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Iso-S-petasin, a hypotensive sesquiterpene from *Petasites formosanus*, depresses cardiac contraction and intracellular Ca^{2+} transients in adult rat ventricular myocytes

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Abstract

Petasites formosanus is an indigenous species of the medicinal plant *Petasites* which has been used to treat hypertension. Both *S*-petasin and its isoform *iso-S*-petasin have been shown to be the effective ingredients in *P. formosanus*. However, their effect on heart function has not been revealed. This study was to examine the effect of *iso-S*-petasin on cardiac contractile function at the myocyte level. Ventricular myocytes were isolated from adult rat hearts and were stimulated to contract at 0.5 Hz under 1.0 mM extracellular Ca^{2+}. Contractile properties were evaluated using an IonOptix MyoCam system including peak shortening (PS), time to PS (TPS), time to 90% re-lengthening (TR_{90}) and maximal velocity of shortening/re-lengthening ($\pm$dL/dt). Intracellular Ca^{2+} properties were assessed by fura-2 and presented as Ca^{2+}-induced Ca^{2+} release (CICR) and intracellular Ca^{2+} decay. Acute application of *iso-S*-petasin (10^{-7} to 10^{-4} M) elicited a concentration-dependent inhibition in PS and CICR, with maximal inhibitions of 51.0% and 31.0%, respectively. *Iso-S*-petasin also induced a concentration-dependent inhibition of $\pm$dL/dt without affecting TPS, TR_{90}, baseline intracellular Ca^{2+} level or intracellular Ca^{2+} decay. Elevation of extracellular Ca^{2+} from 1.0 mM to 2.7 mM significantly antagonized the *iso-S*-petasin-induced depression in PS and CICR. These results demonstrated a direct depressant action of *iso-S*-petasin on ventricular contraction, which may work in concert with its antihypertensive action to reduce the cardiac load. The *iso-S*-petasin-induced decrease in CICR may play a role in its cardiac depressant effect.

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Introduction

Herbal medicine is complementary to mainstream medicine, especially when the latter is ineffective or inadequate (Marshall 1994). However, many herbal medicinal compounds are empirical with multiple unidentified components, thus making it difficult to define their pharmacology and mechanism of action. It is therefore crucial to isolate and purify the active ingredients of these medicinal plants and characterize their pharmacological properties. *Petasites* is a medicinal herb with a long history in the treatment of respiratory (Ziolo & Samochowiec 1998), gastrointestinal and urogenital disorders (Brune et al 1993). *P. formosanus*, an indigenous species of *Petasites* found in Taiwan, has been used to treat hypertension. However, the effective ingredients and pharmacological action of *P. formosanus* remain obscure. Among the sesquiterpene compounds extracted from the aerial part of *P. formosanus* (Lin et al 1998) are *S-petasin*, of which the pharmacological properties have recently been reported (Wang et al 2001), and *iso-S*-petasin (Figure 1), an isomer of *S*-petasin with an isopropenyl group at position 7. The aim of this study was to elucidate the effect of *iso-S*-petasin on cardiac contractile function by evaluating cell shortening and intracellular Ca^{2+} properties in isolated ventricular myocytes.

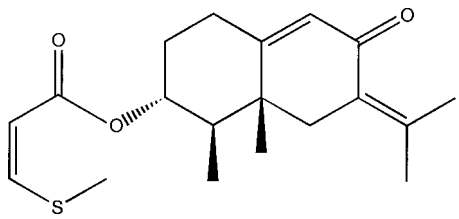

Figure 1 Chemical structure of *iso-S*-petasin.

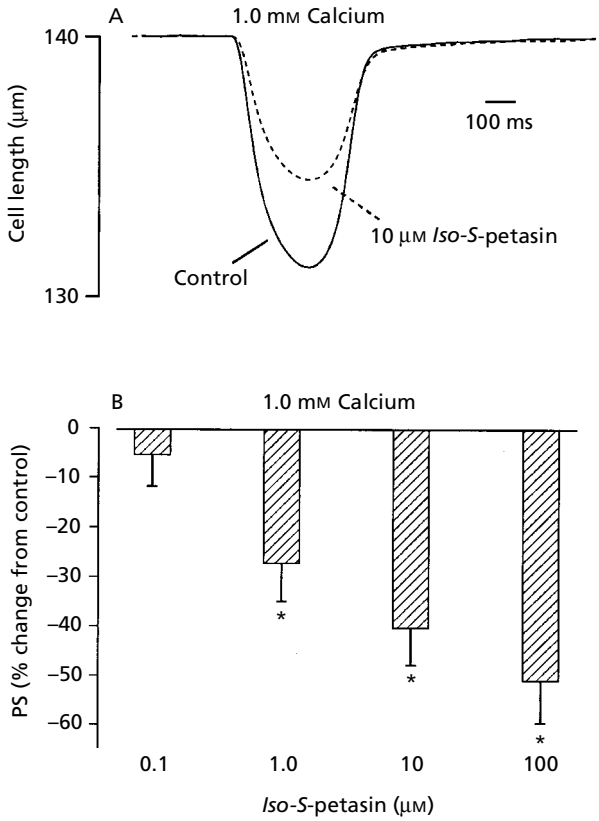

Figure 2 A. Representative trace depicting the effect of *iso-S*-petasin (10^{-5} M) on cell shortening in rat ventricular myocytes. B. Concentration-dependent response of *iso-S*-petasin (10^{-7} to 10^{-4} M) on peak cell shortening (PS). Data are presented as percent change from control PS which was 5.5±1.8%. Mean±s.d., n = 24 cells. Extracellular Ca^{2+} concentration = 1.0 mM. *$P < 0.05$ vs control (without *iso-S*-petasin).

Materials and Methods

Isolation of ventricular myocytes

The experimental procedures described in this study were approved by the Institutional Animal Care and Use Committee of University of North Dakota (Grand Forks, ND). Single ventricular myocytes were isolated from adult male Sprague–Dawley rats, 200–225 g, as described previously (Ren 2002). Briefly, hearts were rapidly removed and perfused (at 37°C) with oxygenated (5% CO_2–95% O_2) Krebs–Henseleit bicarbonate (KHB) buffer (mM: NaCl 118, KCl 4.7, $CaCl_2$ 1.25, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, *N*-[2-hydro-ethyl]-piperazine-*N*′-[2-ethanesulfonic acid] (HEPES) 10, glucose 11.1, pH 7.4). Hearts were subsequently perfused with a nominally Ca^{2+}-free KHB buffer for 2–3 min followed by a 20-min perfusion with Ca^{2+}-free KHB containing 223 U mL^{-1} collagenase (Worthington Biochemical Corporation, Freehold, NJ) and 0.1 mg mL^{-1} hyaluronidase (Sigma Chemical, St Louis, MO). After perfusion, the left ventricle was removed, minced and further digested with trypsin (Sigma) before being filtered through a nylon mesh (300 μm) and collected by centrifugation. Cells were initially washed with Ca^{2+}-free KHB buffer to remove remnant enzyme and extracellular Ca^{2+} was added incrementally back to 1.25 mM.

Myocyte shortening and re-lengthening

Mechanical properties of ventricular myocytes were assessed by an IonOptix Myocam detection system (IonOptix Incorporation, Milton, MA). Cells were placed in a chamber mounted on the stage of an inverted microscope and superfused (at 25°C) with a buffer containing (in mM): 131 NaCl, 4 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 10 HEPES, at pH 7.4. In some studies, 2.7 mM instead of 1.0 mM extracellular Ca^{2+} was used. The cells were field stimulated at a frequency of 0.5 Hz. Cell shortening and relengthening were assessed using the following indices: peak shortening (PS), time to 90% PS (TPS), time to 90% relengthening (TR_{90}) and maximal velocity of shortening (+dL/dt) and re-lengthening (− dL/dt) (Ren 2002). To test the effect of *iso-S*-petasin on cardiac contraction, cell shortening was recorded before and 5 min after its administration under either 1.0 mM or 2.7 mM extracellular Ca^{2+} concentration.

Intracellular Ca^{2+} fluorescence measurement

Myocytes were loaded with fura-2/AM (0.5 μM) for 10 min and fluorescence measurements were recorded with a dual-excitation fluorescence photomultiplier system (Ionoptix) as described by Ren (2002). Myocytes were plated on glass cover slips on an Olympus IX-70 inverted microscope and imaged through a Fluor 40× oil objective. Cells were exposed to light emitted by a 75-W lamp and passed through either a 360- or a 380-nm filter (band widths±15 nm), while being stimulated to contract at 0.5 Hz. Fluorescence emissions were detected at 480–520 nm after first illuminating cells at 360 nm for 0.5 s then at 380 nm for the duration of the recording protocol (333 Hz sampling rate). The 360-nm excitation scan was repeated at the end of the protocol. Qualitative changes in intracellular Ca^{2+} levels were inferred from the ratio of the fluorescence intensity at two wavelengths (360/380) and were used to determine Ca^{2+}-induced Ca^{2+} release (change of fura-2 fluorescent intensity, CICR). Intracellular Ca^{2+} removal was evaluated as the rate of fluorescence decay. To test the effect of *iso-S*-petasin on CICR, intracellular Ca^{2+} transients were recorded before and 5 min after its adminis-

tration under either 1.0 mM or 2.7 mM extracellular Ca^{2+} concentration.

Data analysis

Data were presented as mean±s.d. Statistical significance ($P < 0.05$) for each variable was estimated by analysis of variance or *t*-test, where appropriate.

Results

Effect of *iso-S*-petasin on myocyte shortening (PS) under 1.0 mM extracellular Ca^{2+}

The average cell length used in this study was 146.4±31.4 μm (n = 47). Acute exposure to *iso-S*-petasin did not affect resting myocyte cell length over the range of concentrations tested. A representative trace depicting the effect of *iso-S*-petasin (10^{-5} M) on myocyte shortening (PS) in the presence of 1.0 mM extracellular Ca^{2+} is shown in Figure 2A. At the end of a 5-min exposure to this concentration of *iso-S*-petasin, PS was decreased by 38.6%. *Iso-S*-petasin exhibited little effect on the duration of shortening (TPS) and re-lengthening (TR_{90}). *Iso-S*-petasin (10^{-7} to 10^{-4} M) elicited a concentration-dependent depression of PS, with a maximal inhibition of 51.0%. The threshold of inhibition was between 10^{-7} M and 10^{-6} M (Figure 2B). *Iso-S*-petasin-induced inhibition on cell shortening was maximal within 4 min of exposure and was reversible upon washout (data not shown). The inhibitory effect of *iso-S*-petasin was associated with depressed maximal velocity of shortening/re-lengthening (±dL/dt) with little response on TPS and TR_{90} (Table 1).

Effect of *iso-S*-petasin on intracellular Ca^{2+} transients under 1.0 mM extracellular Ca^{2+}

To determine whether *iso-S*-petasin-induced inhibition of PS was due to reduced availability of intracellular Ca^{2+}, the effect of *iso-S*-petasin on CICR was examined under the extracellular Ca^{2+} concentration of 1.0 mM. *Iso-S*-petasin (10^{-7} to 10^{-4} M) elicited concentration-dependent inhibition of CICR, with a maximal inhibition of 31.0%. The threshold of inhibition was between 10^{-7} M and 10^{-6} M (Figure 3), consistent with that of the cell shortening. The inhibitory response of CICR suggests that a decrease in intracellular free Ca^{2+} is likely to be responsible for *iso-S*-petasin-induced depressive action on myocyte shortening. Neither baseline intracellular Ca^{2+} level nor the intracellular Ca^{2+} decay rate was affected by *iso-S*-petasin (Table 1).

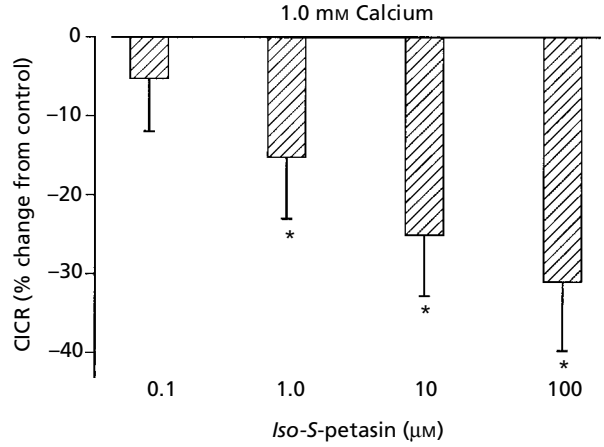

Figure 3 Concentration-dependent response of *iso-S*-petasin (10^{-7} to 10^{-4} M) on intracellular Ca^{2+}-induced Ca^{2+} release (CICR). Data are presented as percent change from respective basal CICR value. Mean±s.d., n = 21 cells. Extracellular Ca^{2+} concentration = 1.0 mM. *$P < 0.05$ vs control (without *iso-S*-petasin).

Effect of *iso-S*-petasin on myocyte shortening and intracellular Ca^{2+} in the presence of elevated extracellular Ca^{2+}

Our study using vascular smooth muscle cells suggested that *iso-S*-petasin inhibits voltage-dependent Ca^{2+} channels (VDCC) (Wang et al 2002). To examine if this Ca^{2+}-channel-blocking property is playing a role in the cardiac response to *iso-S*-petasin, the effect of *iso-S*-petasin on

Table 1 Effect of *iso-S*-petasin on duration and maximal velocity of myocyte shortening and re-lengthening as well as intracellular Ca^{2+} properties in adult rat ventricular myocytes under 1.0 mM extracellular Ca^{2+}.

Iso-S-petasin	TPS (ms)	TR_{90} (ms)	+dL/dt (μm s^{-1})	−dL/dt (μm s^{-1})	Ca^{2+} baseline (360/380 ratio)	Ca^{2+} decay rate (ms)
0	168±59	281±93	81.9±27.9	−76.6±46.1	1.26±0.13	605±362
10^{-7} M	175±73	278±93	67.7±26.0	−67.8±41.2	1.28±0.09	613±349
10^{-6} M	154±59	258±127	64.4±35.8	−61.3±37.7	1.24±0.13	635±362
10^{-5} M	163±69	240±81	54.1±35.8*	−54.1±40.7*	1.25±0.09	698±389
10^{-4} M	166±64	249±81	47.0±44.6*	−44.3±46.1*	1.24±0.09	734±358

TPS, Time to peak shortening; TR_{90}, time to 90% re-lengthening; ±dL/dt, maximal velocity of shortening and re-lengthening. Data represent mean±s.d., n = 20–24 cells; *$P < 0.05$ vs control value.

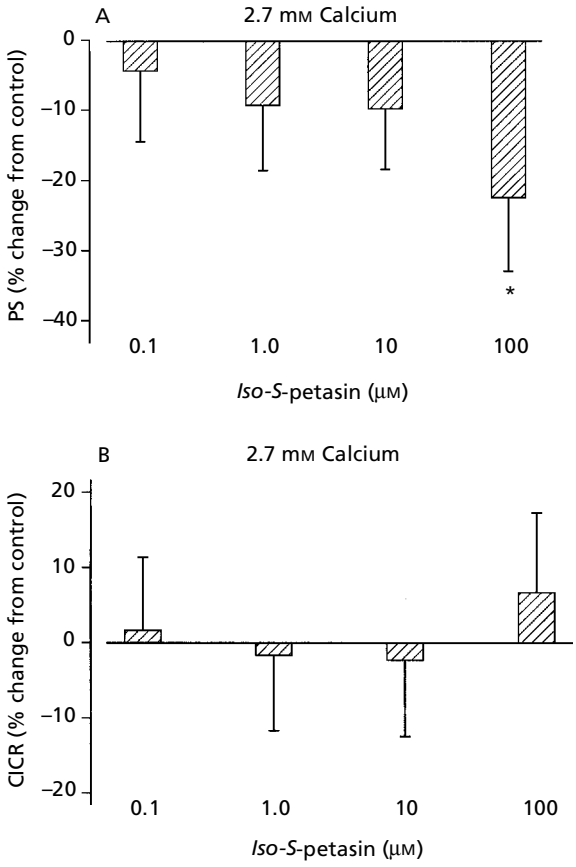

Figure 4 Effect of *iso-S*-petasin (10^{-7} and 10^{-4} M) on cell shortening (PS; A) and Ca^{2+}-induced Ca^{2+} release (CICR; B) under an extracellular Ca^{2+} concentration of 2.7 mM. Mean±s.d., n = 22–23 cells per data point, $*P < 0.05$ vs control (without *iso-S*-petasin).

myocyte shortening was re-examined in the presence of elevated extracellular Ca^{2+} (2.7 mM). As shown in Figure 4, the *iso-S*-petasin-induced decrease in PS and CICR was greatly attenuated by elevation of extracellular Ca^{2+} (except for PS at 10^{-4} M), suggesting a role of Ca^{2+}-channel blockade in the *iso-S*-petasin-induced cardiac depressive response.

Discussion

This study demonstrates that the sesquiterpene extract of *P. formosanus*, namely *iso-S*-petasin, depressed peak shortening, maximal velocity of shortening/re-lengthening and CICR in a concentration-dependent manner in isolated ventricular myocytes. This cardiac-depressant property may allow *iso-S*-petasin to work synergistically with its vasodilatory effect to reduce the overall pre-load, afterload and energy expenditure in the heart (Wang et al 2002). Our results also provide information regarding the clinical application of *iso-S*-petasin in that certain caution must be taken in patients with already compromised heart conditions such as congestive heart failure. It is worth mentioning that neither the duration of contraction (TPS) nor the duration of relaxation (TR_{90}) was affected by *iso-S*-petasin administration, indicating the existence of potential selectivity of *iso-S*-petasin on cardiac contractile proteins.

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 anaesthetized rats elicited a dose-dependent hypotensive response without reflex tachycardia (Wang et al 2002). The direct hypotensive action of *iso-S*-petasin favours its role as one of the effective ingredients in *P. formosanus*. Our results indicate that *iso-S*-petasin may depress cardiac contractile function through intracellular Ca^{2+} accumulation. Although the mechanism(s) of action behind the *iso-S*-petasin-induced reduction of CICR is not clear at this time, the fact that elevating the extracellular Ca^{2+} concentration from 1.0 mM to 2.7 mM abolished cardiac depression induced by *iso-S*-petasin suggests that *iso-S*-petasin may interfere with VDCC in cardiac myocytes. *Iso-S*-petasin is known to inhibit VDCC in vascular smooth muscle cells (Wang et al 2002). Extracellular Ca^{2+} entry through VDCC is essential in triggering the intracellular Ca^{2+} release from the sarcoplasmic reticulum (Bers 2002), which is the ultimate determinant of excitation–contraction coupling in cardiac myocytes. Drugs that block the cardiac VDCC have been proven clinically effective in the treatment of a multitude of cardiovascular disorders including congestive heart failure and hypertension (Opie 2001). Although *iso-S*-petasin may inhibit VDCC, the possibility that the reduced intracellular Ca^{2+} release may also be due to the translocation of the intracellular Ca^{2+} to places like the sarco(endo)plasmic reticulum, or was combined with other substances, could not be ruled out. Whether *iso-S*-petasin directly inhibits VDCC or affects other intracellular Ca^{2+}-regulating machineries in ventricular myocytes warrants further investigation.

Conclusions

Our study demonstrates a direct cardiac depressive response to *iso-S*-petasin at the ventricular myocyte level, possibly through inhibition of VDCC. The precise nature of the cardiac contractile effects of *iso-S*-petasin is still far from clear. Future studies should focus on its direct action on cardiac excitation–contraction coupling including membrane ion channels. These approaches should be essential to the understanding of the cellular actions and pharmacological profiles of this herbal compound in the cardiovascular system.

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