Mechanisms of Relaxant Action of *S*-Petasin and *S*-Isopetasin, Sesquiterpenes of *Petasites formosanus*, in Isolated Guinea Pig Trachea

Wun-Chang Ko[1,*], Chien-Bang Lei[1], Yun-Lian Lin[2], Chieh-Fu Chen[2]

[1] Graduate Institute of Medical Sciences, Taipei Medical College, Taipei, Taiwan, R.O.C.
[2] National Research Institute of Chinese Medicine, Taipei, Taiwan, R.O.C.

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Abstract: We investigated the mechanisms of action of *S*-petasin and *S*-isopetasin, from *Petasites formosanus* Kitamura which is used as a folk medicine for treating hypertension, tumors, and asthma in Taiwan. The tension changes of tracheal segments were isometrically recorded on a polygraph. *S*-Petasin and *S*-isopetasin non-competitively inhibited cumulative histamine-, and carbachol-induced contractions with an exception that *S*-isopetasin produced a parallel, rightward shift of the concentration-response curve of carbachol in a competitive manner. *S*-Petasin also non-competitively inhibited cumulative Ca^{2+}-induced contractions in depolarized (K^+, 60 mM; histamine, 100 μM; or carbachol, 10 μM) guinea-pig tracheas. *S*-Isopetasin did in depolarized (K^+, 60 mM) trachea too. The nifedipine (10 μM)-remaining tension of carbachol (0.2 μM)-induced precontraction was further relaxed by *S*-petasin or *S*-isopetasin, suggesting that no matter whether either blocked VDCCs or not, *S*-petasin or *S*-isopetasin may have other mechanisms of relaxant action. The relaxant effect of *S*-petasin or *S*-isopetasin was unaffected by the presence of propranolol (1 μM), 2′,5′-dideoxyadenosine (10 μM), methylene blue (25 μM), glibenclamide (10 μM), N^{ω}-nitro-L-arginine (20 μM), or α-chymotrypsin (1 U/ml). However, *S*-petasin (100 – 300 μM), but not *S*-isopetasin, significantly inhibited cAMP-, but not cGMP-dependent PDE activity of the trachealis. The above results reveal that the mechanisms of relaxant action of *S*-petasin and *S*-isopetasin may be primarily due to its non-specific antispasmodic and antimuscarinic effects, respectively.

Key words: *S*-Petasin, *S*-isopetasin, *Petasites formosanus*, Asteraceae, guinea-pig trachea, calcium release, calcium influx, cAMP-dependent PDE.

Abbreviations:

ROCCs:	receptor-operated calcium channels
VDCCs:	voltage dependent calcium channels
cAMP:	adenosine 3′,5′-cyclic monophosphate
cGMP:	guanosine 3′,5′-cyclic monophosphate
PDE:	phosphodiesterase
IBMX:	3-isobutyl-1-methylxanthine

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Introduction

In 1993, Brune et al. (1) reported the extract of *Petasites hybridus* L. (Compositae), a therapeutically spasmolytic agent for gastrointestinal tract spasm and for asthmatic attacks in the late middle ages in Europe, to have gastro-protective effects. Bickel et al. (2) identified two main compounds, petasin and isopetasin, in this species, and reported isopetasin and oxopetasan esters to have inhibitory effects on the biosynthesis of vasoconstrictive peptido-leukotrienes. However, petasin has been known to have a spasmolytic effect, although its action mechanism remains unclear, and has been quantitatively analyzed from this plant by Wildi et al. (3). *Petasites formosanus* Kitamura, a perennial herb and the only indigenous *Petasites* species in Taiwan, is used as a folk medicine for treating hypertension, tumors, and asthma in Taiwan (4). Recently, Lin et al. (5), (6) have reported that it contains several new eremophilane-type sesquiterpenes, together with six known compounds, including *S*-petasin, *S*-isopetasin, petasin, and isopetasin. The contents of *S*-petasin, *S*-isopetasin, petasin, and isopetasin in the aerial part of the plant have been reported to be 0.068%, 0.024%, 0.026%, and 0.005%, respectively (6). The content of *S*-petasin is the most abundant among these four. *S*-Petasin (IC_{50} < 10 μM) has been proven to be the most potent in relaxing guinea-pig trachea precontracted by histamine, carbachol, KCl, or leukotriene D_4, although *S*-isopetasin ($IC_{50} \cong 10\,\mu M$) has a similar relaxing potency on carbachol and KCl, but almost has no effect on histamine and leukotriene D_4 (7). In the present study, we investigated the mechanisms of action of *S*-petasin and *S*-isopetasin.

Materials and Methods

Reagents and drugs

S-Petasin and *S*-isopetasin (Fig. **1**) were isolated as previously described (5) from the aerial parts of *Petasites formosanus* Kitamura, and identified by spectral methods, including IR, MS, 1D- and 2D-NMR spectroscopic techniques. The purity of *S*-petasin or *S*-isopetasin was over 99%. The optical rotation values of *S*-petasin and *S*-isopetasin were $[\alpha]_D^{25}$ +58.0° (*c* 1.0, MeOH) and $[\alpha]_D^{25}$ +38.5° (*c* 1.0, $CHCl_3$), respectively. Atropine, aminophylline, carbachol, histamine, propranolol, 2′,5′-dideoxyadenosine, methylene blue, glibenclamide, N^{ω}-nitro-L-arginine (L-NNA), α-chymotrypsin, nifedipine, indomethacin, ethylene gly-

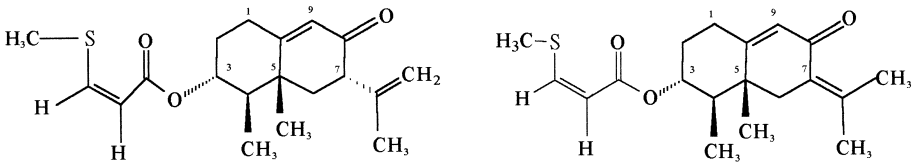

Fig. 1 Chemical structures of *S*-petasin and *S*-isopetasin.

col-bis(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid (EGTA), Trizma base, DL-dithiothreitol, β-mercaptoethanol, cyclic AMP, cyclic GMP, calmodulin, Dowex resin, and *Ophiophagus hannah* snake venom, etc. were purchased from Sigma Chemical, St. Louis, MO, USA. [^3H]cAMP and [^3H]cGMP were purchased from DuPont, Boston, MA, USA. 3-Isobutyl-1-methylxanthine (IBMX) was purchased from Aldrich Chem., Milwaukee, WI, USA. All reagents, including KCl, were of analytical grade. Glibenclamide was dissolved in dimethyl sulfoxide (DMSO), *S*-petasin or nifedipine was dissolved in ethyl alcohol : DMSO (1 : 1), indomethacin was dissolved in ethyl alcohol, and other drugs were dissolved in distilled water. The final concentration of DMSO or ethyl alcohol was less than 0.1 % and did not significantly affect the contraction of the trachea.

Guinea-pig trachea

Male Hartley guinea pigs weighing 250 to 450 g were killed by cervical dislocation and the tracheas were removed. Each trachea was cut into six segments. Each segment consisted of three cartilage rings. All segments were cut open opposite the trachealis. After the segments were randomized to minimize regional variability, they were tied at one end to holders via silk suture, placed in 5 ml of normal or Ca^{2+}-free Krebs solution containing indomethacin (2.8 μM), gassed with a 95 % O_2 – 5 % CO_2 mixture at 37 °C, and attached by the other end of each segment to force displacement transducers (Grass FT03) for the isometric recording of tension changes on a polygraph (Gould RS3200). The composition of the normal Krebs solution was (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, and dextrose 10.1. The isotonic high K^+, Ca^{2+}-free Krebs solution consisted of the above composition without $CaCl_2$, but 60 mM NaCl was replaced by 60 mM KCl. There were three Ca^{2+}-free Krebs solutions prepared by omitting $CaCl_2$ with 2 mM or 0.02 mM EGTA, and without EGTA. The tissues were suspended in normal Krebs solution under an initial tension of 1.5 g and allowed to equilibrate for at least 1 h with washing at 15-min intervals. Either histamine or carbachol was then cumulatively added to the normal or to the Ca^{2+}-free Krebs solution with 0.02 mM EGTA, and the procedure was repeated until the contraction reached constancy after washout. Then, cumulative concentration-response curves were constructed. Maximal contractions of the tracheas without incubation of drugs or their vehicles were set as 100 %. After the tissues were preincubated with *S*-petasin (10 – 200 μM), *S*-isopetasin (10 – 200 μM) or their vehicles for 15 min, these two contractile agonists were also cumulatively added again in normal Krebs solution. When the antagonistic effects of *S*-petasin or *S*-isopetasin on these cumulative concentration-response curves were measured in a non-competitive manner, aminophylline was used as a positive control, and their antagonistic potencies were expressed as pD_2' values. In contrast, when the antagonistic effect of *S*-isopetasin on the cumulative concentration-response curves of carbachol was measured in a competitive manner, atropine was used as a positive control, and their antagonistic potencies were expressed as pA_2 values. In the case of isotonic high K^+ (60 mM)-, histamine (100 μM)-, or carbachol (10 μM)-depolarized tracheal preparations, normal Krebs solution was replaced after equilibration by Ca^{2+}-free Krebs solution without EGTA, and washed with the Ca^{2+}-free solution with 2 mM EGTA after tracheal contraction reached constancy and then incubated for 5 min. After repeating the above procedure until no contraction was observed, cumulative Ca^{2+} (0.01 – 10 mM) was added and contractions were elicited in the depolarized trachealis. The maximal contractile response elicited by Ca^{2+} (10 mM) was taken as 100 %, and the cumulative concentration-response curve was constructed. The inhibitory effects of *S*-petasin or *S*-isopetasin on cumulative Ca^{2+}-induced contractions in isotonic high K^+ (60 mM)-, histamine (100 μM)-, or carbachol (10 μM)-depolarized tracheas were expressed by -log IC_{50} values. The tracheal relaxant effects of cumulative *S*-petasin (0.1 – 300 μM) or *S*-isopetasin (0.1 – 300 μM) to histamine (10 μM)-induced precontraction were allowed to reach a steady state at each concentration. At the end of the experiment without washout, 1 mM of aminophylline was added to standardize the maximal tissue relaxation (100 %). All antagonists or their vehicles were incubated after the precontraction reached a steady state for 15 min prior to the first addition of *S*-petasin or *S*-isopetasin. In a similar manner, nifedipine (10 μM) was added after carbachol (0.2 μM)-induced precontraction reached a steady state, at 15 min prior to the addition of *S*-petasin (100 μM), *S*-isopetasin (100 μM) or their vehicle. At the end of the experiment, 1 mM of aminophylline was also added to standardize maximal tissue relaxation.

Phosphodiesterase activity

The isolated trachealis was homogenized with a glass/teflon homogenizer (Glas-Col, Terre Haute, IN, USA) in 20 volumes of cold medium (pH 7.4) containing 100 mM Tris-HCl, 2 mM $MgCl_2$, and 1 mM dithiothreitol, cAMP- and cGMP-dependent phosphodiesterase (PDE) activities in the homogenate were measured by a modification of the method of Cook et al. (8). The homogenate was centrifuged at 9500 rpm for 15 min, and the upper layer was decanted. Twenty-five microliters of the upper layer were taken for determination of enzyme activity in a final volume of 100 μl containing 40 mM Tris-HCl (pH 8.0), 2.5 mM $MgCl_2$, 3.75 mM mercaptoethanol, 0.1 unit calmodulin (PDE activator), 10 μM $CaCl_2$, and either 1 μM cAMP with 0.2 μCi [^3H]-cAMP or 1 μM cGMP with 0.2 μCi [^3H]-cGMP. In tests of enzyme inhibition, the reaction mixture contained various concentrations of *S*-petasin (30 – 300 μM), *S*-isopetasin (30 – 300 μM) or IBMX (100 – 300 μM), a positive control. The reagents and homogenate were mixed on ice, and the reaction was initiated by transferring the mixture to a water bath at 37 °C. Following a 30-min incubation, the reaction was stopped by transferring the reaction vessel to a bath of boiling water for 3 min. After cooling on ice, 20 μl of a 1 mg/ml solution of *Ophiophagus hannah* venom were added to the reaction mixture, and the mixture was incubated at 37 °C for

10 min. Unreacted [^3H]-cAMP or [^3H]-cGMP was removed by the addition of 500 μl of 1-in-1 Tris-HCl (40 mM) buffer suspension of Dowex resin (1 × 8 – 200) with incubation on ice for 30 min. Each tube was then centrifuged for 2 min at 6000 rpm, and 150 μl of the supernatant was removed for liquid scintillation counting. Less than 15 % of the tritiated cyclic nucleotide was hydrolyzed in this assay.

Statistical analysis

The antagonistic effects of *S*-petasin or *S*-isopetasin on these cumulative concentration-response curves were calculated and expressed as pA_2 or pD_2' values, according to the method described by Ariëns and van Rossum (9), when the antagonism was competitive or non-competitive, respectively. Accordingly, $pA_2 = pA_x + \log (x - 1)$, where pA_x is negative logarithm of the molar concentration of *S*-isopetasin and x is ratio between concentration of agonist in the presence of *S*-isopetasin and that in the absence of *S*-isopetasin; whereas $pD_2' = pD_x' + \log (x - 1)$, where pD_x' is negative logarithm of the molar concentration of *S*-petasin or *S*-isopetasin and x is ratio between maximal effect of agonist in the absence of *S*-petasin or *S*-isopetasin and that in the presence of *S*-petasin or *S*-isopetasin (10). The $-\log IC_{50}$ value was considered to be equal to the negative logarithm of the molar concentrations of *S*-petasin or *S*-isopetasin at which a half-inhibitory effect on Ca^{2+} (10 mM)-induced contraction was observed. The IC_{50} value was calculated by linear regression. All values are shown as means ± SEM. The differences among these values were statistically calculated by one-way analysis of variance (ANOVA), then determined by least significant difference (LSD). The difference between two values, however, was determined by use of Student's unpaired t-test. The differences were considered statistically significant if the P-value was less than 0.05.

Results

S-Petasin (20 – 200 μM) concentration-dependently, but *S*-isopetasin (100 – 200 μM) concentration-independently, inhibited concentration-response curves of cumulative histamine in a non-competitive manner (Figs. **2A**, **C**). The pD_2' values were

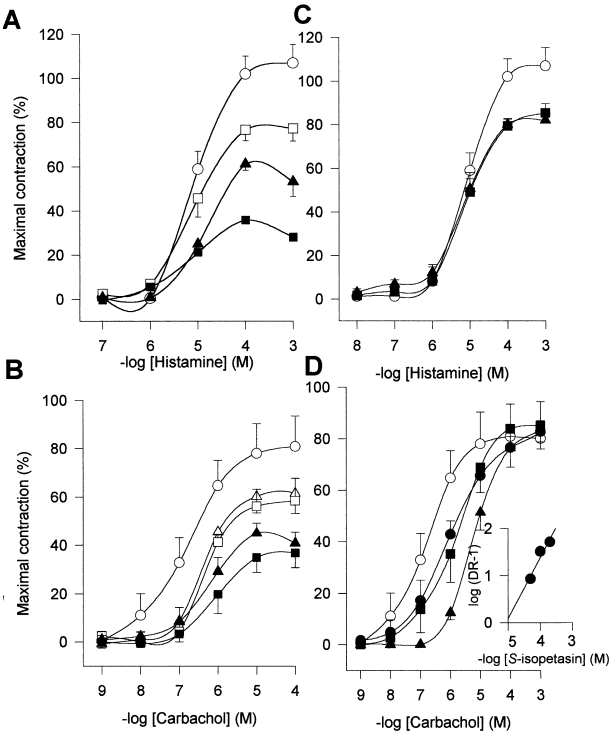

Fig. 2 The inhibitory effects of *S*-petasin (**A**, **B**) and *S*-isopetasin (**C**, **D**) (○, vehicle; △, 10 μM; □, 20 μM; ●, 50 μM; ▲, 100 μM; ■, 200 μM) on cumulative histamine (**A**, **C**)-, and carbachol (**B**, **D**)-induced contractions in guinea-pig trachealis in normal Krebs solution. Each point represents the mean ± SEM of 4 – 11 experiments. The relationship between –log concentration of *S*-isopetasin and log (DR-1), where DR is the dose ratio, is shown in the inset.

4.10 ± 0.08 (n = 18), and 3.15 ± 0.11 (n = 14), respectively which are significantly different from each other (Table **1**). *S*-Petasin (10 – 200 μM) concentration-dependently inhibited concentration-response curves of cumulative carbachol in a non-competitive manner (Fig. **2B**). However, *S*-isopetasin (50 – 200 μM) produced a parallel, rightward shift of the concentration-response curve of carbachol in a competitive manner

Table 1 pD_2', pA_2 and $-\log IC_{50}$ values of *S*-petasin and *S*-isopetasin in non-depolarized and depolarized guinea-pig trachealis

	Non-depolarized preparation				Depolarized preparation		
	Normal Ca^{2+} (2.5 mM)		Ca^{2+}-free (0.02 mM EGTA)		K^+ (60 mM)	His (100 μM)	CCh (10 μM)
	His	CCh	His	CCh	Ca^{2+}	Ca^{2+}	Ca^{2+}
S-petasin							
pD_2'	4.10 ± 0.08 (18)	3.95 ± 0.11 (20)$^{\#\#\#}$	4.20 ± 0.17 (12)$^{\#}$	4.74 ± 0.16 (14)			
$-\log IC_{50}$					4.50 ± 0.31 (6)	3.76 ± 0.32 (6)	4.05 ± 0.07 (5)$^{\#}$
S-isopetasin							
pA_2		5.36 ± 0.09 (25)***					
pD_2'	3.15 ± 0.11 (14)***		ND	ND			
$-\log IC_{50}$					4.82 ± 0.15 (6)	ND	ND
Atropine							
pA_2		8.92 ± 0.08 (7)$^{\$\$\$}$					
Aminophylline							
pD_2'	3.76 ± 0.10 (12)**	3.57 ± 0.12 (17)*					

Values are presented as means ± SEM (n); n is the number of experiments.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared with the corresponding pD_2' value of *S*-petasin.
$^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ when compared with the pD_2' value of *S*-petasin against CCh in Ca^{2+}-free Krebs solution with 0.02 mM EGTA.
$^{\$\$\$}P < 0.001$ when compared with the corresponding pA_2 value of *S*-isopetasin.
ND: not determined.
His: histamine.
CCh: carbachol.

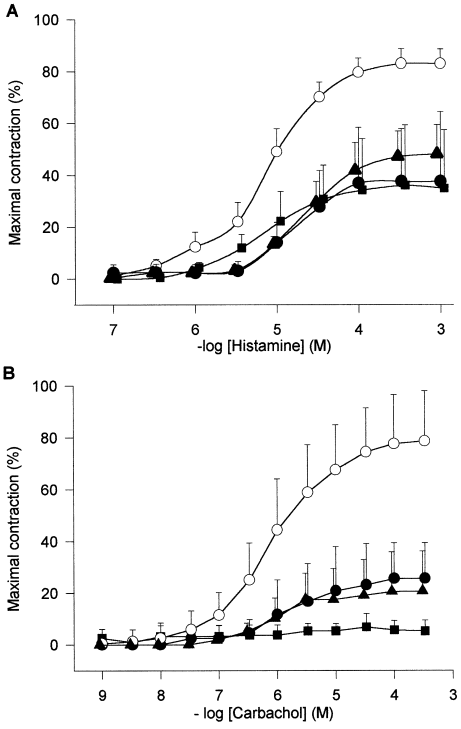

Fig. 3 The inhibitory effects of *S*-petasin (○, vehicle; ●, 50 μM; ▲, 100 μM, ■, 200 μM) on cumulative (**A**) histamine- and (**B**) carbachol-induced contractions in guinea pig trachealis in Ca^{2+}-free medium with 0.02 mM EGTA. Each point represents the mean ± SEM of 4 – 6 experiments.

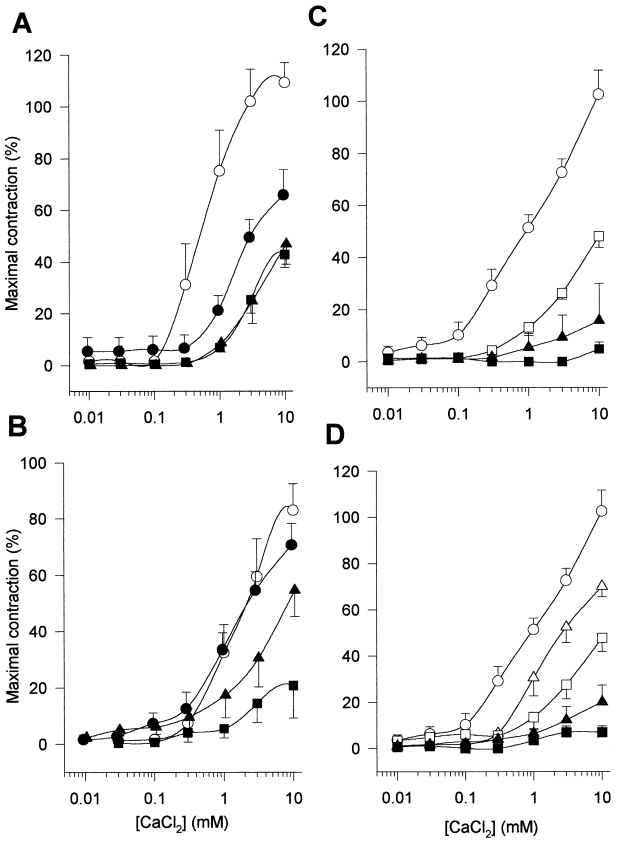

Fig. 4 The inhibitory effects of *S*-petasin (**A**, **B**, **C**, **D**) and *S*-isopetasin (**D**) (○, vehicle; △, 10 μM; □, 20 μM; ●, 50 μM; ▲, 100 μM; ■, 200 μM) on cumulative calcium-induced contractions in guinea pig trachealis depolarized by (**A**) histamine 100 μM, (**B**) carbachol 10 μM, and (**C**, **D**) KCl 60 mM in Ca^{2+}-free medium without EGTA. Each point represents the mean ± SEM of 4 – 15 experiments.

(Fig. **2D**). The pD_2' value of *S*-petasin was 3.95 ± 0.11 (n = 20), but the pA_2 value of *S*-isopetasin was 5.36 ± 0.09 (n = 25), respectively, which are significantly different from each other (Table **1**). The Schild regression equation for *S*-isopetasin is y = 6.57 + 1.30x (r = 0.9626). The slopes [1.299 ± 0.232 (n = 6)] of Schild plots were not significantly different from unity. The pA_2 value of atropine, a positive control, against carbachol was 8.92 ± 0.08 (n = 7), which was significantly greater than that of *S*-isopetasin (Table **1**).

In Ca^{2+}-free Krebs solution with 0.02 mM EGTA, *S*-petasin (50 – 200 μM) also inhibited concentration-response curves of cumulative histamine and carbachol in a non-competitive manner (Fig. **3**). The pD_2' values were 4.20 ± 0.17 (n = 12) and 4.74 ± 0.16 (n = 14), respectively, which significantly differ from each other (Table **1**). The pD_2' value against carbachol in Ca^{2+}-free Krebs solution was also significantly greater than that in normal Krebs solution (Table **1**).

In isotonic Ca^{2+}-free high K^+-, histamine- and carbachol-depolarized tracheas, *S*-petasin concentration-dependently inhibited concentration-response curves of cumulative Ca^{2+} (0.01 – 10 mM) in a non-competitive manner (Figs. **4A**, **B**, **C**). The –log IC_{50} values were 4.50 ± 0.31 (n = 6), 3.76 ± 0.32 (n = 6) and 4.05 ± 0.07 (n = 5), respectively, which are not significantly different from each other (Table **1**). The –log IC_{50} value of *S*-isopetasin against cumulative Ca^{2+}-induced contractions in isotonic Ca^{2+}-free high K^+-depolarized tracheas was 4.82 ± 0.15 (n = 6) (Fig. **4D**, Table **1**), which was not significantly different from the corresponding value of *S*-petasin.

Nifedipine (10 μM) only relaxed 24 ± 10% (n = 6) of carbachol (0.2 μM)-elicited submaximal precontraction [1.22 ± 0.16 g (n = 6)] in normal Krebs solution. Similarly, nifedipine (10 μM) relaxed 28 ± 9% (n = 6) of the precontraction [2.01 ± 0.16 g (n = 6)]. The nifedipine-remaining tension was further relaxed by *S*-petasin (100 μM) or *S*-isopetasin (100 μM) to 80 ± 9% (n = 6) or 72 ± 8% (n = 6), respectively. Finally, aminophylline (1 mM) completely relaxed the trachea (Fig. **5**).

However, none of the antagonists used, such as propranolol (1 μM), 2′,5′-dideoxyadenosine (10 μM), methylene blue (25 μM), glibenclamide (10 μM), L-NNA (20 μM), and α-chymotrypsin (1 U/ml), affected the log concentration-relaxing response curves of cumulative *S*-petasin or *S*-isopetasin to histamine (10 μM)-induced precontraction in normal Krebs solution (data not shown).

S-Petasin at 100 and 300 μM, but not *S*-isopetasin, significantly inhibited 33.9 ± 6.1% (n = 5) and 33.2 ± 4.4% (n = 6) of cAMP-, but not cGMP-dependent PDE activity, respectively. The comparative drug, IBMX (30 – 300 μM) as a positive control, however, inhibited both enzyme activities except IBMX (30 μM) on cGMP-PDE activity (Fig. **6**).

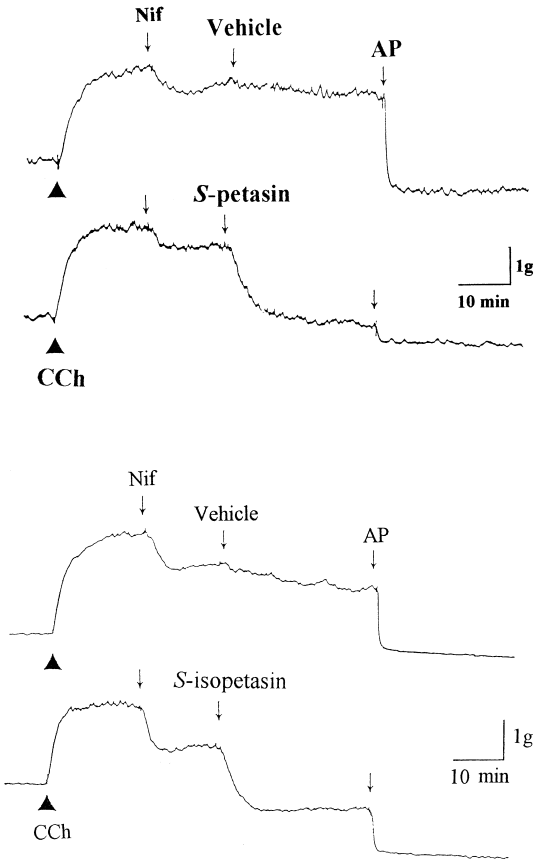

Fig. 5 The tracing graph of relaxant effects of *S*-petasin and *S*-isopetasin on carbachol (CCh, 0.2 μM)-induced precontraction in guinea-pig trachealis in normal Krebs solution. *S*-Petasin (100 μM) or *S*-isopetasin (100 μM), compared to their vehicle, further relaxed nifedipine (Nif, 10 μM)-remaining tension. At the end of the experiment, aminophylline (AP, 1 mM) was added to completely relax the trachealis.

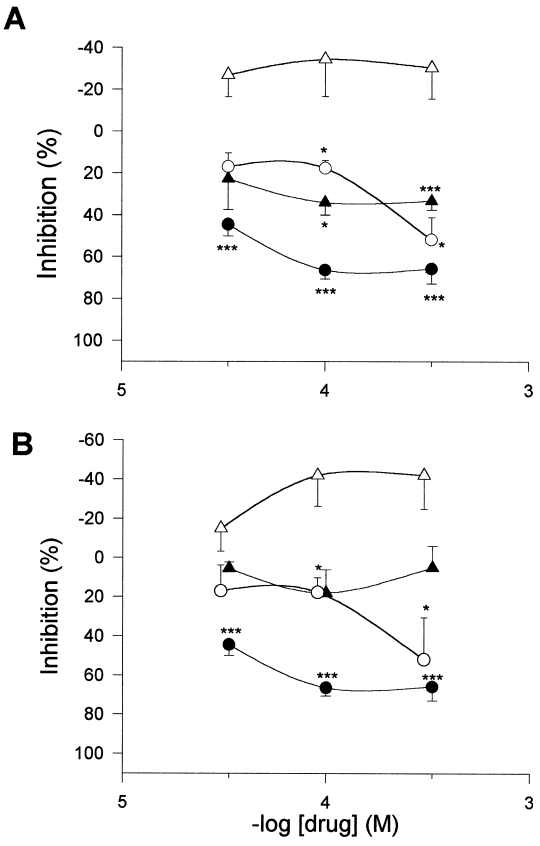

Fig. 6 The log concentration-inhibitory effects of *S*-petasin (**A**), *S*-isopetasin (**B**) (▲, △) and IBMX (●, ○) on cAMP (▲, ●)- and cGMP (△, ○)-dependent phosphodiesterase activities. The inhibitory effects do not include those of their vehicle. Each point represents the mean ± SEM of 4–7 experiments. $^{*}P < 0.05$, $^{***}P < 0.001$ when analyzing the difference between drugs and their vehicles by Student's unpaired t-test.

Discussion

The log concentration-relaxing response curves of cumulative *S*-petasin and *S*-isopetasin to histamine (10 μM)-induced precontraction was not affected by propranolol (1 μM), a non-selective β-adrenoceptor blocker (12), suggesting that the relaxant effect of both is not via the activation of β-adrenoceptor. 2′,5′-Dideoxyadenosine, an adenylate cyclase inhibitor (13), (14) and methylene blue, a soluble guanylate cyclase inhibitor (15), also did not affect the log concentration-response curves of *S*-petasin and *S*-isopetasin. This reveals that the relaxant effect of both is neither via the activation of adenylate cyclase nor via that of guanylate cyclase. Glibenclamide, an ATP-sensitive potassium channel blocker (16), also did not affect the log concentration-response curves of *S*-petasin and *S*-isopetasin, suggesting that the relaxant effect of both is not via the opening of ATP-sensitive potassium channels (17). L-NNA (20 μM), a nitric oxide (NO) synthase inhibitor (18), did not affect the log concentration-response curves of *S*-petasin and *S*-isopetasin, suggesting that the relaxant effect of both is unrelated to NO formation. α-Chymotrypsin (1 U/ml), a peptidase, also did not affect the log concentration-response curves of *S*-petasin and *S*-isopetasin, suggesting that the relaxant effect of both is unrelated to the neuropeptides.

S-Petasin (20–200 μM) and *S*-isopetasin (10–200 μM) concentration-dependently and non-competitively inhibited cumulative Ca^{2+}-induced contractions in the depolarized (K^+, 60 mM) trachealis. At the highest concentration, *S*-petasin and *S*-isopetasin almost blocked these contractions, therefore they may inhibit Ca^{2+} influx via voltage-dependent calcium channels (VDCCs) opened by 60 mM KCl. For example, nifedipine, a selective VDCCs blocker (19), at concentrations below 1 μM, also inhibits those contractions in a non-competitive manner. Nifedipine at 1 μM can further completely inhibit those contractions (11). In the present study, nifedipine (10 μM) only (24–28%) relaxed the carbachol-induced precontraction in normal Krebs solution. The nifedipine-remaining tension was further (72–80%) relaxed by *S*-petasin or *S*-isopetasin a 100 μM suggesting that no matter whether either blocked the VDCCs or not, either may have other relaxant action mechanisms.

S-Isopetasin (30–300 μM) did not significantly inhibit either cAMP- or cGMP-dependent PDE activity. Therefore, the tracheal relaxant action mechanisms of *S*-isopetasin may be due to its antimuscarinic and VDCCs blocking effects on the trachealis. The antimuscarinic effect of *S*-isopetasin is signifi-

cantly less than that of atropine in potency, but significantly greater than the non-specific antispasmodic effect of *S*-petasin against carbachol in potency (Table **1**).

S-Petasin concentration-dependently relaxed the histamine (10 μM)-, carbachol (0.2 μM)-, KCl (30 mM)-, and leukotriene D_4 (10 nM)-induced precontractions. Their –log IC_{50} values did not significantly differ from each other (7). This suggests that the relaxant effects of *S*-petasin are equally effective to any of these four contractile agents, and that *S*-petasin non-selectively and non-specifically inhibits calcium influx via VDCCs and/or receptor-operated calcium channels (ROCCs) induced by these four contractile agents. The non-specific antispasmodic effects of *S*-petasin, like some well known phosphodiesterase (PDE) inhibitors such as aminophylline and papaverine, may be due to its inhibitory effect on the activity of PDE. *S*-Petasin, in this present study, at 100 and 300 μM significantly inhibited 34% and 33% of cAMP-, but not cGMP-dependent PDE activity, respectively. Although the inhibitory effect on this enzyme was slight, the content of cAMP may increase. The increased cAMP subsequently activates cAMP-dependent protein kinase which may phosphorylate and inhibit myosin light-chain kinase, thus inhibiting contraction (20). The precise mechanism by which relaxation is produced by this second-messenger pathway is not known, but it may result from decreased intracellular Ca^{2+} ($[Ca^{2+}]_i$). The decrease of $[Ca^{2+}]_i$ may be due to reduced influx of Ca^{2+}, enhanced Ca^{2+} uptake into the sarcoplasmic reticula, or enhanced Ca^{2+} extrusion through the cell membrane (20). The decreasing effect of *S*-petasin on $[Ca^{2+}]_i$ was also preliminarily reported in rat thoracic aorta (21).

In conclusion, therefore, the mechanisms of tracheal relaxant action of *S*-petasin and *S*-isopetasin may be primarily due to its non-specific antispasmodic and antimuscarinic effects, respectively.

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Prof. Wun-Chang Ko

Graduate Institute of Medical Sciences Taipei Medical College
250 Wu-Hsing St.
Taipei 110
Taiwan, R.O.C.
E-mail: wc_ko@tmc.edu.tw
Fax: +886-2-2377-7639