 tubing (Clay Adams, Parsippany, NJ) for the monitoring of pulse pressure and mean arterial pressure (MAP), and for i.v. bolus administration of S-petasin, respectively. To record changes in blood pressure, the arterial cannula was connected to a Gould 2400 polygraph (Valley View, OH) via a P23XL pressure transducer (Viggo-Spectramed, Oxnard, CA). Changes in MAP after S-petasin administration were compared with those after the injection of the same volume of vehicle.

**In Vitro Vascular Tension Study**

The details of the experimental procedures have been described previously (Wang et al., 1999). Briefly, isolated aortic rings 3 to 4 mm in length were fixed isometrically in organ chambers under passive tension of 1 g for 60 min. After equilibration, near maximal contraction was induced by phenylephrine (0.3 \(\mu\)M). When the rings achieved a stable contractile tension, acetylcholine (1 \(\mu\)M) was added to the bath to assess endothelial integrity. In some preparations, the intima was gently frayed with a cotton swab to disrupt the endothelium. The absence of acetylcholine-induced relaxation indicated successful endothelial denudation.

**Relaxation of Agonist-Induced Contraction.** For the evaluation of relaxation, S-petasin (0.01–100 \(\mu\)M) or vehicle was added in a cumulative manner during the tonic phase of contraction induced by KCl (60 mM) in both endothelium-intact and -denuded aortic rings. A 10-min time interval was required to obtain the maximal effect with each concentration of S-petasin. The construction of concentration-response curves for S-petasin was based on the percentage of relaxation of the agonist-induced contraction. A 100% relaxation was considered attained when the precontracted rings returned to the baseline position. A second assessment of the vasorelaxing effect of S-petasin was also carried out in endothelium-denuded aortic rings precontracted by the L-type VCDD activator Bay K 8644 (50 nM). Because a partial depolarization of the cell membranes is required to obtain responses to Bay K 8644 (Schramm et al., 1983), contractions to this \(\text{Ca}^{2+}\) agonist were obtained in a medium that contained 15 mM KCl.

**Effects of Endothelial Mediators on the Vasorelaxation of S-Petasin.** To investigate the possible involvements of prostacyclin, NO, and guanylyl cyclase in the vasorelaxing effects of S-petasin, endothelium-intact aortic rings were preincubated separately with the cyclooxygenase inhibitor indomethacin (10 \(\mu\)M), the NO synthase inhibitor L-NNA (100 \(\mu\)M), and the guanylyl cyclase inhibitor methylene blue (10 \(\mu\)M) for appropriate periods. Cumulative concentrations of S-petasin (0.01–100 \(\mu\)M) were then applied during the sustained phase of phenylephrine (0.3 \(\mu\)M)-induced contraction. The effects of the various inhibitors were studied by comparing the degrees of vasorelaxation induced by S-petasin in the absence or presence of these inhibitors. The concentrations of the inhibitors used had been reported to be adequate to produce the necessary prostacyclin (Garcia-Cohen et al., 2000), NO (Pieper and Siebeneich, 1997), and guanylyl cyclase (Terluk et al., 2000) inhibition.

**Inhibition of KCl-Induced Contraction.** The contraction generated by cumulative concentration of KCl (15–90 mM) was first recorded in endothelium-denuded preparations. Following washing and recovery for 60 min, the tissue was then treated with S-petasin (1–100 \(\mu\)M) or vehicle for 10 min and finally application of the same concentration of KCl again. Only one S-petasin concentration was tested per tissue. The treatment time of 10 min was chosen based on the maximal relaxation relative to each concentration. Concentration-response curves of S-petasin were constructed and compared.

**Effects of Extracellular Ca\(^{2+}\) on S-Petasin’s Modulation of KCl-Induced Contraction.** An aortic ring depolarized and contracted by \(\text{Ca}^{2+}\) was chosen as the model to investigate the effects of S-petasin on the contraction dependent on \(\text{Ca}^{2+}\) influx from VDCC. Experiments were carried out under \(\text{Ca}^{2+}\)-free conditions after equilibration. Subsequent to the addition of K\(^+\) (60 mM) to depolarize the membrane potential, cumulative concentrations of \(\text{Ca}^{2+}\) (0.1–3 mM) were applied. The stepwise increments in tension represented the vasoconstriction dependent on extracellular \(\text{Ca}^{2+}\) influx induced by K\(^+\). The aortic rings were then washed and equilibrated for 60 min, followed by repetition of the experiment in the presence of S-petasin (1–100 \(\mu\)M) or vehicle for 10 min. Only one S-petasin concentration was tested per tissue. Concentration-response curves to the added \(\text{Ca}^{2+}\) were constructed and compared.

**In Vitro Whole-Cell Patch-Clamp Recording**

**Cell Culture.** VSMCs were isolated by collagenase-elastase dissociation from the rat thoracic aorta using previously published procedures developed in our laboratory (Wang et al., 1996). Before being used in studies, the cells were incubated in trypsin solution for 1 to 2 min, washed with Hank’s balanced salt solution, and divided into small groups ranging in number from 1 to 30 cells for each dish. After dispersion, cells were allowed to reattach to the culture dish with only one experiment conducted per dish. VSMCs were used within 10 to 24 h after they were plated and were of passage between

**S-Petasin Blocks Voltage-Dependent Ca\(^{2+}\) Channels**

![Fig. 1. Chemical structure of S-petasin.](image-url)
3 and 6. The limited time after isolation helped to maximize Ca²⁺ current amplitudes of the cells.

**Electrophysiology.** Ca²⁺ channel activity was determined in single VSMCs by the whole-cell version of the patch-clamp technique as described previously (Wang et al., 1999). In all experiments, Ba²⁺ was used as the charge carrier. Because the inward Ba²⁺ currents were small and the series resistance was less than 0.1 ohms, the series resistance compensation was not usually used. The currents were monitored through digital oscilloscope (Nicolet Instrument Co., Madison, WI) and filtered with a low pass filter (Axon Instruments, Inc., Foster City, CA) at 1 kHz. The software pCLAMP and a labmaster interface were used for the generation of test pulses and storage and analysis of data. Leakage and capacitive currents were subtracted during analysis while simultaneously slow records were taken on an SC 284 chart recorder (Gould). All recording was done at room temperature (20–22°C).

**Effects of S-Petasin on Ca²⁺ Channel Activity in VSMCs.** To generate current-voltage (I-V) curves, the Ba²⁺ current through the Ca²⁺ channels was elicited by depolarizing the VSMCs from a test pulse of −30 mV to more positive test potentials at a frequency of 0.1 Hz. The duration of the depolarizing test pulses was 250 ms at intervals of 5 s. Peak currents were attained for constructing the I-V relationships. Only cells showing stable channel activity for at least 5 min were used to test the effects of S-petasin. The I-V relationships were measured repeatedly for 5 min after the addition of S-petasin (1–50 μM) or vehicle in the medium.

**[Ca²⁺]i Measurement in Individual VSMCs**

[Ca²⁺]i was measured with the ratiometric fluorescent dye Fura-2/AM by a method modified from that of Wang et al. (1996). Briefly, VSMCs were seeded on a sterile glass coverslip at an appropriate density to allow imaging of 10 to 20 single cells. After 24 h, these attached cells were loaded with Fura-2/AM (2 μM) for 40 min in a dark place at room temperature. The dye-loaded cells were gently washed three times with a medium containing 145 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 10 mM glucose, 1 mM CaCl₂, 0.5 mM NaH₂PO₄, and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4). The cells were kept in medium for a further 20 min to allow the hydrolysis of Fura-2/AM into Ca²⁺-sensitive free acid form (Fura-2) by cell esterases. The coverslip with attached cells was then transferred to a 1-ml thermoregulated chamber (22°C) on the stage of an Olympus IX-70 inverted microscope (Tokyo, Japan) and viewed unfiltered to a 1-ml thermoregulated chamber (22°C) on the stage of a PerkinElmer Life Science, Cambridge, UK) connected to a cooled charge-coupled device camera (Kodak KAF 1600, Rochester, NY) for digital imaging of the changes of [Ca²⁺]i in individual cells. The advantages of this system are that multiple cells can be examined simultaneously and that the cells under investigation can be imaged throughout the experiment. Data were analyzed for [Ca²⁺]i, changes by measurement of the 340- and 380-nm excitation signals and emission signal at 510 nm. Maximal and minimal fluorescence were obtained by adding ionomycin (10 μM) and EGTA (5 mM) sequentially at the end of the experiment. Ratio values were converted to an estimate of [Ca²⁺]i, as described previously (Gryniewicz et al., 1985) assuming a K₅₀ of 155 nM. All procedures and experiments were performed at room temperature to minimize compartmentalization and cell extrusion of the dye.

**Effects of S-Petasin on [Ca²⁺]i in VSMCs.** To study the effect of S-petasin on Ca²⁺ influx from VDCC, the VSMCs were challenged with KCl (60 mM) in the presence of S-petasin (10, 100 μM) or vehicle for 10 min, and the changes in [Ca²⁺]i were recorded.

**Drugs**

The following drugs were used: S-petasin (mol. wt. = 334) was isolated and purified by the National Research Institute of Chinese Medicine (Taipei, Taiwan, Republic of China) (Lin et al., 1998); acetylcholine, indomethacin, methylene blue, L-NNA, and phenylephrine were purchased from Sigma Chemical Co. (St. Louis, MO); and Fura-2/AM was obtained from Molecular Probes (Eugene, OR). Indomethacin was dissolved in absolute ethanol. Fura-2/AM was dissolved in dimethyl sulfoxide, whereas the rest of the drugs were dissolved in distilled water and kept at −20°C with the exception of S-petasin. S-Petasin was dissolved in dimethyl sulfoxide, ethanol, and medium mixture (0.05:0.5:1.0:44) to make 0.1 to 100 mM stock solutions. The final concentration of the vehicle in the solution did not exceed 0.1%, and it had no effects on vascular tension, magnitude/kinetics of the inward current and fluorescence imaging of VSMCs.

**Statistical Analyses**

The data are presented as mean ± S.E. and n represents the number of experiments. In line graphs, S.E. values are indicated by error bars (in some cases the error bars were so small they were obliterated by the line symbols). For representation of the Ca²⁺ current data, the peak inward currents were used in most cases. Statistical analyses were carried out by Student’s paired or unpaired t tests when applicable. P values of less than 0.05 were considered to be significant.

**Results**

**Effect of S-Petasin on MAP.** The mean MAP before S-petasin treatment in 10 anesthetized rats was 105 ± 3 mm Hg. Mean body weight for this experiment was 288 ± 6 g. Figure 2 demonstrates the dose dependence of the effect of S-petasin (0.1–1.5 mg/kg) on MAP. Although the vehicle alone slightly decreased MAP, the changes were significantly higher in the presence of S-petasin at doses of 0.1 mg/kg and higher. Within seconds of injection of S-petasin the MAP fell (−34 ± 2 mm Hg) and remained lower than the preinjection value for the next 2 to 3 min at the maximal dose of 1.5 mg/kg.

**Relaxation of Agonist-Induced Contraction.** S-Petasin given alone did not alter the baseline tension of the aortic rings (data not shown). In KCl (60 mM)-precontracted aortic rings, S-petasin (0.01–100 μM) produced concentration-dependent vasorelaxation compared with the vehicle-treated group (data not shown). The vasorelaxing effect of S-petasin on precontracted aortic rings showed no significant difference in the presence or absence of endothelium, implying that S-petasin acted directly on the arterial smooth muscle.

![Fig. 2](image-url)
were 4.2 ± 0.8 μM and 96%, respectively. S-Petasin also induced vasorelaxation in Bay K 8644-precontracted endothelium-denuded aortic rings (Fig. 4). The IC$_{50}$ and the maximal relaxation obtained by 100 μM S-Petasin were 4.2 ± 0.8 μM and 96%, respectively.

**Effect of Endothelial Mediators in S-Petasin-Induced Vasorelaxation.** In the rat thoracic aorta, phenylephrine (0.3 μM) caused an initial phasic and then a tonic contraction, which lasted for at least 30 min. During the tonic contraction induced by phenylephrine, endothelium-intact aortic rings showed a significant relaxation in response to acetylcholine (95 ± 4%) (data not shown). The concentration-response curves for cumulative S-petasin (0.01–100 μM) treatment in endothelium-intact aortic rings, before and after treatment with indomethacin (10 μM), L-NNA (100 μM), or methylene blue (10 μM) are illustrated in Fig. 5. The results indicated that treatment with these inhibitors did not significantly affect either the basal vascular tone or the relaxing effect of S-petasin in endothelium-intact aortic rings.

**Inhibition of KCl-Induced Contraction.** In endothelium-denuded aortic preparations, the cumulative concentration-effect curves for KCl (15–90 mM) in the absence and presence of five concentrations of S-petasin (1–100 μM) are shown in Fig. 6. Pretreatment with S-petasin (3–100 μM) for 10 min suppressed the cumulative concentration contractions induced by KCl. The maximal inhibition obtained with 100 μM S-petasin was approximately 89%. Vehicle treatment had no significant effects.

**Effects of Extracellular Ca$^{2+}$ on S-Petasin’s Modulation of KCl-Induced Contraction.** In Ca$^{2+}$-free, high K$^+$ (60 mM) solution, the cell membrane of aortic smooth muscle was depolarized and VDCCs were activated. The lack of Ca$^{2+}$ entry was verified by the failure of KCl to produce vasoconstriction in the aortic rings in the absence of extracellular Ca$^{2+}$ (data not shown). Figure 7 shows that cumulative addition of Ca$^{2+}$ (0.1–3 mM) caused a stepwise increase of contraction of the rat aorta, apparently caused by Ca$^{2+}$ entering the depolarized cell through VDCC. The maximal tension attained at 3 mM Ca$^{2+}$ was 1.64 ± 0.12 g in the presence of vehicle and was taken to be 100%. When the aortic ring was treated with S-petasin at 1 to 100 μM, 10 min before KCl, the KCl-induced contraction was attenuated in a concentration-dependent manner, suggesting that Ca$^{2+}$ influx through VDCC was probably inhibited by S-petasin. The IC$_{50}$ value was calculated to be 8.2 ± 0.6 μM at a Ca$^{2+}$ concentration of 3 mM.

**Effects of S-Petasin on Ca$^{2+}$ Channel Activity in VSMCs.** VSMCs were depolarized from −30 to 60 mV with the ramp protocol to investigate the channel openings. Ba$^{2+}$ currents through L-type VDCC were observed in VSMCs. During a 5-min application of the vehicle alone, no significant changes (±0.5 ± 1.2%) in the kinetics and I-V relationship of L-type VDCC current occurred (data not shown). Figure 8 shows that a 5-min application of S-petasin (1–50 μM) reduced the L-type VDCC current to below the immediately preceding current measured in vehicle-treated specimen. The decrease in the magnitude of L-channel currents induced by S-petasin was evident within 2 to 3 min and reached a steady-state level within 5 min. Figure 9 summarizes the results from several experiments. The maximal reduction caused by S-petasin was 75.45 ± 13.08%.

**Fig. 3.** Vasorelaxing effect of S-petasin on endothelium-intact (●) and endothelium-denuded (○) Sprague-Dawley rat thoracic aortic rings contracted with KCl (60 mM). The tensions developed in the absence of vehicle and S-petasin in intact and denuded rings were 1.50 ± 0.17 and 1.75 ± 0.15 g, respectively (considered as 100%). Values are mean ± S.E.; n = 7 to 8 for each group.

**Fig. 4.** Comparison the vasorelaxing effects of S-petasin in endothelium-denuded aortic rings between KCl (60 mM, □) and Bay K 8644 (50 nM, △)-induced contractions. The tensions developed by KCl and Bay K 8644 in the absence of S-petasin in denuded rings were 1.75 ± 0.15 and 1.81 ± 0.13 g, respectively (considered as 100%). Values are mean ± S.E.; n = 8 to 10 for each group.

**Fig. 5.** Effects of indomethacin (10 μM, ▲), L-NNA (100 μM, □), and methylene blue (10 μM, △) treatments on S-petasin (0.01–100 μM)-induced relaxation in endothelium-intact (●) thoracic aorta isolated from Sprague-Dawley rats. Aortic rings were precontracted with phenylephrine, and the change in tension is expressed as a percentage of the active tension originally generated by phenylephrine. The tension developed in the absence of S-petasin and agents in endothelium-intact rings was 1.92 ± 0.03 g. Values are mean ± S.E.; n = 9 to 10 for each group.
Fig. 6. Inhibitory effect of S-petasin (1–100 μM) on the contraction induced by KCl (15–90 mM) in Sprague-Dawley rat thoracic aortic rings with denuded endothelium. Aorta was preincubated with vehicle (○) or S-petasin (1 μM, ●; 3 μM, ▲; 10 μM, ▼; 30 μM, ◆; 100 μM, ■) at 37°C for 10 min, and then cumulative concentrations of KCl (15–90 mM) were used to trigger the contraction. The mean maximal contractile responses induced by KCl (90 mM) in the absence of S-petasin in endothelium-denuded rings was 1.46 ± 0.07 g. Values are mean ± S.E.; n = 10 to 12 for each group. * , **Statistically significant difference (P < 0.05, P < 0.01, respectively) between the vehicle and the S-petasin-treated group.

Fig. 7. Inhibitory effect of S-petasin (1–100 μM) on the contraction, dependent on extracellular Ca²⁺ influx, induced by KCl (60 mM) in Ca²⁺-free Krebs’ solution of endothelium-denuded thoracic aortic rings from Sprague-Dawley rats. Aorta was preincubated with vehicle (○) or S-petasin (1 μM, ●; 3 μM, ▲; 10 μM, ▼; 30 μM, ◆; 100 μM, ■) at 37°C for 10 min, and then cumulative concentrations of Ca²⁺ (0.1–3.0 mM) were used to trigger the contraction. The mean maximal contractile responses induced by Ca²⁺ (3.0 mM) in the absence of S-petasin in endothelium-denuded rings was 1.84 ± 0.12 g. Values are mean ± S.E.; n = 10 to 12 for each group. * , **Statistically significant difference (P < 0.05 and P < 0.01, respectively) between the vehicle and the S-petasin-treated group.

**Effects of S-Petasin on [Ca²⁺]i in Individual VSMCs.**

The average basal [Ca²⁺]i in single VSMCs was 112.06 ± 2.52 nM. [Ca²⁺]i increased to 149.68 ± 2.66 nM when the VSMCs were stimulated by KCl (60 mM). S-Petasin (10, 100 μM) suppressed the KCl-induced increase of [Ca²⁺]i by 39.6 ± 2.43 and 66.8 ± 1.97%, respectively (Fig. 10). S-Petasin itself did not alter the basal [Ca²⁺]i in VSMCs (data not shown).

**Discussion**

Although folk medicines are popular in many parts of the world and in certain cases can ably complement or supplement mainstream medicine where ineffectual, inadequate, or low therapeutic indices exist (Marshall, 1994). Unfortunately in many cases the claims are shaky or inadequately substantiated, the mechanisms of action undefined, and the pharmacology mystified, impeding their acceptance and development. Complicating further their therapeutic application is the fact that many folk medicines are empirical with multiple components and lack of batch-to-batch consistency, although the claim is that the interactions of these components may be beneficial. The first goal of the present study was to identify the active ingredients. Following chemical isolation and identification, the major component of P. formosanus turned out to be the sesquiterpene of S-petasin. Intravenous administration of S-petasin in anesthetized rats produced a dose-dependent hypotensive effect. However, no accompanying
reflex tachycardia was observed. Related studies involving isolated atria and cell cultures are in progress and will be reported in due course. Preliminary indications are that S-petasin may exert direct cardiac depressant effects, which may even be beneficial if S-petasin were to be developed to be an antihypertensive agent. For the present purpose, S-petasin appeared to be the or one of the hypertensive principles in *P. formosanus*. Mechanistic studies focusing on S-petasin thus followed.

In the vascular tension studies, the S-petasin-induced relaxation in endothelium-denuded or -intact aorta precontracted by KCl or Bay K 8644, suggesting that vasorelaxation may be a basis for its hypertensive action. The role of the endothelium, being known to be involved in the regulation of cardiovascular functions, was then being examined. Endothelium-intact and -denuded preparations as well as inhibitors of the known vasorelaxing mediators prostacyclin, NO, and guanylyl cyclase were used. The results indicated that the vasorelaxing actions of S-petasin were not affected even in the presence of indomethacin, l-NAME, or methylene blue. Indomethacin is known to block the generation of prostacyclin, whereas l-NAME and methylene blue have been reported to inactivate the NO system or inhibit the activation of guanylyl cyclase, respectively (Thorin et al., 1998). It thus appeared that the vasorelaxation caused by S-petasin was endothelium-independent and not mediated by prostacyclin or the NO-guanylyl cyclase pathway but rather acted directly on the arterial smooth muscle. The rest of the experiments were therefore conducted in endothelium-denuded aortic preparations or cultured VSMCs.

An increase in free cytoplasmic Ca\(^{2+}\) levels is required for excitation-contraction coupling of vascular smooth muscle. Vasoconstrictors can increase the [Ca\(^{2+}\)], by activating several different pathways. VDCC represents the principal route by which Ca\(^{2+}\) enters vascular smooth muscle cells (Bolton, 1979) and plays an essential role in the sustained phase of contraction (Cauvin and Malik, 1984). Drugs that block the Ca\(^{2+}\) channel have proven clinically effective for the treatment of a multitude of cardiovascular disorders. It has been reported that increased KCl depolarizes smooth muscle cells, leading to the opening of VDCC, with subsequent Ca\(^{2+}\) influx and contraction (Karaki and Weiss, 1979).

In the present study, pretreatment with S-petasin suppressed, in a concentration-dependent manner, the aortic contractile response to high K\(^+\). The maximum inhibition produced by S-petasin at 100 μM was about 89%. When S-petasin was cumulatively added during the tonic contraction induced by high K\(^+\), it exerted 100% vasorelaxation. Similar results were also obtained as the aortic preparations were challenged with the VDCC activator Bay K 8644. These observations suggested that S-petasin might interfere with these Ca\(^{2+}\) channels in the aortic smooth muscle, possibly resulting in the decrease of Ca\(^{2+}\) influx and contraction. Furthermore, in Ca\(^{2+}\)-depleted and high K\(^+\) medium, the cell membrane of aortic smooth muscle was depolarized and the VDCCs were activated but without contraction due to the lack of extracellular Ca\(^{2+}\). Addition of Ca\(^{2+}\) produced sustained contraction that was produced by the Ca\(^{2+}\) influx through VDCC. Preincubation with S-petasin could effectively antagonize, in a concentration-dependent manner, Ca\(^{2+}\)-induced contraction, implying that S-petasin probably blocked Ca\(^{2+}\) influx through VDCC in isolated aortic smooth muscle cells. However, the antagonism was noncompetitive in nature because there was a nonparallel shift to the right and suppression of the maximal response. A plausible reason is probably that much higher concentrations of Ca\(^{2+}\) would be needed to achieve the maximal contraction because Ca\(^{2+}\) in high concentrations can be autoinhibitory and decrease the permeability of the cell membrane for Ca\(^{2+}\).

With K\(^+\) and Na\(^+\) channels blocked, whole-cell patch clamp was studied in isolated cultured VSMCs. These data provide strong evidence that S-petasin inhibited Ca\(^{2+}\)-generated currents in the L-type VDCC, the predominant Ca\(^{2+}\) channels in VSMCs. The fact that S-petasin can inhibit VDCC activity suggests that S-petasin may reduce the increase in [Ca\(^{2+}\)], elicited by KCl, resulting in decreased Ca\(^{2+}\) entry and [Ca\(^{2+}\)]. In our FurA-2 studies, the measurement of [Ca\(^{2+}\)] in cultured VSMCs confirmed this interpretation. S-Petasin indeed produced a significant reduction in [Ca\(^{2+}\)], induced by KCl, which indicated that the direct effect of S-petasin on blood vessels was probably related to interference with Ca\(^{2+}\) transport and consequently the contraction. Taken together, the vasorelaxant action induced by S-petasin in KCl-contracted aortic rings appeared to be mediated via direct inhibition of VDCC activity, leading to decreased Ca\(^{2+}\) entry and [Ca\(^{2+}\)]. The attenuation of KCl-induced Ca\(^{2+}\) transients by S-petasin may explain its observed hypotensive effects in vivo. It thus seems that S-petasin exerts its hypotensive action by decreasing vascular reactivity to endogenous pressor agents, at least in part, through inhibition of the VDCC activity and the net inward flow of Ca\(^{2+}\).

In conclusion, the present studies identified S-petasin as the principal active ingredient in *P. formosanus* and verified its hypotensive effect. Mechanistic studies suggested vasorelaxation via inhibition of Ca\(^{2+}\) being the likely underlying mechanism. Vascular endothelium and related vasorelaxation mediators play small roles. These findings may be helpful in the establishment of S-petasin as a potential antihypertensive agent, elucidation of its pharmacological actions and its further development as a therapeutic agent.

Acknowledgments

We thank Professor Peter Pang of the University of Hong Kong, Faculty of Medicine, for proofreading the manuscript and Shu-Jen...
Huang for excellent technical assistance in the performance of some of these studies.

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