

Yu-Cheng Lu · Hwei-Hsien Chen · Chien-Hsin Ko
Yi-Ruu Lin · Ming-Huan Chan

The mechanism of honokiol-induced and magnolol-induced inhibition on muscle contraction and Ca^{2+} mobilization in rat uterus

Received: 26 March 2003 / Accepted: 19 August 2003 / Published online: 18 September 2003

© Springer-Verlag 2003

Abstract The effects of honokiol and magnolol extracted from the *Magnolia officinalis* on muscular contractile responses and intracellular Ca^{2+} mobilization were investigated in the non-pregnant rat uterus. Honokiol and magnolol (1–100 $\mu\text{mol/l}$) were observed to inhibit spontaneous and uterotonic agonists (carbachol, $\text{PGF}_{2\alpha}$, and oxytocin)-, high K^+ -, and Ca^{2+} channel activator (Bay K 8644)-induced uterine contractions in a concentration-dependent manner. The inhibition rate of honokiol on spontaneous contractions appeared to be slower than that of magnolol-induced response. The time periods that were required for honokiol and magnolol, at 100 $\mu\text{mol/l}$, to abolish 50% spontaneous contractions were approximately 6 min. Furthermore, honokiol and magnolol at 10 $\mu\text{mol/l}$ also blocked the Ca^{2+} -dependent oscillatory contractions. Consistently, the increases in intracellular Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) induced by $\text{PGF}_{2\alpha}$ and high K^+ were suppressed by both honokiol and magnolol at 10 $\mu\text{mol/l}$. After washout of these treatments, the rise in $[\text{Ca}^{2+}]_i$ induced by $\text{PGF}_{2\alpha}$ and high K^+ was still partially abolished. In conclusion, the inhibitory effects of honokiol and magnolol on uterine contraction may be mediated by blockade of external Ca^{2+} influx, leading to a decrease in $[\text{Ca}^{2+}]_i$. Honokiol and magnolol may be considered as putative Ca^{2+} channel blockers and be of potential value in the treatment of gynecological dysfunctions associated with uterine muscular spasm and dysmenorrhea.

Keywords Honokiol · Magnolol · Ca^{2+} · Smooth muscle · Rat uterus

Introduction

The crude drugs containing magnolia bark (*Magnolia officinalis* or *Magnolia obovata*) are usually prescribed as therapeutic herbal agents for acute pain, headache, diarrhea, allergy, asthma and gynecological disorders in traditional oriental medicine (Chen 1986; Haraguchi et al. 1997). Honokiol and magnolol, the isomers of phenolic compounds, are the major bioactive components purified from the medicinal plant magnolia bark (Fujita et al. 1973). Several previous studies have described the pharmacological activities of honokiol and magnolol, including an anxiolytic effect, antimicrobial activity, anti-platelet aggregation, anti-inflammation, and potential antioxidation (Teng et al. 1988; Wang et al. 1992; Chan et al. 1996; Kuribara et al. 2000). Recent studies from our laboratory demonstrated that honokiol and magnolol could attenuate smooth muscle contractile responses in swine trachea (Ko et al. 2003). Consistently, magnolol was reported to inhibit the vascular contractility induced by norepinephrine and high K^+ (Teng et al. 1990). Numerous mechanisms, including stimulation of relaxing factor release, blockade of receptor-operated activation, and alteration in intracellular Ca^{2+} mobilization might be involved in their inhibitory effects on smooth muscle contractions.

Although the bark of *Magnolia officinalis* has been used for more than 1,000 years as a folk medicine in Asia, its action on uterine complications and underlying mechanisms are still unclear. The purpose of the present study was to investigate the pharmacological effects of honokiol and magnolol on rat uterus oscillatory contractions. The underlying mechanisms of honokiol and magnolol on spontaneous and agonist-induced uterine contractions and intracellular Ca^{2+} mobilization were also studied.

Y.-C. Lu
Department of Pharmacy,
Buddhist Dalin Tzu Chi General Hospital, Chia Yi, Taiwan

Y.-C. Lu · H.-H. Chen · M.-H. Chan (✉)
Institute of Pharmacology and Toxicology, Tzu Chi University,
No. 701, Section 3, Chung Yang Road, 970 Hualien, Taiwan
Tel.: +886-3-8565301 ext. 7197, Fax: +886-3-8561465,
e-mail: ming@mail.tcu.edu.tw

Y.-R. Lin · M.-H. Chan
Institute of Medical Sciences, Tzu Chi University,
Hualien, Taiwan

C.-H. Ko
Department of Chinese Medicine,
Buddhist Tzu Chi General Hospital, Hualien, Taiwan

Methods

Uterine preparations

Female virgin Sprague–Dawley rats, weighing 220–280 g, from the animal facilities at Tzu Chi University were used throughout this study. The experimental protocol was approved by the Committee of Tzu Chi University for the Use of Animal Subjects. Rats were given standard laboratory chow and tap water and housed in group cages under automatically controlled temperature and light cycle conditions in the animal facilities at Tzu Chi University. The rat at estrus stage, confirmed by microscope examination of a vaginal smear, was killed by decapitation, and both uterine horns were surgically removed, rinsed in normal saline, and placed in a Petri dish containing *N*-(2-hydroxyethyl)piperazine-*N*-(2-ethanesulphonic acid) (HEPES) buffer solution. After removal of adherent fat and mesenteric attachments, each uterine horn was divided by a transverse cut into equal-length segments (~15 mm), which were used for the measurement of muscle tension under isometric conditions.

Intracellular calcium imaging studies were performed on the longitudinal fiber layer of the myometrium. The uterine segment was opened longitudinally along the muscle fiber bundles, on a dissection tray. After the connective tissue had been carefully removed, the longitudinal myometrium strips were prepared and incubated in HEPES buffer solution containing 0.2% protease (type XIV, Sigma) under rotation (80 rpm) at 37°C for 20 min. The solution was then replaced with HEPES buffer solution containing 0.2% collagenase and 0.2% trypsin inhibitor under rotation (80 rpm) and incubated for 60 min at 37°C. The supernatant was strained through a 70-nm nylon mesh, and the cells were purified by centrifugation. The isolated myometrial cells were seeded on round glass cover slips (20 mm) and incubated in Dulbecco's modified Eagle's medium (DMEM) and Ham's nutrient mixture F12 (F12) medium (1:1, vol/vol) containing 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C. After ~24-h incubation, the dissociated myometrial cells were put onto glass cover slips, and the intracellular calcium mobilization was measured.

Measurements of uterine oscillatory contraction

The muscle segments from uterine horns were obtained as described above. Each segment was mounted vertically in a 10-ml water-jacketed organ bath that had been filled with Krebs–bicarbonate buffer and gassed with a mixture of 95% O₂ and 5% CO₂ at 37°C. The bottom end of the segment was fixed to an L-shaped hook, and the top end was tied to a stainless-steel wire attached to a force displacement transducer (Grass FT.03) for monitoring changes in isometric tension, which was measured with an isometric force transducer interfaced to the computer via an A/D converter. Each segment was subjected to a load of 1 g and was allowed to equilibrate for 60 min before the agents were added to the organ baths. This was determined to be optimal for force generation and spontaneous oscillatory contractions. The mean isometric tension values were obtained by the measurement of the mean amplitude of all the oscillatory contractions recorded over a 10-min period.

Calcium imaging

The fluorescent probe fura 2-AM (Fluka Chemical Corporation, Milwaukee, Wis., USA) was used for the monitoring of intracellular calcium concentrations. Uterine myometrium seeded on round glass cover slips (20 mm) were initially washed with buffer solution and then incubated with 10 µmol/l fura 2-AM at 37°C for 60 min. After the free fura 2-AM had been washed out, the cover slip was mounted in a homemade perfusion chamber and then on the stage of an inverted epifluorescence microscope (Olympus IX70, Japan) equipped with a 40× fluorescence oil objective (Dapo 40, Olympus, Japan). Cells were visualized with a Quantix EEV57 intensified charged-coupled device (CCD) video-camera and image intensifier

system (Photometrics, Munich, Germany). Data were collected and stored with the image-acquisition program Imaging Workbench 4 (Axon Instruments, USA). The data were also analyzed with Imaging Workbench 4 software, which allowed the regions of interest (ROIs) drawn around each cell to be moved between frames. The size and shape of the ROIs were not changed at any point for any given cell.

Once mounted in the microscope, myometrium cells were incubated with buffer solution for 20–30 min to allow for complete hydrolysis of intracellular fura 2-AM to fura 2. During the subsequent calcium imaging experiments, those cells were continuously superfused with buffer solution at room temperature (20–25°C). The fura 2 cytosolic calcium measurements were performed with the video-based digital calcium imaging system, set up for dual excitation at 340 and 380 nm and emission at 510 nm. The results of the cytosolic calcium analyses were presented as the relative fluorescent intensities of the 340 and 380 nm images and as the 340:380 ratios (F340/F380).

Drugs and solutions

All dilutions of drugs were prepared on the day of the experiments. Stock solution of magnolol and honokiol (Nacalai Tesque, Japan) was dissolved in dimethylsulfoxide (DMSO), and the final concentration of DMSO in buffer solution was less than 0.1%, which had no effect on spontaneous contractions and the oscillations evoked by stimuli. DMSO was purchased from J.T. Baker (Phillipsburg, N.J., USA), whereas other reagents were obtained from the Sigma Chemical Company (St. Louis, Mo., USA).

The HEPES buffer solution consisted of the following (in millimoles per liter): NaCl 140; KCl 5; CaCl₂ 2; glucose 5.5; HEPES 10, and the pH was adjusted to 7.4. The Krebs–bicarbonate buffer was composed of (in millimoles per liter): NaCl 113; KCl 4.8; CaCl₂ 2.5; NaHCO₃ 18; KH₂PO₄ 1.2; MgSO₄ 1.2; glucose 5.5; mannitol 30, and the pH was adjusted to 7.4.

Statistical analysis

The amplitude of spontaneous contractions and contractions elicited by stimulants were analyzed. We averaged 10-min consecutive contractions after initiating the recording as values for the baseline and peak tension. Unless indicated otherwise, the data are given as percentage contraction induced by applied agents compared with mean amplitude of spontaneous contractions, taken as 100%. All data are expressed as means ± SEM. Statistical significance of difference between groups was determined by one-way ANOVA followed by a Student–Newman–Keuls post-hoc test. A *P* value less than 0.05 was considered to be statistically significant.

Results

Effects of honokiol and magnolol on spontaneous uterine contractility

It was observed that honokiol and magnolol (1–100 µmol/l) concentration dependently decreased the spontaneous uterine contractions in amplitude (Fig. 1). Fig. 1C showed the rate of inhibition from the decline in amplitude of spontaneous oscillatory contractions affected by honokiol and magnolol. The inhibition rate of magnolol on spontaneous uterine contractions was greater than that of the honokiol-elicited response. The time periods required for honokiol, at 10, 30, and 100 µmol/l, to cause a 50% decline in amplitude of oscillatory contractions were 21.4±3.9, 15.7±1.7, and 6.1±1.1 min, respectively. The latency for 10, 30, and 100 µmol/l magnolol to inhibit 50% spontaneous con-

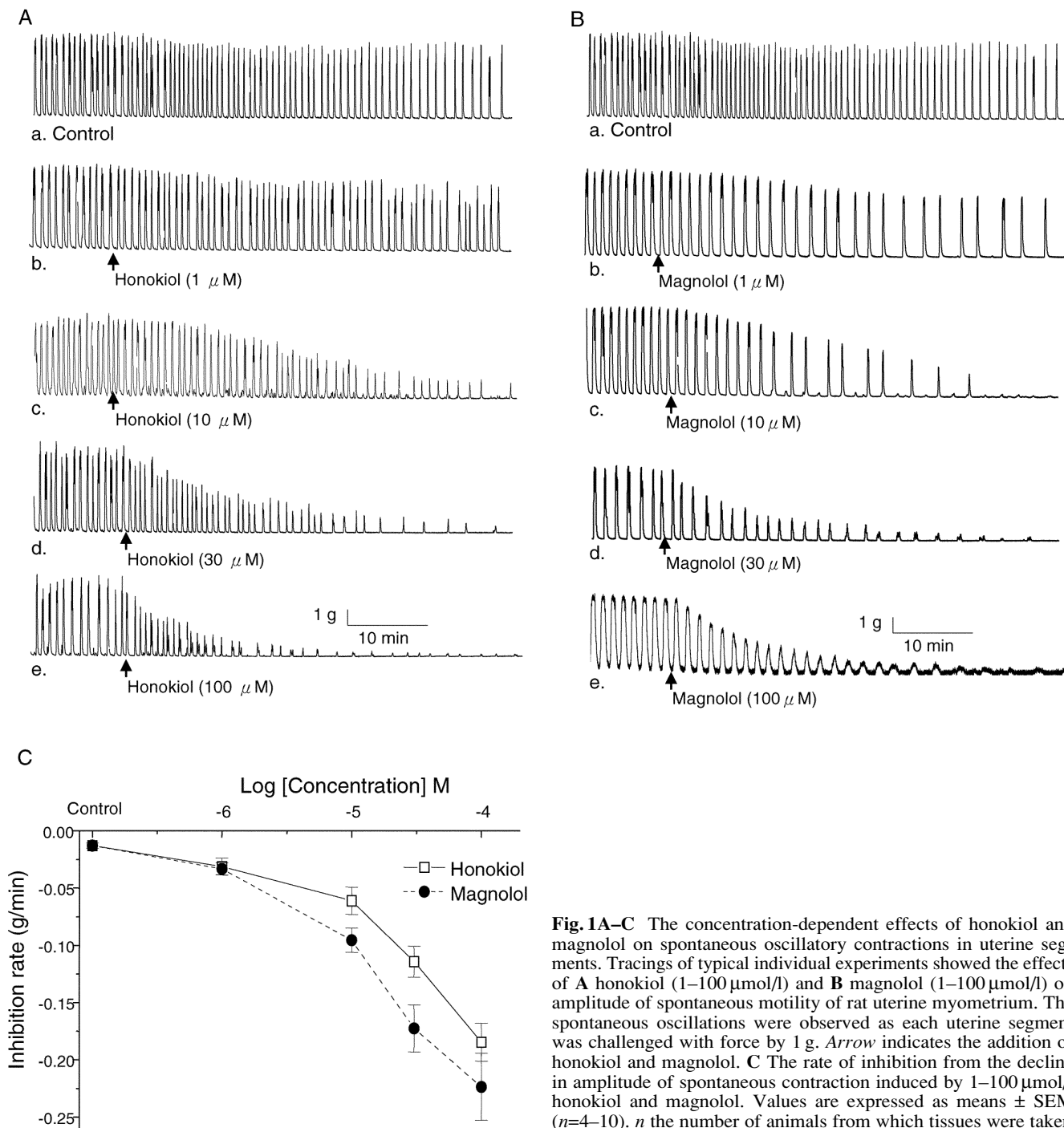


Fig. 1A–C The concentration-dependent effects of honokiol and magnolol on spontaneous oscillatory contractions in uterine segments. Tracings of typical individual experiments showed the effects of **A** honokiol (1–100 $\mu\text{mol/l}$) and **B** magnolol (1–100 $\mu\text{mol/l}$) on amplitude of spontaneous motility of rat uterine myometrium. The spontaneous oscillations were observed as each uterine segment was challenged with force by 1 g. Arrow indicates the addition of honokiol and magnolol. **C** The rate of inhibition from the decline in amplitude of spontaneous contraction induced by 1–100 $\mu\text{mol/l}$ honokiol and magnolol. Values are expressed as means \pm SEM ($n=4-10$). n the number of animals from which tissues were taken

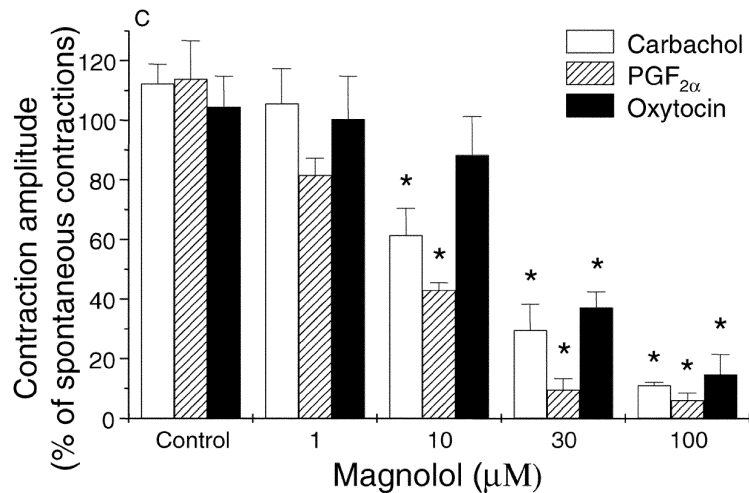
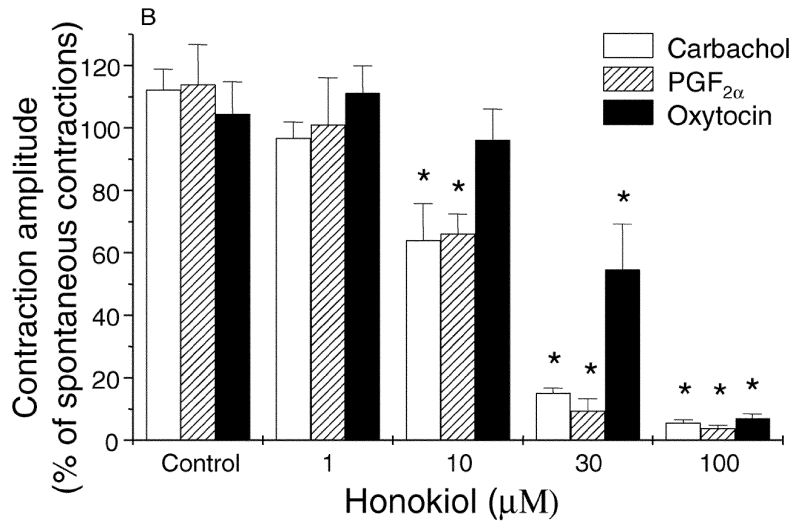
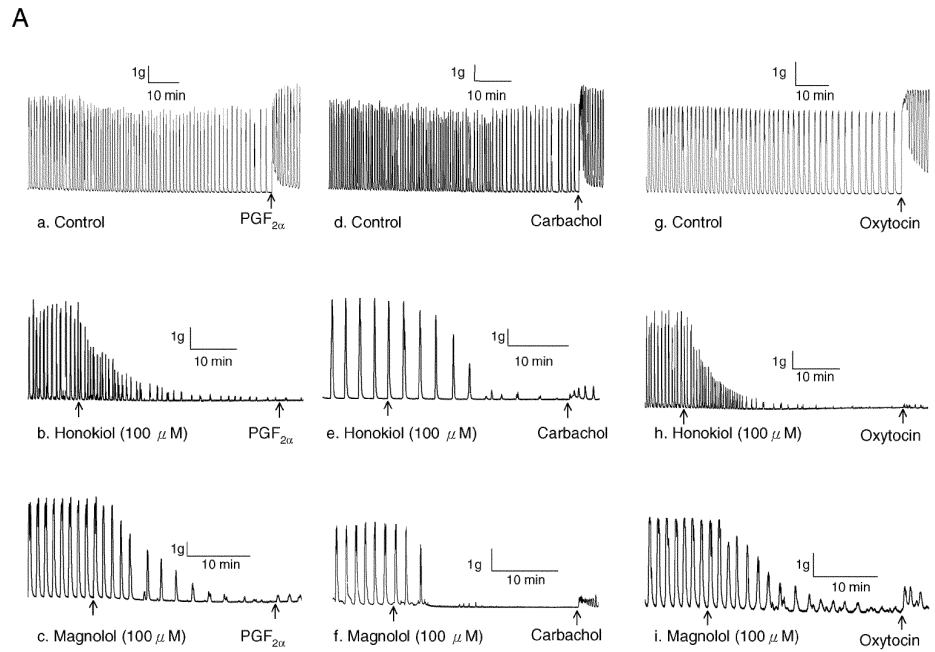
contractions was 20.2 ± 3.1 , 9.8 ± 0.9 , and 6.2 ± 1.3 min, respectively. Control uterine strips exposed to vehicle (DMSO) did not exhibit significant changes in the force and frequency of contractions over the observation time (~ 60 min).

Effects of honokiol and magnolol on uterotonic agent-induced contraction

As shown in Fig. 2, uterotonic agents such as carbachol (10 $\mu\text{mol/l}$), $\text{PGF}_{2\alpha}$ (0.1 $\mu\text{mol/l}$), and oxytocin (10 mU) all

potentiated the spontaneous oscillatory contractions by enhancing contractile amplitude. Exposure of muscle segment to honokiol and magnolol (1–100 $\mu\text{mol/l}$) for 30 min, concentration dependently, decreased the spontaneous contractions and also reduced the subsequent stimulant-evoked increase in oscillatory contractions (Fig. 2). Honokiol and magnolol at 10–100 $\mu\text{mol/l}$ significantly decreased the uterine contractility in response to carbachol and $\text{PGF}_{2\alpha}$. The concentrations of honokiol and magnolol required to decrease the oxytocin-induced contractile responses were not less than 30 $\mu\text{mol/l}$. Thus, both compounds seemed to

Fig. 2A–C Concentration-dependent effects of honokiol and magnolol on uterine contractions stimulated by uterotonic agents. **A** Representative recordings showed the uterine spontaneous contraction and responses to various agents. The muscle segments were pretreated with different concentrations of honokiol and magnolol for ~30 min before carbachol (1 $\mu\text{mol/l}$), $\text{PGF}_{2\alpha}$ (0.1 $\mu\text{mol/l}$), and oxytocin (10 mU) stimulation. Summary of the inhibitory effects of **B** honokiol (1–100 $\mu\text{mol/l}$) and **C** magnolol (1–100 $\mu\text{mol/l}$) on the amplitude of uterine contractions induced by carbachol, $\text{PGF}_{2\alpha}$, and oxytocin, expressed as mean percentages of the respective responses to spontaneous contractions. Values are presented as means \pm SEM ($n=5$). *Significant at $P<0.05$ vs the respective vehicle groups



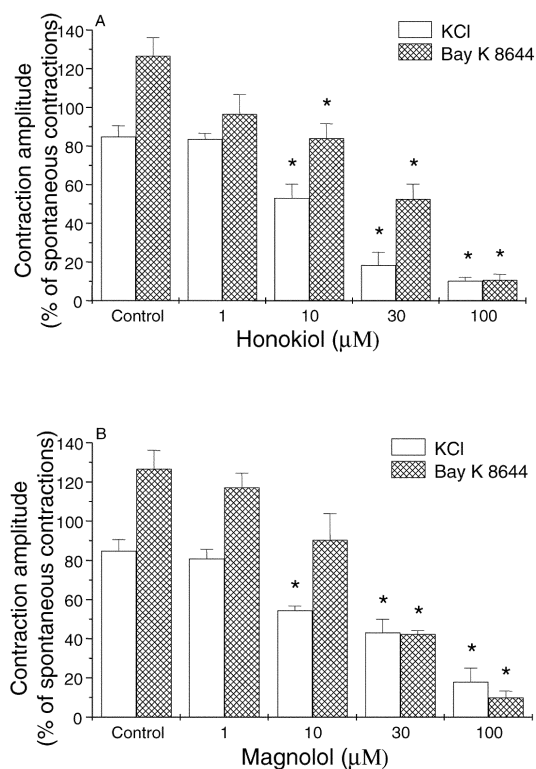


Fig. 3A, B Effects of honokiol and magnolol on KCl-induced and Bay K 8644-induced muscle contractions in rat uterus. The muscle segments were initially pretreated with **A** honokiol (1–100 μmol/l) or **B** magnolol (1–100 μmol/l) for ~30 min and then KCl (60 mmol/l) or Bay K 8644 (1 μmol/l) was applied to induced uterine contractions. The inhibitory effects of honokiol and magnolol on the amplitude of uterine contractions evoked by KCl and Bay K 8644 are expressed as mean percentages of the respective responses to spontaneous contractions. Values are means ± SEM ($n=4$). *Significant at $P<0.05$ vs the respective vehicle groups

be more potent against carbachol and $\text{PGF}_{2\alpha}$ than oxytocin. Furthermore, the generation of uterine oscillatory contractions by these stimulants was also attenuated by subsequent addition of honokiol and magnolol (≥ 10 μmol/l).

Effects of honokiol and magnolol on Ca^{2+} -dependent contractions

As noted previously, Ca^{2+} channel activator Bay K 8644 has been reported to induce uterine contractions. Consistent with this reported effect, Bay K 8644 enhanced the mean amplitude of oscillatory contractions (Fig. 3). Depolarization produced by elevation of external $[\text{K}^+]$ markedly altered the pattern of uterine contractile activity compared with spontaneous oscillations, since high K^+ evoked the stable contractions. After 30 min incubation, the peak tension of muscle contraction was slightly increased by high K^+ , but it was less than or similar to the amplitude of initial spontaneous oscillatory contractions. However, there were no significant differences in the amplitude of muscle contractions. Pretreatment of honokiol at 10–100 μmol/l and magnolol at 30–100 μmol/l for 30 min significantly de-

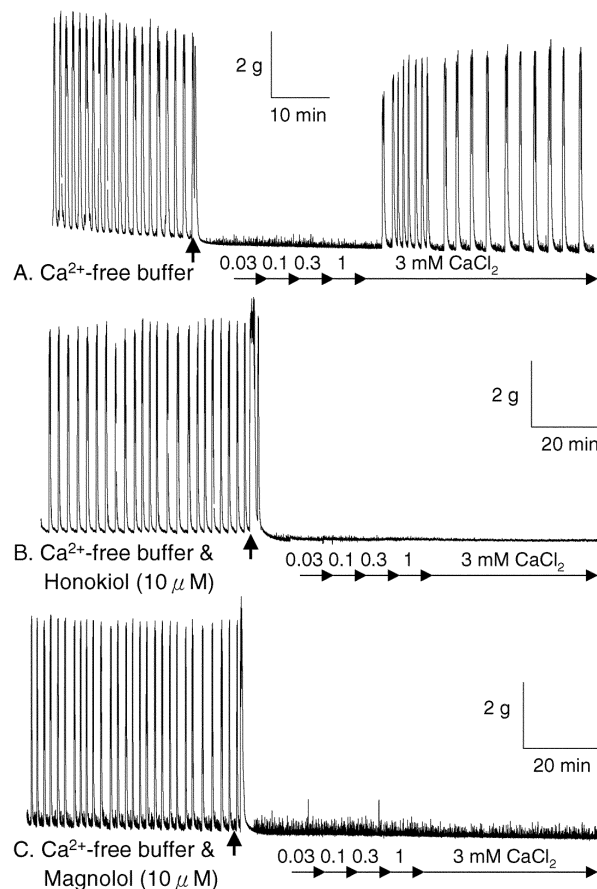


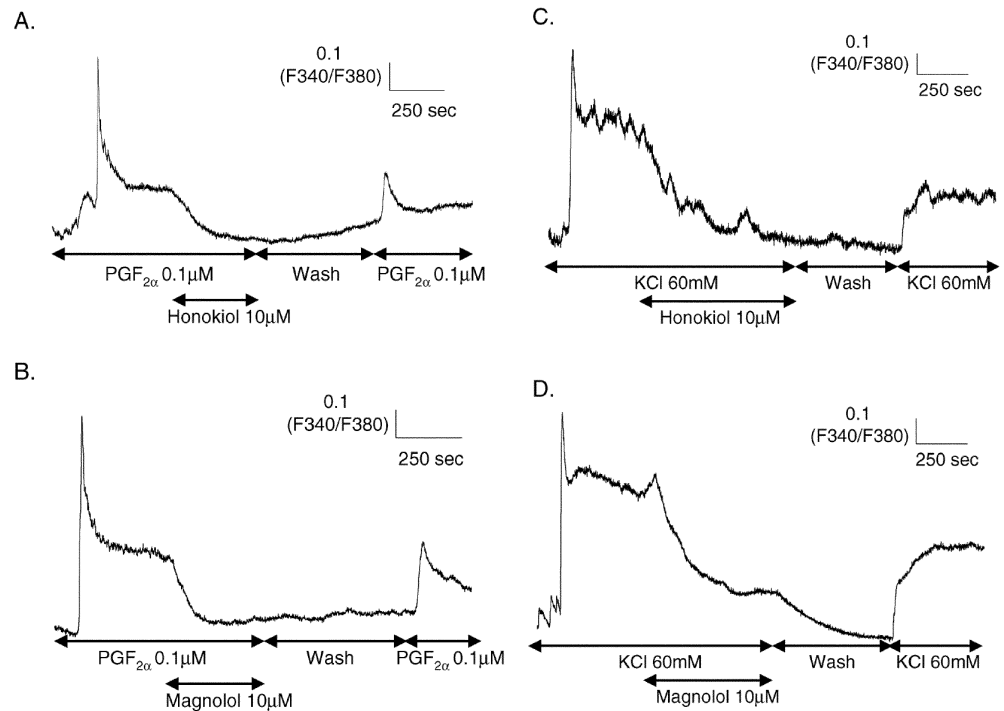
Fig. 4A–C Inhibitory actions of honokiol and magnolol on Ca^{2+} -dependent contractile responses. Muscle segments were initially pretreated with Ca^{2+} -free medium containing **A** vehicle, **B** honokiol (10 μmol/l), or **C** magnolol (10 μmol/l), and then calcium (0.03–3 mmol/l) was cumulatively applied to trigger muscle contraction. These results are representative of records from five experiments

creased the Bay K 8644-induced uterine oscillatory contractions. Consistently, honokiol and magnolol at 10–100 μmol/l decreased the high K^+ (60 mmol/l)-induced uterine contractile responses (Fig. 3). To investigate whether the inhibition of oscillatory contractions by honokiol and magnolol is due to blockade of external Ca^{2+} entry, we performed the experiments in free external Ca^{2+} . In the absence of external Ca^{2+} , the spontaneous contractions were abolished. Then, the increasing application of Ca^{2+} from 0.03 to 3 mmol/l restored the spontaneous oscillatory contractions (Fig. 4A). However, when this buffer solution contained, additionally, 10 μmol/l honokiol or magnolol, the Ca^{2+} -evoked uterine oscillatory contractions could not be observed (Fig. 4B, C).

Effects of honokiol and magnolol on intracellular Ca^{2+} concentration

In order to determine whether honokiol-induced and magnolol-induced alterations in intracellular Ca^{2+} mobilization were consistent with muscular contraction, we used single longitudinal myometrial cells to measure intracellu-

Fig. 5A–D Inhibition of $\text{PGF}_{2\alpha}$ -induced and KCl -induced increases in $[\text{Ca}^{2+}]_i$ by honokiol and magnolol in myometrial cells. The relative changes in concentration of cytosolic Ca^{2+} were indicated as ratiometric (F340/F380) fluorescence of fura 2 loaded cells. Myometrial cell was initially superfused with HEPES buffer solution containing $0.1 \mu\text{mol/l}$ $\text{PGF}_{2\alpha}$ (A, B) or 60 mmol/l KCl (C, D); subsequently, addition of $10 \mu\text{mol/l}$ honokiol (A, C) or $10 \mu\text{mol/l}$ magnolol (B, D) to superfusion medium resulted in inhibition of the $\text{PGF}_{2\alpha}$ -induced and high K^+ -induced responses. After washout of treatments, a second exposure of cells to $\text{PGF}_{2\alpha}$ ($0.1 \mu\text{mol/l}$) or KCl (60 mmol/l) produced a rise in $[\text{Ca}^{2+}]_i$ with a smaller peak amplitude when compared with previous stimulation. These graphs are representative of records from six separate experiments



lar Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) by Ca^{2+} imaging. Application of $0.1 \mu\text{mol/l}$ $\text{PGF}_{2\alpha}$ or high K^+ (60 mmol/l) to myometrial cells caused an initial rapid rise in cytosolic calcium level followed by a sustained increase in $[\text{Ca}^{2+}]_i$ (Fig. 5). The subsequent superfusion of $10 \mu\text{mol/l}$ honokiol and magnolol produced inhibition of $\text{PGF}_{2\alpha}$ -elicited increases in $[\text{Ca}^{2+}]_i$ by $108.9 \pm 15.2\%$ and $94.3 \pm 13.0\%$, respectively, whereas the high K^+ -stimulated responses were reduced by $127.8 \pm 39.6\%$ and $74.2 \pm 14.3\%$, respectively. After washout of honokiol or magnolol treatment, the increases in $[\text{Ca}^{2+}]_i$ induced by a second exposure of $\text{PGF}_{2\alpha}$ and high K^+ were still partially inhibited. Furthermore, the response to stimulant did not recover after several washouts of all previous treatments. However, honokiol and magnolol alone induced an increase in $[\text{Ca}^{2+}]_i$ but they did inhibit subsequent $\text{PGF}_{2\alpha}$ -evoked and high K^+ -evoked rise in intracellular Ca^{2+} (data not shown).

Discussion

In the Orient, the bark of *Magnolia officinalis* is prescribed in many traditional herb medicines for gynecological dysfunction. In the present studies, honokiol and magnolol, isolated from magnolia bark, were first observed markedly to suppress spontaneous and uterotonic agents such as carbachol, $\text{PGF}_{2\alpha}$ and oxytocin that induced uterine oscillatory contractions in a concentration-dependent manner. Of interest, both compounds also inhibited oscillatory contractions stimulated by Ca^{2+} channel activator and depolarization in response to high K^+ . Consistent with their inhibitory effects on myometrial contractions, honokiol and magnolol inhibited $\text{PGF}_{2\alpha}$ -induced and high K^+ -induced elevation in $[\text{Ca}^{2+}]_i$. These results indicate that the actions

of honokiol and magnolol would mimic that of Ca^{2+} channel blockers, which block Ca^{2+} influx through plasma membrane voltage-operated Ca^{2+} channels, resulting in inhibition of uterine contractions. The Ca^{2+} channel blockers with the tocolytic activity are now employed to inhibit muscular contraction and increase uterine blood flow (Ulmsten et al. 1980; Tsatsaris et al. 2001). Thus, our results have provided evidence to support the notion that the bark of *Magnolia officinalis* acts as the traditional remedy for gynecological complications. In this aspect honokiol and magnolol may be of potential value in the treatment of gynecological disorders linked with uterine muscular spasm and pain.

Beta-adrenoceptor agonists have been used for tocolysis to reduce uterine muscle hypercontractility and subsequently increase uterine blood flow (Akerlund 1979; Caughey and Parer 2001). However, the inhibitory actions of honokiol and magnolol on uterine contractions were insensitive to the β -adrenoceptor antagonist propranolol (data not shown). Similar responses were also observed in carbachol-evoked and high K^+ -evoked swine tracheal muscle contractions (Ko et al. 2003) and K^+ -stimulated 5-HT release from rat cortex (Tsai et al. 1995). Furthermore, magnolol did not change porcine heart protein kinase A activity (Wang et al. 1999). Thus, it appears that the inhibitory effects of honokiol and magnolol were not mediated by the sympathomimetic and PKA-dependent pathways.

The present results demonstrated that honokiol and magnolol inhibited uterotonic hormone-induced uterine oscillatory contractions and were also effective against high K^+ -elicited and Ca^{2+} channel activator Bay K8644-elicited uterine contractions. It is known that both Ca^{2+} channel activator and membrane depolarization caused by high K^+ can increase Ca^{2+} influx through the voltage-gated Ca^{2+}

channel (Foster et al. 1983). Uterotonic hormones that induce uterine contractions increase $[Ca^{2+}]_i$, via both the release of intracellular stored Ca^{2+} and the influx of extracellular Ca^{2+} through Ca^{2+} channels (Ivorra et al. 1994; Fuchs 1995; Ruttner et al. 2002). Thus, the general spasmolytic activity of honokiol and magnolol on uterine contraction is thought to interfere with receptor-operated cation channels and voltage-operated Ca^{2+} channels. Consistently, the extract of magnolia bark was also reported to inhibit the secretion of catecholamines from bovine adrenal chromaffin cells stimulated by acetylcholine, which activate voltage-operated Ca^{2+} channels and receptor-operated cation channels (Tachikawa et al. 2000).

In our current and previous studies, honokiol and magnolol were found to block the Ca^{2+} -dependent contractile responses in uterine oscillatory contractions and high K^+ -evoked tracheal muscle contractions (Ko et al. 2003). This further indicated that their inhibition might be related to blockade of Ca^{2+} influx through depolarization and/or voltage-operated Ca^{2+} channels. Similar mechanisms may contribute to their inhibition of high K^+ -induced and $PGF_{2\alpha}$ -induced increases in $[Ca^{2+}]_i$, since the increases in $[Ca^{2+}]_i$ evoked by high K^+ and $PGF_{2\alpha}$ were highly external Ca^{2+} -dependent processes (Kimura et al. 1978; Kawarabayashi et al. 1997). Recently, honokiol was reported to decrease acetylcholine-evoked and high K^+ -evoked Ca^{2+} influx in bovine adrenal chromaffin cells (Tachikawa et al. 2000), whereas magnolol inhibited the rise in $[Ca^{2+}]_i$ mediated by high K^+ in rat aorta (Teng et al. 1990) and by IgE in rat basophilic leukemia-2H3 cells (Hamasaki et al. 1999). Similar to the effects of Ca^{2+} channel antagonists, honokiol and magnolol are likely to possess the ability to block Ca^{2+} channels (Yamahara et al. 1986; Ruttner et al. 2002), leading to reduction in $[Ca^{2+}]_i$ and muscle relaxation. Alternatively, regulation of other ion channel activity, such as K^+ channels, can also affect intracellular free $[Ca^{2+}]_i$ that then approaches to control myometrial contractile activity (Word 1995). Anwer et al. (1993) have reported that inhibition of BK_{Ca} channels could promote Ca^{2+} entry and contraction in human myometrium. Recently, the stimulating action of magnolol on BK_{Ca} channels in a human tracheal muscle cell line was demonstrated that might cause inhibition of Ca^{2+} entry and muscular contraction (Wu et al. 2002). In addition to blockade of plasma membrane ion channels, the ability of honokiol and magnolol to block some of the agonist membrane-bound receptors or internal Ca^{2+} channels cannot be ruled out. Thus, their proposed mechanisms in utero requires further study.

Taken together, the spasmolytic effects of honokiol and magnolol on uterine muscle contractions and intracellular Ca^{2+} mobilization may be due to the blockade of receptor-operated cation channels and voltage-operated Ca^{2+} channels, in the rat. Thus, both chemical compounds seem to be of potential value in the treatment of certain diseases, including gynecological disturbances and dysfunctions associated with an increase in uterine muscular activity such as dysmenorrhea and premature deliveries.

Acknowledgments Y.-C. Lu and H.-H. Chen contributed equally to this work. We would like to thank Dr. H.I. Chen for his kind equipment support and S.-C. Jong for her technical assistance. This research was supported by grant TCMRC90210 from the intramural fund of Tzu Chi University, Taiwan.

References

- Akerlund M (1979) Pathophysiology of dysmenorrhea. *Acta Obstet Gynecol Scand Suppl* 87:27–32
- Anwer K, Oberti C, Perez GJ, Perez-Reyes N, McDougall JK, Monga M, Sanborn BM, Stefani E, Toro L (1993) Calcium-activated K^+ channels as modulators of human myometrial contractile activity. *Am J Physiol* 265:C976–985
- Caughey AB, Parer JT (2001) Tocolysis with beta-adrenergic receptor agonists. *Semin Perinatol* 25:248–255
- Chan P, Cheng JT, Tsao CW, Niu CS, Hong CY (1996) The in vitro antioxidant activity of trilinolein and other lipid-related natural substances as measured by enhanced chemiluminescence. *Life Sci* 59:2067–2073
- Chen QS (1986) Pharmacology and applications of Chinese materia medica. In: Chang HM, But PPH (eds) World Scientific, Singapore, vol 2, pp 878–880
- Foster RW, Small RC, Weston AH (1983) The spasmogenic action of potassium chloride in guinea-pig trachealis. *Br J Pharmacol* 80:553–559
- Fuchs AR (1995) Plasma membrane receptors regulating myometrial contractility and their hormonal modulation. *Semin Perinatol* 19:15–30
- Fujita M, Itokawa H, Sashida Y (1973) Studies on the components of *Magnolia obovata* Thunb. 3. Occurrence of magnolol and honokiol in *M. obovata* and other allied plants. *Yakugaku Zasshi* 93:429–434
- Hamasaki Y, Kobayashi I, Zaitu M, Tsuji K, Kita M, Hayasaki R, Muro E, Yamamoto S, Matsuo M, Ichimaru T, Miyazaki S (1999) Magnolol inhibits leukotriene synthesis in rat basophilic leukemia-2H3 cells. *Planta Med* 65:222–226
- Haraguchi H, Ishikawa H, Shirataki N, Fukuda A (1997) Antiperoxidative activity of neolignans from *Magnolia obovata*. *J Pharm Pharmacol* 49:209–212
- Ivorra MD, Chulia S, Noguera MA, D'Ocon MP (1994) Intervention of two voltage-dependent calcium-entry pathways in the contractile response to acetylcholine and KCl in rat uterus. *Pharmacology* 49:33–41
- Kawarabayashi T, Shoji H, Tsukamoto T (1997) Effects of oxytocin, prostaglandin F2 alpha and prostaglandin E2 on intracellular free calcium concentrations of longitudinal muscle cells isolated from term pregnant rat myometrium. *Gynecol Obstet Invest* 43:145–149
- Kimura M, Kimura I, Maekawa M (1978) The mode of action of contractile effects induced by external calcium and its related bivalent cations in the KCl-depolarized rat uterus. *Jpn J Pharmacol* 28:681–692
- Ko CH, Chen HH, Lin YR, Chan MH (2003) Inhibition of smooth muscle contraction by magnolol and honokiol in porcine trachea. *Planta Med* 69:532–536
- Kuribara H, Kishi E, Hattori N, Okada M, Maruyama Y (2000) The anxiolytic effect of two oriental herbal drugs in Japan attributed to honokiol from magnolia bark. *J Pharm Pharmacol* 52:1425–1429
- Ruttner Z, Ivanics T, Slaaf DW, Reneman RS, Toth A, Ligeti L (2002) In vivo monitoring of intracellular free calcium changes during uterine activation by prostaglandin f(2alpha) and oxytocin. *J Soc Gynecol Invest* 9:294–298
- Tachikawa E, Takahashi M, Kashimoto T (2000) Effects of extract and ingredients isolated from *Magnolia obovata* thunberg on catecholamine secretion from bovine adrenal chromaffin cells. *Biochem Pharmacol* 60:433–440

- Teng CM, Chen CC, Ko FN, Lee LG, Huang TF, Chen YP, Hsu HY (1988) Two antiplatelet agents from *Magnolia officinalis*. *Thromb Res* 50: 757-765
- Teng CM, Yu SM, Chen CC, Huang YL, Huang TF (1990) EDRF-release and Ca^{2+} -channel blockade by magnolol, an antiplatelet agent isolated from Chinese herb *Magnolia officinalis*, in rat thoracic aorta. *Life Sci* 47:1153-1161
- Tsai TH, Lee TF, Chen CF, Wang LC (1995) Modulatory effects of magnolol on potassium-stimulated 5-hydroxytryptamine release from rat cortical and hippocampal slices. *Neurosci Lett* 186:49-52
- Tsatsaris V, Papatsonis D, Goffinet F, Dekker G, Carbonne B (2001) Tocolysis with nifedipine or beta-adrenergic agonists: a meta-analysis. *Obstet Gynecol* 97:840-847
- Ulmsten U, Andersson KE, Wingerup L (1980) Treatment of premature labor with the calcium antagonist nifedipine. *Arch Gynecol* 229:1-5
- Wang JP, Hsu MF, Raung SL, Chen CC, Kuo JS, Teng CM (1992) Anti-inflammatory and analgesic effects of magnolol. *Naunyn Schmiedebergs Arch Pharmacol* 346:707-712
- Wang JP, Hsu MF, Raung SL, Chang LC, Tsao LT, Lin PL, Chen CC (1999) Inhibition by magnolol of formylmethionyl-leucyl-phenyl alanine-induced respiratory burst in rat neutrophils. *J Pharm Pharmacol* 51:285-294
- Word RA (1995) Myosin phosphorylation and the control of myometrial contraction/relaxation. *Semin Perinatol* 19:3-14
- Wu SN, Chen CC, Li HF, Lo YK, Chen SA, Chiang HT (2002) Stimulation of the BK(Ca) channel in cultured smooth muscle cells of human trachea by magnolol. *Thorax* 57:67-74
- Yamahara J, Miki S, Matsuda H, Fujimura H (1986) Screening test for calcium antagonists in natural products. The active principles of *Magnolia obovata*. *Yakugaku Zasshi* 106:888-893