Use-Dependent Block of Voltage-Gated \( \text{Ca}_{2.1} \) \( \text{Ca}^{2+} \) Channels by Petasins and Eudesmol Isomers

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Received January 20, 2009; accepted April 14, 2009

ABSTRACT

Migraine is a frequent and often disabling disease. Treatment is unsatisfactory in many patients. A disturbed dynamic balance between excitatory and inhibitory signal processing with enhanced cortical activity probably underlies common forms of migraine. Presynaptic voltage-gated \( \text{Ca}^{2+} \) channels are critical determinants of neurotransmitter release and also contribute to trigeminovascular signal transduction. Because clinical evidence exists for migraine-prophylactic actions of Petasites hybridus extracts, we investigated whether petasins comprising the main constituents of the extract inhibit currents evoked through presynaptic \( \text{Ca}_{2.1} \) channels expressed in Xenopus laevis oocytes. \( P. \) hybridus extract (0.02 mg/ml), petasin, neopetasin, isopetasin, S-petasin, and iso-S-petasin (50 \( \mu \)M) were weak tonic blockers of \( \text{Ca}_{2.1} \)-mediated barium currents \( (I_{\text{Ba}}) \) during infrequent depolarizations (0.1 Hz), but their inhibitory potency increased at higher stimulation rates (1 Hz), indicating preferential block of open and/or inactivated channels. Sulfur-containing compounds (S-petasin, Iso-S-petasin) were the most potent significantly promoting the accumulation of \( \text{Ca}_{2.1} \) channel in inactivated states during pulse trains \( (I_{\text{Ba}} \text{ decrease during 1-Hz pulse trains: control, 45\%; S-petasin, 79\%; iso-S-petasin, 80\%}) \). For the Eucalyptus williamsiania sesqui-terpenes \( \alpha- \) and \( \gamma \)-eudesmol, a comparable use-dependent inhibition was found in addition to a tonic block component. \( \alpha \)-Eudesmol and petasins accelerated the voltage-dependent inactivation of \( \text{Ca}_{2.1} \) channels during depolarizations. We demonstrate that S-petasin, iso-S-petasin, and eudesmol are \( \text{Ca}_{2.1} \) channel inhibitors preferentially acting as use-dependent channel blockers and with the sulfur-containing substituent in position 3 of the petasins serving as important functional feature. The \( \text{Ca}_{2.1} \)-inhibitory properties of these petasins may contribute to migraine-prophylactic properties described for \( P. \) hybridus extracts.

Depolarization-induced \( \text{Ca}^{2+} \) ion influx through voltage-gated \( \text{Ca}^{2+} \) channels (VGCCs) supports many physiological processes in electrically excitable tissues. This includes muscle contraction, cardiac pacemaking, neurotransmitter and hormone release, synaptic plasticity, and sensory cell function (Koschak and Striessnig, 2008). Five different types of VGCCs with different pharmacological properties, with different inactivation rates (Koschak and Striessnig, 2008). The pharmacological inhibition of non-LTCCs also appears as an attractive therapeutic concept. For example, the N-type (\( \text{Ca}_{2.2} \)) channel blocker ziconotide is licensed to treat neu-

ABBREVIATIONS: VGCC, voltage-gated \( \text{Ca}^{2+} \) channel; LTCC, L-type (\( \text{Ca}_{1.1} \)) \( \text{Ca}^{2+} \) channel; HPLC, high-performance liquid chromatography; \( I_{\text{Ba}} \), inward \( \text{Ba}^{2+} \) current; DMSO, dimethyl sulfoxide; \( \tau_{\text{fast}} \), \( \tau_{\text{slow}} \), time constants of fast and slowly inactivating current components; MS-222, tricaine (ethyl 3-aminobenzoate) methanesulfonate.
ropic pain. Unfortunately, therapy is limited by neurological side effects. These may be caused by its state-indepen-
dent (Feng et al., 2003) inhibitory effects blocking both slowly and rapidly firing neurons. Use-dependent N-type channel blockers are currently developed that are likely to exhibit a more favorable side-effect profile by preferential inhibition of rapidly firing neurons in the pain pathway.

Migraine headache represents a frequent pain disorder in which primary central nervous system dysfunction leads to trigeminovascular activation, central sensitization, and enhanced pain sensation (Pietrobon and Striessnig, 2003; Bur-stein et al., 2004; Goodasy, 2007). Although the neurobiology and genetics of common migraine are not fully understood, rare inherited forms of migraine indicate that changes in signal transduction that increase synaptic glutamatergic activity can favor cortical spreading depression known to underlie migraine aura (Pietrobon, 2003; Goodasy, 2007). Although other mechanisms leading to disturbances in the fine-tuning of the dynamic balance between excitatory and inhibitory signal processing probably underlie common forms of migraine with and without aura (Pietrobon and Striessnig, 2003; Coppola et al., 2007; Brighina et al., 2009), experimental evidence exists suggesting that presynaptic P/Q- (Ca_{2.1}) and N-type Ca^{2+} channels contribute to nociceptive synaptic transmission of trigeminovascular neurons (Akerman et al., 2003; Shields et al., 2005; Xiao et al., 2008). Moreover, excitatory and inhibitory neurotransmitter release in the brain critically depends on the activity of these presynaptic Ca^{2+} channels. Therefore, it is possible that P/Q- and/or N-type channel inhibitors reduce nociceptive signaling of the trigeminovascular neurons and perhaps also counteract enhanced cortical activity observed in patients with common forms of migraine, thus exerting antimigraine effects.

Because of the frequent lack of efficacy and adverse events of existing migraine medications, alternative treatments, including the identification of natural compounds, are sought. Although data about their efficacy and safety from long-term clinical studies are limited, some evidence points to short-term migraine-preventive effects of herbal preparations from clinical studies are limited, some evidence points to short-term migraine-preventive effects of herbal preparations from

Materials and Methods

Materials. HPLC-grade solvent (acetonitrile) was purchased from Acros Organics (Fairlawn, NJ) and Merck (Darmstadt, Germany). HPLC-grade water was obtained from a Nanopure system (Dubuque, IA), and organic solvents were distilled according to standard procedures. Salts for the oocyte culture media, recording solution, and microelectrode filling solution were purchased from Sigma-Aldrich (St. Louis, MO).

Plant Material. P. hybridus compounds were isolated from a fluid CO_{2} extract of P. hybridus foliage free of pyrrolizidine alkaloids and rich in petasins and α-eudesmol obtained from a standardized, commercially available source. α-, β-, and γ-Eudesmol were isolated from Eucalyptus williamssonia essential oil (eudesmols accounting for approximately 70% of the oil), a kind gift from Dr. J. Brophy (University of New South Wales, Sydney, NSW, Australia). Pure compounds were isolated as described previously (Debrunner and Neuenschwander, 1994). Purity of all isolated compounds was determined by HPLC or gas chromatography and was >90% for isopeta-
sins, >95% for petasins and S-petasins, and >98% for β- and γ-eu-
desmol. Purity of α-eudesmol was 86% and contained 10% γ-eudesmol.

Expression of Ca_{2.1} VGCCs in Xenopus laevis Oocytes. Capped run-off poly(A)^+ cRNA transcripts from XbaI-, HindIII-, and NotI-linearized cDNA templates, respectively, were synthesized according to Krieg and Melton (1984). Oocytes were harvested from an MF 1 x male field. Oocytes were injected with 200 ng of various cRNA fragments into X. laevis (5–10 ng) together with β_{3} (1–3 ng) and α_{3}β_{3} (3–6 ng) subunits as described previously (Kraus et al., 1998; Wappl et al., 2002). Oocytes were incubated at 18°C in ND96 solution (1.8 mM CaCl_{2}, 1 mM MgCl_{2}, 2 mM KCl, 96 mM NaCl, 5 mM HEPES, pH 7.5, with NaOH) before recording.

Electrophysiological Recordings. Two-electrode voltage-clamp experiments were performed as described previously (Kraus et al., 1998; Wappl et al., 2002). One to 2 days after cRNA injection, a Ba^{2+} inward current through Ca_{2.1} channel complexes (I_{Ba}) was recorded at room temperature using a Turbo Tec OIC amplifier (NPI Electronic GmbH, Tamm, Germany) and Digitida 1322 digitizer (Molecular Devices, Sunnyvale, CA). Data acquisition and analysis were performed with the pClamp software package version 9.2 (Axon Instruments). Contribution of endogenous I_{Ba} was quantified using β_{3}/α_{3}β_{3}-injected oocytes. Ca_{2.1}α_{1}/β_{3}/α_{3}β_{3}-injected oocytes were only used for analysis if peak I_{Ba} was at least 10 times larger than endogenous currents and did not exceed 1.9 μA. Recording electrodes had resistances between 0.5 and 2 MΩ and were filled with solution containing 2.8 M CaCl_{2}, 0.2 M CaOH, 10 mM HEPES, and 10 mM EGTA (pH adjusted to 7.3 with HCl). The recording solution contained 10 mM BaOH_{2}, 95 mM NaOH, 2 mM CaOH, and 5 mM HEPES, pH 7.4 (adjusted with methanesulfonic acid; 229 mM). Stock solutions of the purified compounds were prepared in dimethylsulfoxide (DMSO) and diluted to the indicated final concentrations in recording solution. Two milligrams of plant extract was dissolved in 0.1 ml of DMSO and diluted 100-fold in recording solution. The highest concentration of DMSO employed in our assay [1% (v/v)] did not affect channel currents.

The voltage dependence of activation was determined by 150-ms step depolarization from a holding potential of −80 mV to various test potentials (10–40 mV increments, every 15 s). I-V curves were fitted to equation: I = G_{max} (V - V_{rev}/1 + exp [(V - V_{0.5,act}/k_{act})] + C, where V_{rev} is the extrapolated reversal potential of I_{max}, V is the membrane potential, I is the peak current, G_{max} is the maximal conductance, V_{0.5,act} is the voltage for half-maximal activation, k_{act} is the slope factor of the Boltzmann equation, and C is an offset factor. Voltage-dependent activation was calculated during 3–4 pulses from a holding potential of −80 mV to the voltage of maximal current (V_{max}). Traces were best fitted to a biexponential decay yield-
ing time constants for a fast \( \tau_{\text{fast}} \) and slowly \( \tau_{\text{slow}} \) inactivating component. Drug effects were measured either during 0.1-Hz depolarizations of 100-ms pulses applied from a holding potential of \(-80\) mV to \( V_{\text{max}} \) (preferential block of resting channels) or during 1-Hz pulse trains of 15 consecutive 100-ms pulses to \( V_{\text{max}} \) from a holding potential of \(-60\) mV (inducing additional use-dependent block; Kraus et al., 1998). \( I_{\text{Ba}} \) was stable during 0.1-Hz depolarizations. Drug effects were quantified from \( I_{\text{Ba}} \) amplitudes at the end of 100-ms test pulses, which also take into account drug effects on \( I_{\text{Ba}} \) inactivation during depolarization. In some experiments, 1-Hz pulse trains were applied in the absence of drug after stabilization of \( I_{\text{Ba}} \) during preceding 0.1-Hz depolarizations. Cells were then again stimulated at 0.1 Hz to allow \( I_{\text{Ba}} \) recovery before the drug was added; after inhibition at 0.1 Hz was complete, another 1-Hz pulse train was applied.

**Statistics.** All data are presented as means ± S.E. Statistical calculations (statistical tests are indicated in the figure legends or in the text) were performed in Prism 4.03 (GraphPad Software Inc., San Diego, CA). Statistical significance was set at \( p < 0.05 \).

**Results**

The inhibition of a P/Q-type current component by 15 and 45 μM α-eudesmol has been reported previously in rat cerebellar Purkinje cells. However, α-eudesmol also inhibits α-agatoxin IVA-insensitive N- and L-type current components in neuronal NG108-15 cells (Asakura et al., 1999). To demonstrate unequivocally the modulation of Ca\(_{\text{2.1}}\) currents by α-eudesmol and by different petasins isolated from a plant extract with antimigraine efficacy (Fig. 1), we heterologously expressed Ca\(_{\text{2.1}}\) channel complexes in \( X. \text{laevis} \) oocytes and quantified drug effects using the two-electrode voltage-clamp technique (Kraus et al., 1998). As illustrated in Fig. 2, 50 μM α-eudesmol caused an approximately 50% inhibition of \( I_{\text{Ba}} \) when channels were stimulated infrequently (0.1 Hz) from \(-80\) mV to \( V_{\text{max}} \). Inhibition by α-eudesmol occurred over the whole voltage range without shifting the current-voltage relationship (Fig. 2A, inset) and was approximately complete after 20 sweeps (Fig. 2B). This inhibition must reflect mostly tonic inhibition of resting Ca\(_{\text{2.1}}\) channels, although some drug-induced acceleration of current inactivation (Fig. 2B, inset) also indicates interaction with open or inactivated channel states (see below). γ-Eudesmol was of similar potency, whereas β-eudesmol caused significantly less inhibition (\( p < 0.05 \); see legend to Fig. 2A). \( P. \text{hybridus} \) extract (0.02 mg/ml), petasin, neopetasin, isopetasin, S-petasin, and iso-S-petasin (all 50 μM) caused significantly smaller tonic block than α-eudesmol (for statistics, see legend to Fig. 2A). Like for α-eudesmol, all compounds reduced \( I_{\text{Ba}} \) at all voltages examined without significant changes of \( V_{\text{max}} \) values compared with control (data not shown). Tonic inhibition of Ca\(_{\text{2.1}}\) currents by α-eudesmol was concentration-dependent and significant at concentrations \( > 10 \mu M \) (for statistics, see Fig. 2C). These data demonstrate that α-eudesmol causes a tonic inhibition of Ca\(_{\text{2.1}}\) channels in a stereoselective manner and that \( P. \text{hybridus} \) sesquiterpenes are less potent inhibitors of resting Ca\(_{\text{2.1}}\) channels.

Next, we tested whether these natural compounds exert an additional use-dependent inhibitory component unmasked during more frequent stimulations from a slightly more depolarized holding potential (−60 mV) as described previously (Kraus et al., 1998). This protocol favors the availability of open and/or inactivated channels. A typical experimental time course is illustrated in Fig. 3A for α-eudesmol. After stable \( I_{\text{Ba}} \) was obtained (0.1-Hz depolarizations), drug was applied during the 0.1-Hz protocol yielding the expected tonic inhibition. After 20 sweeps, a 1-Hz pulse train was applied, causing further fast and strong reduction in \( I_{\text{Ba}} \). Note that in the absence of drug, the same protocol caused only an approximately 50% decrease of the \( I_{\text{Ba}} \) remaining at the end of the 0.1-Hz protocol, as evident from the representative current traces in Fig. 3B (left) and the statistical data illustrated in Fig. 3, C and D. To quantify the additional drug-induced
use-dependent inhibitory effect, current amplitudes during the 1-Hz train were normalized to the Ib decrease by approximately 50% during the 1-Hz train. Instead, 50 µM α-, β-, and γ-eudesmol induced 70 to 80% inhibition of Ib (Fig. 3C) in addition to their tonic block (as quantified in Fig. 2). The sulfur-containing compounds S-petasin and iso-S-petasin, which were only weak tonic blockers (Fig. 2A), were strong use-dependent inhibitors, similar to the eudesmols and significantly more potent than petasin (Fig. 3D). In contrast, petasin, P. hybridus extract, neopetasin, and isopetasin were significantly (p < 0.001) less potent use-dependent blockers than eudesmol (for statistics, see legend to Fig. 3, C and D).

The concentration dependence of the use-dependent component for α-eudesmol and petasin (which was commonly used to standardize P. hybridus extracts) is illustrated in Fig. 3E. With no α-eudesmol added, Ib declined to 55.5 ± 1% during the pulse train (Fig. 3E). In the presence of 3 µM α-eudesmol, which caused no tonic inhibition (Fig. 2C), significant channel inhibition was induced (for statistics, see legend to Fig. 3E). α-Eudesmol (10 µM), which caused 15% tonic block, inhibited approximately half of the current that remained after tonic block (remaining Ib, 10 µM: 31.8 ± 2%). Use-dependent inhibition by 50 µM α-eudesmol (Fig. 3E) was also larger than tonic block (50% inhibition, Fig. 2A and C), clearly indicating that drug action occurred in a state-dependent manner. Considering tonic and use-dependent block together (as illustrated in Fig. 3A), it can be calculated that mean Ib decreased to 27% (10 µM) and 8% (50 µM) in the presence of α-eudesmol compared with 55% in the absence of drug. The same calculation revealed an overall Ib decrease by iso-S-petasin to 19.3% (tonic, 3.5 ± 2.7%
Inhibition of Ca_{2.1} VGCCs by eudesmols and petasins during 1-Hz pulse trains. A, representative experiment for Ca_{2.1} current inhibition by α-eudesmol (50 μM). Tonic inhibition of I_{Ba} during 0.1-Hz stimulation (31% remaining current); additional I_{Ba} decrease was induced during 1-Hz pulse trains. Cell: 11050_6_0082/83. B, use-dependent Ca_{2.1} current inhibition during 1-Hz pulse trains. Representative traces before (left) and after (right) application of 50 μM S-petasin. The compound induced only weak inhibition during the 0.1-Hz protocol (see Fig. 2A) but strong inhibition of I_{Ba} during pulse trains in comparison with control. Cells: 12050_6_0050/52; 19010_7_0002/5). C, I_{Ba} decay during the 1-Hz pulse train was normalized to the I_{Ba} amplitude at the end of the 0.1-Hz protocol. Relative I_{Ba} remaining at the end of the pulse train was as follows: control, 0.49 ± 0.01 (n = 29); α-eudesmol, 0.17 ± 0.02 (n = 17; p < 0.001); β-eudesmol, 0.16 ± 0.02 (n = 10; p < 0.001); and γ-eudesmol, 0.22 ± 0.07 (n = 5, p < 0.001). The p values indicate significant differences of remaining I_{Ba} in the presence versus in the absence of drug (control) at the end of the 1-Hz train (two-way ANOVA, Bonferroni post-test). Eudesmol isomers inhibited with indistinguishable potencies (p > 0.05). Data are means ± S.E. For some data points, error bars are smaller than symbol size. D, same as in C. Control, 0.55 ± 0.01 (n = 51); Petasites extract, 0.33 ± 0.02 (n = 12, p < 0.001); petasin, 0.36 ± 0.02 (n = 18, p < 0.001); isopetasin, 0.37 ± 0.04 (n = 7, p < 0.001); neopetasin, 0.44 ± 0.03 (n = 12, p < 0.001); S-petasin, 0.21 ± 0.02 (n = 6, p < 0.001); iso-S-petasin, 0.20 ± 0.01 (n = 4, p < 0.001). Iso-S-petasin (p < 0.05) and S-petasin (p < 0.01) were significantly more potent than petasin. Data are means ± S.E. E. Concentration-dependent inhibition by α-eudesmol and petasin during 1-Hz pulse trains. Normalized I_{Ba} at the end of the 1-Hz pulse train obtained in the absence (control) or presence of the indicated concentrations of α-eudesmol and petasin. Statistically significant difference to control (0 μM): ***, p < 0.001; statistically significant difference between concentrations, +++, p < 0.001 (one-way ANOVA and Bonferroni post-test). Means ± S.E. are shown for the indicated number of experiments.

Inhibition, followed by 79.6 ± 1.9% use-dependent inhibition and to 35% by the less potent petasin (50 μM).

Figure 4 illustrates that both drugs (50 μM) also significantly accelerated I_{Ba} inactivation during prolonged (3-s) depolarizations to V_{max}. α-Eudesmol significantly decreased the time constants for the slow (τ_{slow}, control, 2015 ± 237 ms, n = 11; +α-eudesmol, 730 ± 74 ms, n = 8, p = 0.0003; unpaired Student’s t-test) and fast (τ_{fast}, control, 221 ± 9 ms, n = 11; +α-eudesmol, 103 ± 15 ms, n = 8, p = 0.0001) components of the biexponential time course but did not change their relative contribution to the inactivation process (% τ_{slow}, control, 13.1 ± 0.79%, n = 11; +α-eudesmol, 12.2 ± 1.31%, n = 8, p = 0.56; % τ_{fast}, control, 82.2 ± 1.59%, n = 11; +α-eudesmol, 84.9 ± 1.67%, n = 8, p = 0.27). A smaller but consistent acceleration of both inactivation time constants was also observed for petasin (Fig. 4B; τ_{slow}, 1133 ± 84 ms, p < 0.05; τ_{fast}, 157 ± 20 ms, p = 0.005; n = 4). These effects are also in agree-
ment with interaction of both natural compounds with open and/or inactivated channel states.

Discussion

Here, we demonstrate that the sesquiterpene α-eudesmol and its β- and γ-isomers block recombinant Ca_{2.1} channels at micromolar concentrations. Inhibition was observed during infrequent stimulation, which mostly reflects drug action on resting channels. However, an additional use-dependent component was revealed when 1-Hz pulse trains were elicited from a slightly more depolarized membrane potential that both favors availability of open and/or inactivated channels. We also found that petasins, the main constituents of the antimigraine herb *P. hybridus*, block Ca_{2.1} channels. They were only weak blockers of Ca_{2.1} channels at low stimulation rates. However, their inhibitory effects were unmasked during the 1-Hz protocol. We identified the sulfur-containing derivatives, S-petasin and iso-S-petasin, as the most active compounds. Although tonic block was almost absent, they were potent use-dependent inhibitors, comparable with α-eudesmol when Ca_{2.1} channels were depolarized frequently. The more pronounced decrease of I_{Ba} during 1-Hz pulse trains can be explained by enhanced inactivation during pulses or by slowing of recovery from inactivation between pulses or both (Kraus et al., 1998). We have observed an acceleration of inactivation during depolarizing pulses, suggesting that this contributed to inhibition by α-eudesmol and petasins during frequent stimulation.

S-Petasin (Wang et al., 2001) and iso-S-petasin (Wang et al., 2002) were found recently to inhibit smooth and cardiac muscle contraction by inhibiting L-type VGCCs (presumably of the Ca_{1.2} isoform, which is predominant in these tissues; Sinnegger-Brauns et al., 2004) within a concentration range similar to the Ca_{2.1} inhibition reported here. This indicates that these petasins are not isoform-selective blockers of VGCCs. This broader pharmacological profile of S-petasin and iso-S-petasin resembles the calcium channel blocker flunarizine, a piperazine derivative inhibiting ω-agatoxin IVA-sensitive P/Q-type channels (Geer et al., 1993) and N-type (Tytgat et al., 1991), L-type, and T-type VGCCs (Tytgat et al., 1996). Flunarizine is widely used as effective treatment for the prophylaxis of migraine attacks (Goadsby et al., 2002). Although the precise mechanism of its antimigraine activity is unknown, it is likely that its ability to inhibit several ion channels controlling neuronal excitability contributes to its antimigraine effects. It is thought that P/Q-, N-, and L-type VGCCs mediate calcitonin gene-related peptide release from trigeminal nerve fibers (and subsequent blood vessel dilation as observed during migraine attacks; Akerman et al., 2003) and nociceptive transmission to central neurons (in the trigemino-vascular complex; Shields et al., 2005). Therefore, the combined block of both P/Q- and L-type channels by sulfur-containing petasins could represent an advantage for inhibition of processes involved in triggering and/or sustaining migraine attacks. If preferential activity of S-petasins on channels opening frequently and from slightly depolarized resting potentials would be beneficial for therapy cannot be answered because the pharmacological effects of systemically applied nonpeptide P/Q-type channel blockers have not yet been reported. However, like for N-type channel block in neuropathic pain (see Introduction), use-dependent properties of P/Q-type channel blockers may facilitate the inhibition of neurotransmitter release in rapidly firing trigeminal neurons.

Our findings prompt further electrophysiological studies investigating the modulatory capacity of these compounds on other ion channels implicated in migraine pathophysiology, such as voltage-gated Na⁺ channels (Dichgans et al., 2005). This may encourage future preclinical studies to evaluate the pharmacokinetic and therapeutic actions of sulfur-containing petasins in migraine.

In our *P. hybridus* preparation, petasin, isopetasin, and neopetasin comprise approximately 35% of the extract, and only approximately 6% are sulfur-containing petasins. Therefore, the calculated approximate concentration of petasins in the diluted extract (20 µg/ml) is approximately 30 µM. The extent of tonic and use-dependent Ca_{2.1} inhibition by the extract was similar to the more abundant petasins. Therefore, the pharmacological effect of the extract can be explained by the combined effect of petasins and sulfur-containing petasins. It appears unlikely that other potent Ca_{2.1} channel inhibitors of reasonable abundance are present in the extract.

In contrast to previous studies on L-type VGCCs, we compared different isoforms of the sesquiterpenes eudesmol and petasin. Neither the structural differences between the eudesmol isomers nor between petasin and isopetasin (Fig. 1) caused major differences in their channel-inhibitory properties. However, replacement of the angeloyl group of petasin and isopetasin with a 3-methylthiopropanoyl moiety significantly increased the potency as use-dependent blockers of Ca_{2.1} VGCCs. This suggests that a sulfur-containing substituent in position 3 of the petasins (Fig. 1) is an important feature for binding of these compounds to the open and/or inactivated state of the Ca_{2.1} channel. Based on these observations, a more systematic analysis of the structure activity relationship of substituents in position 3 is justified to investigate whether compounds with higher potency blockers can be obtained or perhaps derivatives that could serve as lead compounds for the development of isoform-selective inhibitors of VGCCs.
Acknowledgments
We thank Elisabeth Kaltenegger and Sonja Sturm for providing purified eudesmols and petasins, Stefan Schweiger for helping with HPLC methods, Katrin Watschinger for discussions, and Gilda Pelster and Jasmin Aldrian for expert technical assistance.

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