ELSEVIER

European Journal of Pharmacology 445 (2002) 239–245

www.elsevier.com/locate/ejphar

Ca^{2+} channel blocking effect of *iso-S*-petasin in rat aortic smooth muscle cells

Guei-Jane Wang [a,*], Xi-Chen Wu [b], Yun-Lian Lin [a], Jun Ren [c], Andrew Yau-Chik Shum [d], Yu-Yuan Wu [e], Chieh-Fu Chen [a]

[a] *National Research Institute of Chinese Medicine, Room 355, 155-1, Section 2, Li-Nong Street, Pei-tou District (112), Taipei, Taiwan, ROC*
[b] *Heritage Medical Research Center, Cardiology of Medicine, University of Alberta, Edmonton, Alberta, Canada*
[c] *Department of Pharmacology, Physiology, and Therapeutics, University of North Dakota School of Medicine, Grand Forks, ND 58203, USA*
[d] *Department and Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan, ROC*
[e] *Drug Development Division, Development Center for Biotechnology, Taipei, Taiwan, ROC*

Received 10 January 2002; received in revised form 24 April 2002; accepted 3 May 2002

Abstract

The purpose of the present study was to examine the mechanisms underlying the putative hypotensive actions of *iso-S*-petasin, a sesquiterpene extract of *Petasites formosanus* through both in vivo and in vitro experiments. Intravenous administration of *iso-S*-petasin elicited dose-dependent (0.1–1.5 mg/kg) hypotensive and bradycardiac responses in anesthetized rats. Isometric tension recording in isolated thoracic aorta revealed that *iso-S*-petasin (0.01–100 μM) inhibited KCl- or Bay K 8644 (1,4-dihydro-2,6-dimethyl-5-nitro-4-[2′-(trifluoromethyl)phenyl]-3-pyridinecarboxylic acid methyl ester)-induced vasoconstriction independent of endothelium. *Iso-S*-Petasin also attenuated Ca^{2+}-induced vasoconstriction in a concentration-dependent manner in Ca^{2+}-depleted/high K^+-depolarized ring segments, indicating that *iso-S*-petasin inhibited Ca^{2+} influx into vascular smooth muscle cells. This was confirmed by whole-cell patch-clamp recording in cultured vascular smooth muscle cells where *iso-S*-petasin (10–100 μM) appeared to directly inhibit the L-type voltage-dependent Ca^{2+} channel (VDCC) activity. Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) measurements using the fluorescent probe fura-2/AM (1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2′-amino-5′-methylphenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid pentaacetoxymethyl ester) showed suppression of the KCl-stimulated increase in $[Ca^{2+}]_i$ by *iso-S*-petasin (10, 100 μM). In conclusion, these results suggest that Ca^{2+} antagonism of the L-type VDCC in vascular smooth muscle cells might largely account for the hypotensive action of *iso-S*-petasin. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: *Iso-S*-Petasin; Vascular smooth muscle cell; L-type Ca^{2+} channel; Intracellular Ca^{2+} concentration; Hypotension

1. Introduction

Herbal medicine is popular in many parts of the world and considered complementary to mainstream medicine in certain cases where mainstream therapeutic measures are found to be ineffective or inadequate (Marshall, 1994). However, claims on herbal medicine may often be shaky due to undefined mechanisms of action and mystified pharmacology, thus impeding the acceptance and development of herbal medicinal compounds. Complicating further the therapeutic application of herbal medicine is the fact that many folk medicines are essentially empirical with multiple components and lack of consistency. To remedy these shortcomings, our laboratories, along with collaborating experts in chemical synthesis and isolation, have in recent years been engaged in the isolation and pharmacological characterization of active ingredients from these medicinal plants. *Petasites* is a medicinal plant long used for the treatment of a variety of ailments including asthma, cough (Ziolo and Samochowiec, 1998), gastrointestinal disorders, and urogenital-tract spasm (Brune et al., 1993). *Petasites formosanus*, an indigenous species of *Petasites* in Taiwan, is used in antihypertensive treatment. However, the effective ingredients and hypotensive mechanism of *P. formosanus* remain largely obscure. Among the sesquiterpene com-

* Corresponding author. Tel.: +886-2-2820-1999x3561; fax: +886-2-2378-0159.
E-mail address: jennyw@cma23.nricm.edu.tw (G.-J. Wang).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)01764-8

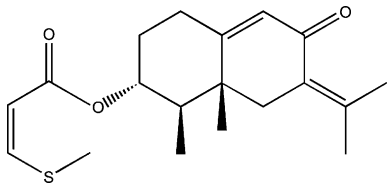

Fig. 1. Chemical structure of *iso-S*-petasin.

pounds that have recently been extracted from the aerial part of *P. formosanus* (Lin et al., 1998) are *S*-petasin, whose pharmacological characterization has recently been reported (Wang et al., 2001) and *iso-S*-petasin (Fig. 1), an isomer of *S*-petasin with an isopropenyl group at position 7, as small structural differences may result in considerable functional differences (Triggle and Rampe, 1989). In the present study, we proceeded to characterize *iso-S*-petasin. We monitored the effects of *iso-S*-petasin on blood pressure and heart rate, agonist-induced vasoconstriction, L-type Ca^{2+} channel activation and intracellular Ca^{2+} mobilization, in both isolated thoracic aorta and cultured vascular smooth muscle cells. Blood pressure, heart rate, vascular tension, L-type voltage-dependent Ca^{2+} channel (VDCC) and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were evaluated using blood pressure monitor, force-transducer, whole-cell patch-clamp and fluorescent dye (fura-2/AM; 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2′-amino-5′-methylphenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid pentaacetoxymethyl ester) imaging, respectively.

2. Materials and methods

All experiments reported herein were in accordance with guidelines of the animal investigation committee of the National Research Institute of Chinese Medicine.

2.1. Blood pressure and heart rate measurement

Arterial blood pressure and heart rate were measured in male Sprague–Dawley rats under sodium pentobarbital anesthesia as previously described (Wang et al., 1996). The right femoral artery and vein were cannulated using PE-50 tubing for mean arterial pressure and heart rate monitoring and intravenous bolus administration of *iso-S*-petasin, respectively. Changes in mean arterial pressure and heart rate from eight rats following *iso-S*-petasin administration were compared with those from another eight rats that received vehicle (dimethyl sulfoxide, ethanol and saline mixture = 0.05:0.51:0.44) only.

2.2. In vitro vascular tension studies

The tension measurement procedures have been described in detail previously (Wang et al., 1999). To evaluate the vasorelaxing effect of *iso-S*-petasin, concentration–response curves were constructed by cumulatively adding *iso-S*-petasin (0.01–100 μM) or vehicle to KCl (60 mM)-precontracted aortic ring, the magnitudes of vasorelaxation being expressed as percentages of the maximal contraction induced by KCl. A 10-min interval was given to allow the development of maximal effect at each concentration of *iso-S*-petasin. As initial observations indicated that endothelium integrity made little difference in these treatments, subsequent experiments were carried out on endothelium-denuded preparations. An alternative assessment of the vasorelaxing effect of *iso-S*-petasin was also constructed in endothelium-denuded rings precontracted by the L-type VDCC agonist Bay K 8644 (50 nM; 1,4-dihydro-2,6-dimethyl-5-nitro-4-[2′-(trifluoromethyl)phenyl]-3-pyridine-carboxylic acid methyl ester; RBI/Sigma, St. Louis, MO). Because a partial depolarization of the cell membranes is required to obtain responses to Bay K 8644 (Schramm et al., 1983), contractions to this Ca^{2+} agonist were obtained in a medium that contained 15 mM KCl. To investigate the inhibitory effect of *iso-S*-petasin, pretreatment on KCl-induced contraction, the tissue preparation was treated with one of the concentrations of *iso-S*-petasin (1–100 μM) or vehicle for 10 min, followed by cumulative addition of KCl (15–90 mM). With the maximal tension attained by vehicle at KCl (90 mM) being considered as 100%, concentration–response curves were constructed. To evaluate Ca^{2+} influx dependency on vasorelaxant response of *iso-S*-petasin, vehicle or *iso-S*-petasin (1–100 μM) were added to the aortic rings in Ca^{2+}-free medium for 10 min, followed by the addition of K^+ (60 mM) for membrane depolarization, and finally cumulative concentrations of Ca^{2+} (0.1–3 mM). Concentration–response curves as functions of the added Ca^{2+} were then constructed and compared.

2.3. Whole-cell patch-clamp recording

Vascular smooth muscle cells were dispersed by collagenase–elastase dissociation from the rat thoracic aorta using our previously described procedures (Wang et al., 1996). VDCC activity was determined in single vascular smooth muscle cell by the whole-cell version of the patch-clamp technique as described previously (Wang et al., 1999). The extracellular solution used for recording Ca^{2+} currents contained 20 mM $BaCl_2$, 5 mM KCl, 5 mM CsCl, 105 mM Tris, 20 mM HEPES, 20 mM glucose, as well as 0.5 μM tetradotoxin. The internal (pipette) solution consisted of 70 mM Cs_2-aspartate, 2 mM ATP-Na_2, 5 mM potassium-succinate, 5 mM potassium–pyruvate, 5 mM $MgCl_2$, 5 mM phosphocreatine-Na_2, 15 mM HEPES, 10 mM EGTA, 25 mM glucose and creatine phosphokinase at a concentration of 50 U/ml. The pH and osmolality of all solutions were adjusted to 7.4 and 320 mOsm, respectively. Ca^{2+} channel currents were isolated from possible interference by Na^+ currents by having tetradotoxin added to Na^+-free extracellular solution

while K_v and K_{Ca} currents were eliminated by filling the detecting micropipette with Cs^+-containing solution, which was introduced into the intracellular milieu upon breaking the cell membrane by suction (Quandt and Narahashi, 1984). Solutions were filtered (0.22 μm) before use.

In all experiments, Ba^{2+} was used as the charge carrier. The holding potential was maintained at −40 mV. To generate current–voltage (I–V) curves, the Ba^{2+} current through the Ca^{2+} channels was elicited by depolarizing the vascular smooth muscle cells 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 the construction of the I–V relationships. Only cells showing stable channel activity for at least 5 min were used for the testing of the effects of *iso-S*-petasin. The I–V relationships were measured before and 5 min after addition of *iso-S*-petasin (1–100 μM) or vehicle in the medium.

2.4. $[Ca^{2+}]_i$ measurement in individual vascular smooth muscle cell

$[Ca^{2+}]_i$ was determined by using the fura-2/AM technique. Vascular smooth muscle cells were used between passages 4 and 6. Cells (approximately 3×10^4) seeded on a sterile glass coverslip were cultured with medium which was changed every 48 h until the cells became elongated and confluent. These cells were loaded with 2 μM fura-2/AM for 40 min in a dark place at room temperature according to our previous method (Wang et al., 1996). The Merlin imaging system (PerkinElmer Life Science, Cambridge, UK) was used for digital imaging of the changes of $[Ca^{2+}]_i$ in individual cells as previously described (Wang et al., 2001). Data were analyzed for $[Ca^{2+}]_i$ changes by measurement of the 340 and 380 nm of excitation signals and emission signal at 510 nm. Ratio values were converted to an estimate of $[Ca^{2+}]_i$ as described previously (Grynkiewicz et al., 1985) assuming a K_d of 155 nM. To study the effect of *iso-S*-petasin on Ca^{2+} influx from VDCC, the vascular smooth muscle cells were challenged with KCl (60 mM) in the presence of *iso-S*-petasin (10, 100 μM) or vehicle for 10 min, and changes in $[Ca^{2+}]_i$ were recorded.

2.5. Statistics

Data are presented as mean ± S.E. and n represents the number of experiments. Statistical significance ($P < 0.05$) for each variable was estimated by Student's paired or unpaired t-tests. The final concentration of the vehicle in the solution never exceeded 0.1% and had no effects on mean arterial pressure, heart rate, vascular tension, magnitude/kinetics of the inward current and fluorescence dye imaging of vascular smooth muscle cells.

3. Results

3.1. Effect of iso-S-petasin on mean arterial pressure and heart rate

The mean body weights of eight anesthetized rats were 238 ± 9 g. The mean arterial pressure and heart rate before *iso-S*-petasin treatment were 101 ± 4 mm Hg and 393 ± 8 bpm, respectively. *Iso-S*-Petasin (0.1–1.5 mg/kg) elicited a dose-dependent decrease in mean arterial pressure (Fig. 2A). Although there appeared to be a slight tendency in lowering mean arterial pressure in the group treated with vehicle alone, the decrease in mean arterial pressure was never statistically significant. In contrast, the drop in mean arterial pressure induced by *iso-S*-petasin at doses of 0.75 mg/kg and higher were significantly different not only from the pretreatment value but also from those treated with corresponding concentrations of the vehicle. Within seconds of injection of *iso-S*-petasin, the mean arterial

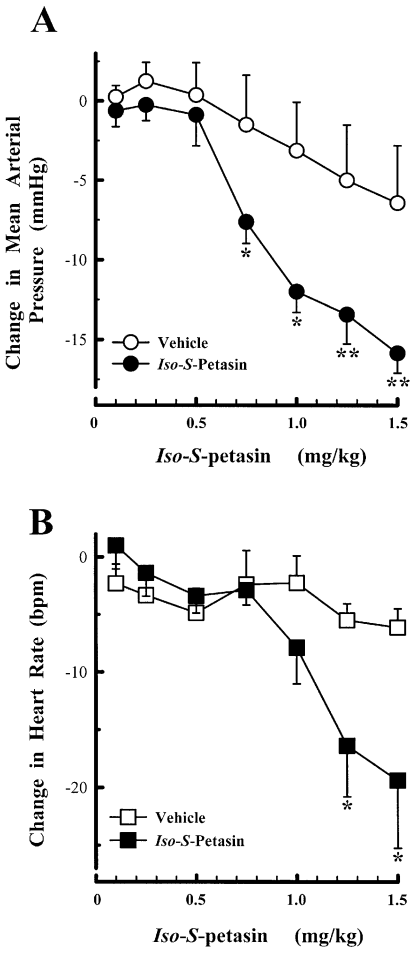

Fig. 2. The dose-dependent decreases in mean arterial pressure (A) and heart rate (B) after intravenous bolus injection of *iso-S*-petasin (0.1–1.5 mg/kg) in anesthetized Sprague–Dawley rats. $n = 8$. * $P < 0.05$, ** $P < 0.01$ vs. vehicle control.

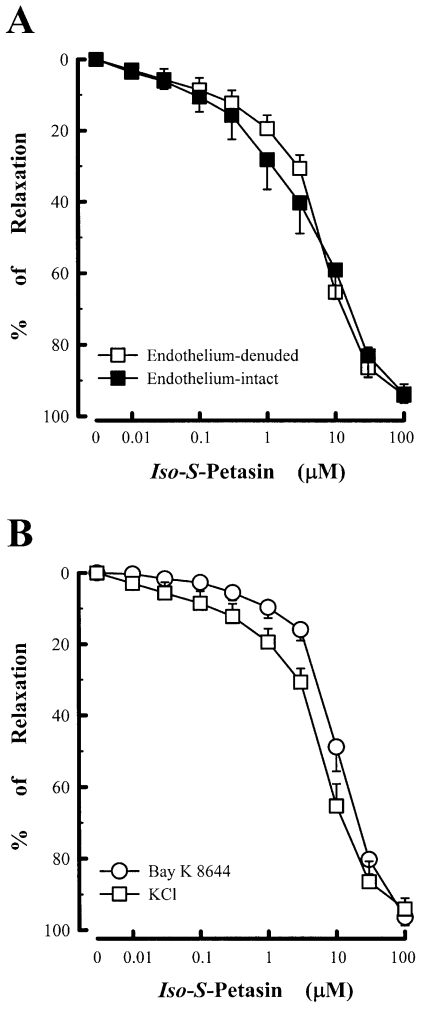

Fig. 3. (A) Vasorelaxing effect of *iso-S*-petasin (1–100 μM) on endothelium-intact and -denuded Sprague–Dawley rat thoracic aortic rings contracted with KCl (60 mM). (B) Comparison of the vasorelaxing effects of *iso-S*-petasin in endothelium-denuded aortic rings between KCl (60 mM)- and Bay K 8644 (50 nM)-induced contractions. $n = 8–9$.

pressure fell (-16 ± 1 mm Hg) and remained lower than the preinjection value for the next 1 to 2 min at the maximal dose of 1.5 mg/kg. Fig. 2B demonstrates the dose-dependence curves for *iso-S*-petasin-induced bradycardiac response. At 1.25 mg/kg, the reduction of heart rate was significantly lower than that induced by vehicle treatment. Within seconds of *iso-S*-petasin injection heart rate fell markedly to reach its lowest value, with the bradycardia persisting for more than 5 min at the maximal dose of 1.5 mg/kg. The maximum bradycardia induced by *iso-S*-petasin was -22 ± 6 bpm while vehicle treatment did not affect heart rate.

3.2. Relaxation of agonist-induced contraction

The tensions developed in the absence of vehicle and *iso-S*-petasin in intact and denuded rings were 1.31 ± 0.16 and 1.62 ± 0.11 g, respectively (considered as 100%). In KCl (60 mM)-precontracted aortic rings, *iso-S*-petasin (0.01–100 μM) elicited comparable concentration-dependent relaxation in ring segments with or without endothelium, indicating that *iso-S*-petasin-induced vasorelaxation may be due to its direct action on the arterial smooth muscle instead of the endothelium (Fig. 3A). The IC_{50} and the maximal relaxation (obtained at 100 μM) of *iso-S*-petasin were 5.47 ± 1.98 μM and 93.8%, respectively. The tensions developed by Bay K 8644 (50 nM) in the absence of *iso-S*-petasin in denuded rings were 1.83 ± 0.18 g. *Iso-S*-Petasin also induced vasorelaxation in Bay K 8644 (50 nM) precontracted endothelium-denuded aortic rings (Fig. 3B). The IC_{50} and the maximal relaxation (obtained at 100 μM) of *iso-S*-petasin were 11.18 ± 1.76 μM and 96.4%, respectively. *Iso-S*-petasin did not alter the baseline tension of the

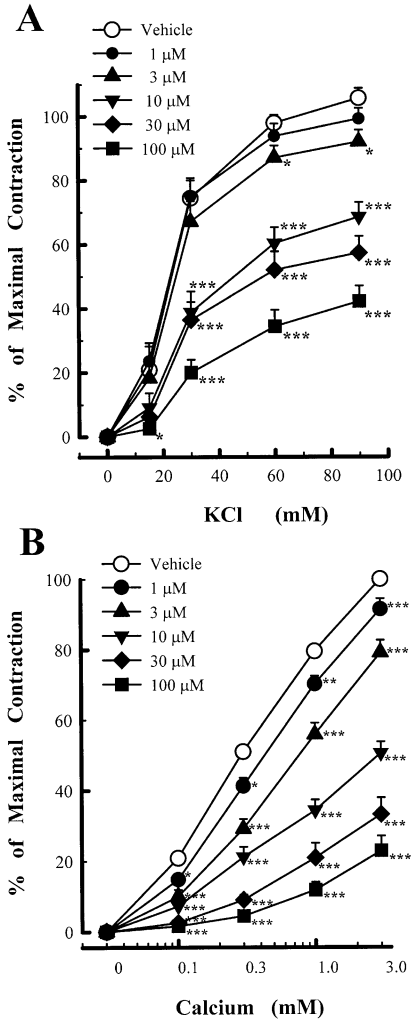

Fig. 4. Inhibitory effect of *iso-S*-petasin (1–100 μM) or vehicle on the contraction induced by KCl (15–90 mM) (A) or by extracellular Ca^{2+} influx in Ca^{2+}-free/high K^+ (60 mM) Krebs' solution (B) in Sprague–Dawley rat aortic rings without endothelium. $n = 10–12$. When no S.E. is shown, it was smaller than the symbol for the mean. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. vehicle control.

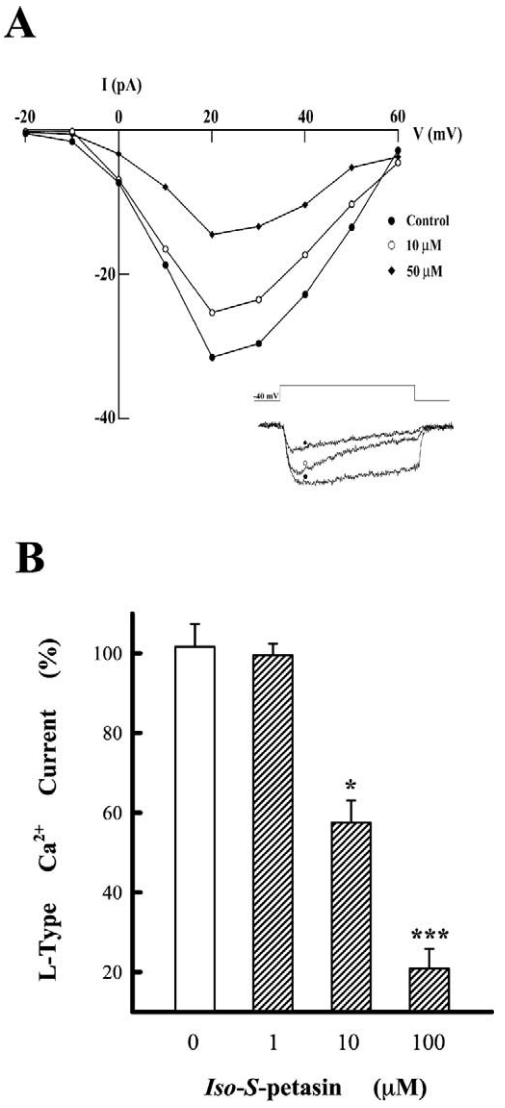

Fig. 5. (A) Representative $I-V$ plots and recordings in single cultured vascular smooth muscle cell. The peak amplitude of L-type VDCC currents, carried by Ba^{2+}, was inhibited by *iso-S*-petasin (10, 50 μM). (B) Inhibitory effect of *iso-S*-petasin (1–100 μM) on inward Ca^{2+} currents recorded at 20 mV in cultured vascular smooth muscle cells. $n=5$. $^{*}P<0.05$, $^{***}P<0.001$ vs. vehicle control.

aortic rings (data not shown). The recovery of the responses to KCl and Bay K 8644 were more than 96%.

3.3. Inhibition of KCl-induced contraction

Fig. 4A shows the concentration-dependent vasoconstricting effect of KCl (15–90 mM) tested in the absence and presence of varying concentrations of *iso-S*-petasin (1–100 μM) in endothelium-denuded aortic preparations. The mean maximal contractile responses induced by KCl (90 mM) in the absence of *iso-S*-petasin was 1.45 ± 0.21 g. Pretreatment with *iso-S*-petasin for 10 min suppressed the KCl-induced vasoconstriction in a concentration-dependent manner. The maximal inhibition obtained with 100 μM *iso-S*-petasin was approximately 57.8%.

3.4. Effects of extracellular Ca^{2+} on iso-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 inferred from the failure of KCl to produce vasoconstriction in the aortic rings in the absence of extracellular Ca^{2+}. Fig. 4B shows that increase in bath Ca^{2+} concentration (0.1–3 mM) exerted a stepwise increase of contraction of the rat aorta, apparently through Ca^{2+} influx into the depolarized cell through VDCC. The maximal tension, attained at 3 mM Ca^{2+} in the presence of the vehicle, was of 1.86 ± 0.06 g. When aortic rings were pretreated with *iso-S*-petasin (1–100 μM) for 10 min prior to KCl application, the Ca^{2+}-dependent-KCl-induced vasoconstriction was attenuated by *iso-S*-petasin in a concentration-dependent manner, suggesting that Ca^{2+} influx through VDCC might be inhibited by *iso-S*-petasin. The IC_{50} value was calculated to be 13.33 μM at a Ca^{2+} concentration of 3 mM.

3.5. Effects of iso-S-petasin on Ca^{2+} channel activity in vascular smooth muscle cells

Vascular smooth muscle cells were depolarized from −30 to 60 mV to examine the L-type VDCC opening characteristics. Vehicle alone had little effects ($-0.5 \pm 1.2\%$) on the kinetics and $I-V$ relationship of the current recorded (data not shown). Fig. 5A shows that a 5-min application of *iso-S*-petasin (10, 50 μM) reduced the L-type VDCC current

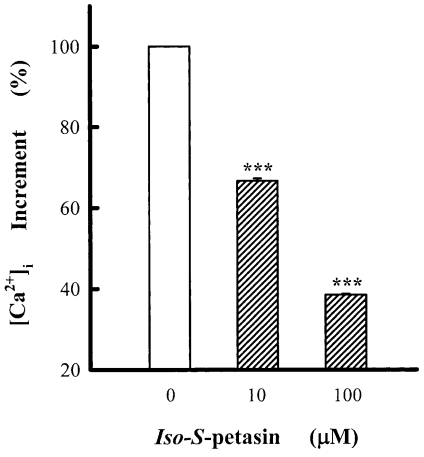

Fig. 6. Inhibitory effect of *iso-S*-petasin on the peak increment of $[Ca^{2+}]_i$ induced by KCl (60 mM) in cultured vascular smooth muscle cells. The cells were challenged by KCl (60 mM) in the presence of *iso-S*-petasin (10, 100 μM) or vehicle for 10 min and the peak increment in $[Ca^{2+}]_i$ was determined. $n=3-6$ separate experiments. $^{***}P<0.001$ vs. vehicle control.

amplitude. The decrease in the magnitude of L-channel currents was evident within 2–3 min and reached a steady-state level within 5 min. Fig. 5B summarizes the results from several experiments. The maximal reduction of currents recorded at 20 mV, a marked negative shift of L-type Ca^{2+} channels, caused by *iso-S*-petasin (100 μM) was 79.2 ± 5.0%.

3.6. Effects of iso-S-petasin on $[Ca^{2+}]_i$ in individual vascular smooth muscle cell

The resting and KCl (60 mM)-stimulated $[Ca^{2+}]_i$ in single vascular smooth muscle cell were 126.6 ± 1.1 and 163.2 ± 1.8 nM, respectively. *Iso-S*-Petasin (10, 100 μM) suppressed the KCl-induced increase in $[Ca^{2+}]_i$ by 33.3 ± 0.6 and 61.5 ± 0.3%, respectively (Fig. 6). *Iso-S*-Petasin itself did not alter the resting $[Ca^{2+}]_i$ in vascular smooth muscle cell (data not shown).

4. Discussion

This study demonstrates that intravenous administration of the sesquiterpene extract of *P. formosanus*, namely *iso-S*-petasin, decreased blood pressure in anesthetized rats. Our in vitro study indicated that *iso-S*-petasin relaxed agonist-induced vasoconstriction in isolated thoracic aorta, independent of endothelium. Further observations revealed that *iso-S*-petasin inhibited L-type VDCC activities and KCl-induced increase of $[Ca^{2+}]_i$ in cultured vascular smooth muscle cells, suggesting that the decrease in the agonist-stimulated increase in $[Ca^{2+}]_i$ in vascular smooth muscle cells was likely attributable to reduced extracellular Ca^{2+} influx through L-type VDCC which may in turn account, at least in part, for the hypotensive action of *iso-S*-petasin.

Chemical isolation and identification have confirmed the sesquiterpene *iso-S*-petasin as one of the major components of *P. formosanus* (Lin et al., 1998). Intravenous administration of *iso-S*-petasin in anesthetized rats elicited a dose-dependent hypotensive response without reflex tachycardia. In another series of experiments, it was discovered that *iso-S*-petasin exerts direct cardiac depressant effects, which could be beneficial if *iso-S*-petasin were to be developed as an antihypertensive agent. The inhibitory effect of *iso-S*-petasin on myocardium will be reported in a separate communication. The direct actions of *iso-S*-petasin on blood pressure and heart rate favor it to be one of the effective ingredients in *P. formosanus*.

The fact that *iso-S*-petasin relaxes agonist-precontracted thoracic aorta, either endothelium-denuded or -intact is consistent with its hypotensive action. Our results showed that the *iso-S*-petasin-induced vasorelaxation was independent of the endothelium, indicating that the vasorelaxing response of *iso-S*-petasin was not likely mediated through the endothelium, but rather due to a direct effect on the arterial smooth muscle.

An increase in free cytoplasmic Ca^{2+} levels is the ultimate determinant of excitation–contraction coupling in vascular smooth muscle cells. Increase in $[Ca^{2+}]_i$, often due to the presence of vasoconstricting agents, may be achieved through several different pathways, with the Ca^{2+} entry through VDCC being the primary mechanism in vascular smooth muscle cells (Bolton, 1979). Extracellular Ca^{2+} entry through VDCC is essential in the maintenance of tonic vascular tone (van Zwieten et al., 1983). Drugs that block the Ca^{2+} channel have proven clinically effective in the treatment of a multitude of cardiovascular disorders including hypertension (Guazzi et al., 1983; van Zwieten et al., 1983). KCl depolarizes smooth muscle cells and leads to the opening of VDCC, Ca^{2+} influx and eventually vasoconstriction (Karaki and Weiss, 1979). In the present study, treatment with *iso-S*-petasin before and following K^+ depolarization both produced suppressant effects although the magnitudes were somewhat different. The maximum inhibition produced by pretreatment with *iso-S*-petasin was 65.6%. When *iso-S*-petasin was cumulatively added during the tonic contraction induced by KCl, it exerted 93.8% vasorelaxation. Similar results were also seen in preparations challenged with the VDCC agonist Bay K 8644. These observations provided evidence that *iso-S*-petasin may interfere with the VDCC in the aortic smooth muscle. Furthermore, in Ca^{2+}-depleted/high K^+ medium, no vascular contraction was induced due to the lack of extracellular Ca^{2+}. Reapplication of Ca^{2+} produced sustained vasoconstriction which was effectively antagonized by *iso-S*-petasin in a concentration-dependent manner, confirming that *iso-S*-petasin probably blocks Ca^{2+} influx through VDCC in aortic smooth muscles.

The whole-cell patch-clamp data in isolated cultured vascular smooth muscle cells confirm the notion obtained from tension studies that *iso-S*-petasin inhibits Ca^{2+} influx through L-type VDCC, which is the predominant Ca^{2+} channel in rat vascular smooth muscle cells. The ability of *iso-S*-petasin to inhibit VDCC activities is supported by our fura-2 measurements that inhibited KCl-induced increase of $[Ca^{2+}]_i$ in cultured vascular smooth muscle cells, suggesting that *iso-S*-petasin might interfere with Ca^{2+} influx through VDCC. Taken together, the vasorelaxant action induced by *iso-S*-petasin in KCl-contracted aortic rings appeared to be mediated via direct inhibition of VDCC activity, leading to decreased Ca^{2+} entry and $[Ca^{2+}]_i$. The attenuation of KCl-induced Ca^{2+} transients by *iso-S*-petasin may explain its observed hypotensive effects in vivo. As the range of doses used in vitro experiments was consistent with the inferred blood concentration which was calculated from total blood volume in vivo studies, findings from in vitro studies may be relevant. It thus seems that *iso-S*-petasin might exert its hypotensive action by decreasing vascular reactivity to 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 confirmed the hypotensive and vasorelaxing effects of *iso-S*-petasin, which may

be responsible for or at least contribute towards the antihypertensive effect of *P. formosanus*. The vasorelaxing effect of *iso*-*S*-petasin is likely to be mediated via inhibition of Ca^{2+} influx through the VDCC. Our data do not favor contributions from vascular endothelium and related vasorelaxation mediators. These findings may be helpful in the establishment of *iso*-*S*-petasin as a potential antihypertensive agent.

Acknowledgements

This work was supported by Grant NSC90-2320-B-077-003 from National Science Council of the Republic of China to G.J.W.

References

Bolton, T.B., 1979. Mechanisms of action of transmitters and other substances on smooth muscle. Physiol. Rev. 59, 606–718.

Brune, K., Bickel, D., Peskar, B.A., 1993. Gastro-protective effects by extracts of *Petasites hybridus*: the role of inhibition of peptido-leukotriene synthesis. Planta Med. 59, 494–496.

Grynkiewicz, G., Poenie, M., Tsien, R.Y., 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J. Biol. Chem. 260, 3440–3450.

Guazzi, M.D., Polese, A., Fiorentini, C., Bartorelli, A., Moruzzi, P., 1983. Treatment of hypertension with Ca^{2+} antagonists. Hypertension 5 (4 Pt 2), II85–II90.

Karaki, H., Weiss, G.B., 1979. Alterations in high and low affinity binding of $^{45}Ca^{2+}$ in rabbit aortic smooth muscle by norepinephrine and potassium after exposure to lanthanum and low temperature. J. Pharmacol. Exp. Ther. 211, 86–92.

Lin, Y.L., Mei, C.H., Huang, S.L., Kuo, Y.H., 1998. Four new sesquiterpenes from *Petasites formosanus*. J. Nat. Prod. 61, 887–890.

Marshall, E., 1994. The politics of alternative medicine. Science 265, 2000–2002.

Quandt, F.N., Narahashi, T., 1984. Isolation and kinetic analysis of inward currents in neuroblastoma cells. Neuroscience 13, 249–262.

Schramm, M., Thomas, G., Towart, R., Franckowiak, G., 1983. Novel dihydropyridines with positive inotropic action through activation of Ca^{2+} channels. Nature 303, 535–537.

Triggle, D.J., Rampe, D., 1989. 1,4-Dihydropyridine activators and antagonists: structural and functional distinctions. Trends Pharmacol. Sci. 10, 507–511.

van Zwieten, P.A., van Meel, J.C., Timmermans, P.B., 1983. Pharmacology of calcium entry blockers: interaction with vascular alpha-adrenoceptors. Hypertension 5 (4 Pt 2), II8–II17.

Wang, G.J., Shan, J., Pang, P.K.T., Yang, M.C.M., Chou, C.J., Chen, C.F., 1996. The vasorelaxing action of rutaecarpine: direct paradoxical effects on intracellular Ca^{2+} concentration of vascular smooth muscle and endothelial cells. J. Pharmacol. Exp. Ther. 76, 1016–1021.

Wang, G.J., Wu, X.C., Chen, C.F., Lin, L.C., Huang, Y.T., Shan, J., Pang, K.T.P., 1999. Vasorelaxing action of rutaecarpine: effects of rutaecarpine on Ca^{2+} channel activities in vascular endothelial and smooth muscle cells. J. Pharmacol. Exp. Ther. 289, 1237–1244.

Wang, G.J., Shum, A.Y.C., Lin, Y.L., Liao, J.F., Wu, X.C., Ren, J., Chen, C.F., 2001. Calcium channel blockade in vascular smooth muscle cells: major hypotensive mechanism of *S*-petasin, a hypotensive sesquiterpene from *Petasites formosanus*. J. Pharmacol. Exp. Ther. 297, 240–246.

Ziolo, G., Samochowiec, L., 1998. Study on clinical properties and mechanisms of action of *Petasites* in bronchial asthma and chronic obstructive bronchitis. Pharm. Acta Helv. 72, 378–380.