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Antispasmodic activity of licochalcone A, a species-specific ingredient of *Glycyrrhiza inflata* roots

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Abstract

Licochalcone A, a species-specific and characteristic retrochalcone ingredient of *Glycyrrhiza inflata* root, has been shown to possess multiple bioactive properties. However, its muscle relaxant activity has not been reported previously. Licochalcone A showed a concentration-dependent relaxant effect on the contraction induced by carbachol (50% effective concentration (EC50) = 5.64 ± 1.61 μM), KCl (EC50 5.12 ± 1.68 μM), $BaCl_2$ (EC50 1.97 ± 0.48 μM) and A23187 (EC50 2.63 ± 2.05 μM). Pretreatment with licochalcone A enhanced the relaxant effect of forskolin, an adenylyl cyclase activator, on the contraction in a similar manner to 3-isobutyl-1-methylxanthine (IBMX), a phosphodiesterase (PDE) inhibitor. Furthermore, the IC50 (22.1 ± 10.9 μM) of licochalcone A against cAMP PDE was similar to that of IBMX (26.2 ± 7.4 μM). These results indicated that licochalcone A may have been responsible for the relaxant activity of *G. inflata* root and acted through the inhibition of cAMP PDE.

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Introduction

Liquorice is one of the most frequently used natural drugs prepared from some *Glycyrrhiza* species roots and contains glycyrrhizin as an important pharmacologically active glycoside (Olukoga & Donaldson 2000). In Japan, liquorice prepared mainly from *G. uralensis* root is used in clinical practice to treat various abdominal spasmodic symptoms (Katsura 1995) as a component of a traditional Chinese formulation ("Shakuyakukanzoto" in Japanese). We have been searching for muscle relaxant components in *G. uralensis* root and have obtained glycycoumarin (Sato et al 2006) and isoliquiritigenin (Sato et al 2007) by assessing carbachol (carbamylcholine)-induced contraction of mouse jejunum.

Previous pharmaceutical studies comparing the relaxant activity of liquorice and roots of some *Glycyrrhiza* species (Nagai et al 2006) provided us with preliminary knowledge about the relaxant activity of *G. inflata* roots. In that study, we examined in detail the relaxant activity of a hot water extract of *G. inflata* root. HPLC analysis (Hatano et al 1991) has shown that *G. inflata* root contains isoliquiritigenin, a relaxant component also found in *G. uralensis* root. Therefore, this paper describes the isolation of active components other than isoliquiritigenin from *G. inflata* root monitored by measurement of their relaxant effects.

Materials and Methods

Chemicals

The grades of carbachol (carbamylcholine) chloride, acetylcholine, $BaCl_2$, KCl, A23187, 3-isobutyl-1-methylxanthine (IBMX), forskolin, papaverine and all other chemicals used in Tyrode solution were identical to those used by Sato et al (2006). Authentic licochalcone A was purchased from EMD Bioscience, Inc. (Darmstadt, Germany). 3′, 5′-Cyclic-nucleotide 5′-nucleotidohydrolase (phosphodiesterase: PDE) from bovine heart was purchased from Sigma-Aldrich Fine Chemicals (St Louis, MO).

Extraction and HPLC analysis

G. inflata roots were kindly provided by Tochimoto Tenkaido Co., Ltd (Osaka, Japan), and their botanical origin was confirmed by the presence of licochalcone A, a species-specific ingredient of *G. inflata*, in their HPLC profile (Yamamoto & Tani 2006). Voucher specimens have been deposited in the department of Kampo Pharmaceutics, Institute of Natural Medicine, University of Toyama.

G. inflata roots (30 g) were extracted with boiled water (600 mL) for 40 min, and then freeze-dried powders (4.8 g) were obtained. After repeating this procedure three times, the total extract (13.7 g) was obtained. The extract powders (5 mg) were dissolved with MeOH (10 mL) for 30 min under ultrasonication and filtered through a 0.45-μm membrane filter. A 10-μL sample of the filtrate was injected into the HPLC system under the conditions described in the legend for Figure 1 (HPLC-profile analysis) and below (licochalcone A quantitative analysis).

For licochalcone A quantitative analysis the following were used: column, YMC-Pack ODS-A (250×4.6 mm i.d.); column temperature, 25°C; pump, 880-PU (Jasco); detector, UVIDEC-100-IV (Jasco); guard column, Develosil Packed Column ODS-MG-5 (4.0/10); mobile phase, 0.05 M ammonium acetate (pH 3.6):CH_3CN=50:50; flow rate, 1.0 mL min^{-1}; wavelength, 351 nm.

The standard curve for determining the concentration of licochalcone A was: y=0.006x+9.66 (r=1.00), where y is the amount of licochalcone A (μg), and x is the peak area of licochalcone A. Detection limit was 250 ng mL^{-1}, and the recovery rate was 97.4%. Reproducibility for licochalcone A was assessed in terms of relative standard deviation (r.s.d.). The r.s.d. for licochalcone A was 4.39% (intraday) and 3.00% (interday).

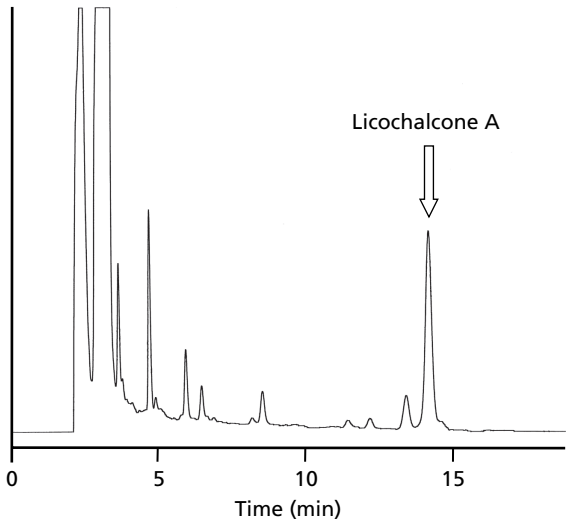

Figure 1 HPLC-profile of the MeOH soluble portion of boiled water extracts of *G. inflata* at 351 nm wavelength. HPLC system and conditions: column, YMC-Pack ODS-A (250×4.6 mm i.d.); column temperature, 40°C; pump, PU-1580 (Jasco); detector, MD-2010 Plus (Jasco); guard column, Develosil Packed Column ODS-MG-5 (4.0/10); mobile phase, 0.05 M ammonium acetate (pH 3.6):CH_3CN=50:50; flow rate, 1.0 mL min^{-1}; wavelength, multi (200–650 nm); column oven, CO-1565 (Jasco); degasser, DG-1580–54 (Jasco).

Isolation of licochalcone A

A 9.6-g sample of the boiled water extract was partitioned into a $CHCl_3$:H_2O (1:1) mixture to obtain the $CHCl_3$-soluble portion (123.8 mg, EC50 7.7 μg mL^{-1}) and water-soluble portion (7.4 g, EC50 205.3 μg mL^{-1}). The $CHCl_3$ extract (100 mg) was separated by HPLC (YMC-Pack ODS-A: 250×4.6 mm i.d., MeOH-H_2O (4:1)) to afford five fractions: A (23.7 mg), B (18.9 mg), C (2.4 mg), D (46.0 mg), and E (1.7 mg). These five fractions were monitored by measurement of their relaxant effects on isolated mouse jejunum to give the relatively active fraction E (EC50 3.3 μg mL^{-1}), which was re-fractionated by HPLC to give an active compound (1.0 mg). The NMR data of the active compound were as follows: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.0 (1H, d, J=12.8 Hz, H-β), 7.99 (2H, d, J=7.3 Hz, H-2′, 6′), 7.59 (1H, d, J=12.8 Hz, H-α), 7.47 (1H, s, H-6), 6.94 (2H, d, J=7.3 Hz, H-3′, 5′), 6.45 (1H, s, H-3), 6.20 (1H, dd, J=14.0, 8.4 Hz, H-2″), 5.39 (1H, d, J=14.0 Hz, H_{trans}-3″), 5.35 (1H, d, J=8.4 Hz, H_{Cis}-3″), 3.87 (3H, s, OCH_3), 1.45 (6H, s, H-4″, 5″). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 27.3 (C-4″, C-5″), 39.7 (C-1″), 55.6 (OCH_3), 101.1 (C-3), 114.0 (C-3″), 115.4 (C-3′, 5′), 116.5 (C-1), 120.2 (C-α), 124.5 (C-5), 128.8 (C-6), 131.1 (C-2′, 6′), 131.5 (C-1′), 141.0 (C-β), 147.8 (C-2″), 158.2 (C-2), 159.6 (C-4), 160.0 (C-4′), 190.2 (C=O). The ^{1}H and ^{13}C NMR spectra were recorded on a UNITY plus 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz, Varian, Inc., USA).

Assay of relaxant effects

All animal experiments were carried out in accordance with the Guidelines of the Animal Care and Use Committee of the University of Toyama, approved by the Japanese Association of Laboratory Animal Care.

Determination of the 50% effective concentration (EC50) of samples on the contractile response induced by carbachol, KCl, $BaCl_2$ or A23187 in isolated mouse jejunum was performed as described by Sato et al (2006). Briefly, 1-cm segments of jejunum were isolated from male ICR mice (7–10 weeks old, purchased from Japan SLC Inc., Japan). The segments were maintained in a bath of Tyrode solution. After maximal contraction had been evoked by acetylcholine (1 μM), the segments were washed and allowed to equilibrate for another 45 min, and then exposed to carbachol (1 μM), KCl (60 mM), $BaCl_2$ (1 mM), or A23187 (6 μM). When the contraction reached a steady-state level, licochalcone A (0.3–30 μM) or other samples were administered in a cumulative manner every 5 min.

Effects on phasic and tonic phase contraction

After maximal contraction had been evoked by acetylcholine (1 μM) or KCl (60 mM), the segments were washed and allowed to equilibrate for 45 min. Licochalcone A (3.0 or 6.0 μM) was then administered. After 10 min, the tissues were again exposed to acetylcholine (1 μM) or KCl (60 mM).

The relaxant effects were measured by comparing the height of contraction induced by acetylcholine or KCl before

and after the pretreatment with licochalcone A. Phasic phase expresses the temporary peak of agent-induced contraction. Tonic phase expresses the lasting contraction at 10 min after administration of the agent.

Assay of cAMP phosphodiesterase (PDE)

The PDE activity was determined using a cyclic nucleotide phosphodiesterase assay kit (BIOMOL International, LP) with cAMP as a substrate, and by measuring the absorbance at 620 nm using a microplate reader (NJ-2100; Intermed, Japan). The IC50 values of the sample compounds were determined from the concentration–cAMP PDE activity curve for each compound.

Statistical analysis

Data are expressed as mean ± s.d. of the number (n) of experiments. Data were analysed statistically by the Kruskal–Wallis test followed by the Dunn test. Differences were considered statistically significant at $P < 0.05$.

Results

Relaxant effects of *G. inflata* root extract

G. inflata root extract was found to relax isolated mouse jejunum contraction induced by carbachol with an EC50 of 96.3 μg mL^{-1}. To obtain active components, the extract was partitioned into water-soluble (EC50 205.3 μg mL^{-1}) and $CHCl_3$-soluble (EC50 7.7 μg mL^{-1}) portions, which were fractionated by HPLC to obtain an active compound. The isolated active compound was identified as licochalcone A (4, 4′-dihydroxy-5-(dimethylallyl)-2-methoxychalcone; Figure 2) by HPLC co-chromatography analysis using the authentic compound and by comparison with the reported spectral data for ^{1}H (Saitoh & Shibata 1975) and ^{13}C NMR (Wang et al 2004).

The remarkable peak of licochalcone A was detected in an HPLC profile (Figure 1) and its yield from the water extract was 0.21 ± 0.06% determined by HPLC quantitative analysis. The HPLC peak corresponding to another relaxant component, isoliquiritigenin (Sato et al 2007), was detected in the water-soluble portion, but it was not isolated or identified due to its very low content.

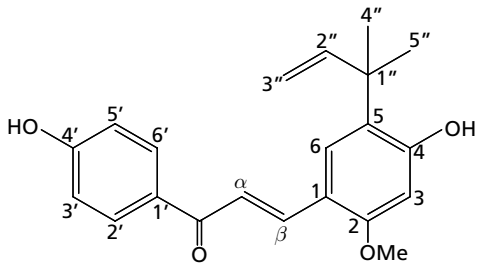

Figure 2 Chemical structure of licochalcone A.

Relaxant effect of licochalcone A

Figure 3 shows the concentration-dependent relaxant effect of licochalcone A on the contraction induced by four stimulants (carbachol, KCl, $BaCl_2$ and A23187). The EC50 values of licochalcone A for carbachol- and KCl-induced contraction (5.64 ± 1.61 and 5.12 ± 1.68 μM, respectively) were weaker than those of papaverine (1.64 ± 0.46 and 0.78 ± 0.51 μM), but the EC50 values for $BaCl_2$- and A23187-induced contraction (1.97 ± 0.48 and 2.63 ± 2.05 μM, respectively) were similar to those (2.35 ± 0.16 and 2.97 ± 0.75 μM) of papaverine.

Mechanism of action of licochalcone A

Pretreatment with licochalcone A significantly relaxed the tonic phase contraction induced by acetylcholine (Figure 4A). The phasic phase contraction was only weakly affected and it was not a dose–response effect. Figure 4B shows that licochalcone A merely relaxed the tonic phase contraction induced by KCl.

Forskolin, an adenylyl cyclase activator, relaxed the contraction induced by carbachol in a concentration-dependent manner (Figure 5). The relaxant effects of forskolin tended to be enhanced by pretreatment with licochalcone A as well as IBMX, a PDE inhibitor. The EC50 of forskolin tended to decrease from 0.94 ± 0.22 to 0.40 ± 0.07 μM (with licochalcone A pretreatment) and to 0.28 ± 0.18 μM (with IBMX pretreatment). These results suggested that licochalcone A relaxed the contractile response of mouse jejunum through inhibition of PDE in a similar manner to IBMX. To understand the

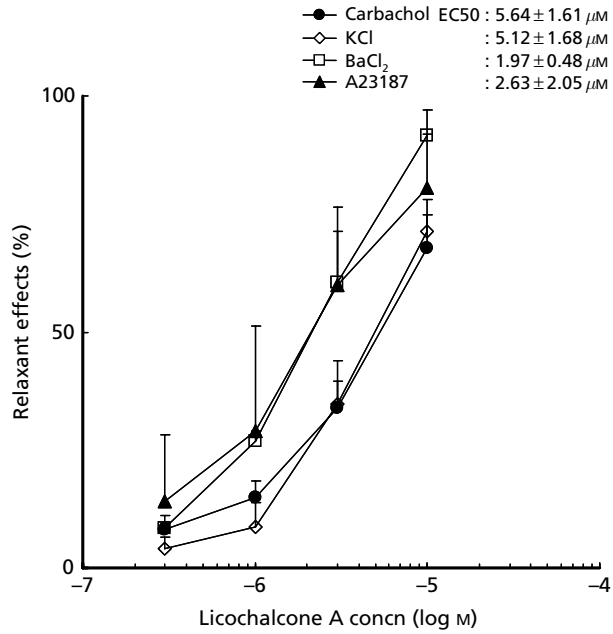

Figure 3 Effect of licochalcone A on the contraction of mouse jejunum induced by carbachol, KCl, $BaCl_2$ and A23187. Each point represents mean ± s.d. (n = 3). After contraction had been induced by carbachol (1 μM), KCl (60 mM), $BaCl_2$ (3 mM), or A23187 (6 μM), lichochalcone A was added cumulatively. The relaxant effects (%) were calculated and expressed as percentage inhibition of the plateau level contraction (maximum contraction) induced by each stimulant.

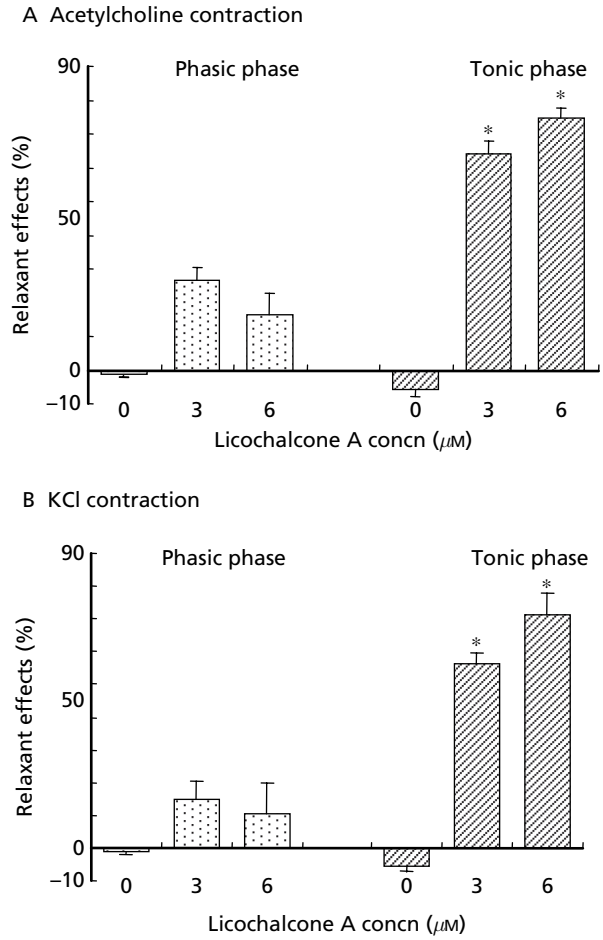

Figure 4 Effect of licochalcone A on the phasic and tonic contraction of mouse jejunum induced by acetylcholine (A) or KCl (B). The relaxant effects (%) were measured by comparing the heights of contractions induced by acetylcholine or KCl before and after pretreatment with licochalcone A. Phasic phase expresses the temporary peak of agent-induced contraction. Tonic phase expresses the lasting contraction at 10 min after administration of the agent. $^*P < 0.05$ vs phasic phase.

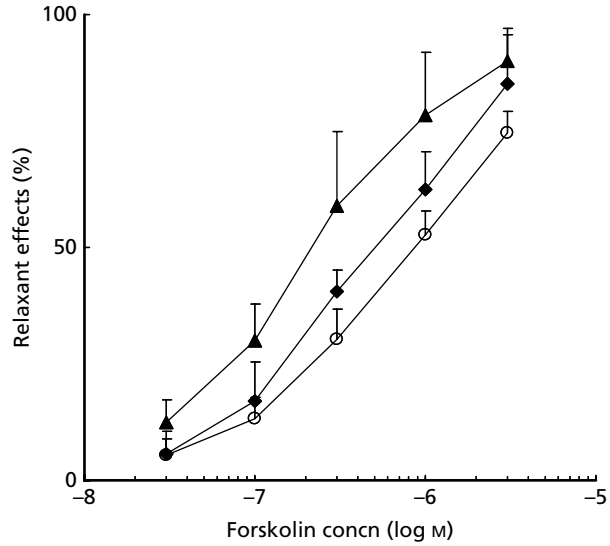

Figure 5 Effect of licochalcone A and IBMX on the relaxant response of forskolin in mouse jejunum precontracted with carbachol. Each point represents mean ± s.d. (n = 3). Under control conditions (○) and in the presence of licochalcone A (1 μM, ♦), or IBMX (1 μM,▲), forskolin was added cumulatively to mouse jejunum precontracted with carbachol (1 μM). The relaxant effects (%) were calculated and expressed as percentage inhibition of the plateau level contraction (maximum contraction) induced by carbachol.

mechanism of the relaxant activity of licochalcone A, its influence on PDE was examined. As shown in Figure 6, the IC50 (22.1 ± 10.9 μM) of licochalcone A against PDE was similar to those of the PDE inhibitors IBMX and papaverine (26.2 ± 7.4 and 31.8 ± 2.0 μM, respectively), and also to that of glycycoumarin (30.3 ± 8.0 μM), a muscle relaxant isolated from *G. uralensis* root. In contrast, isoliquiritigenin showed no influence on PDE activity at a concentration of more than 200 μM.

Discussion

In the course of our continuing pharmaceutical studies of various *Glycyrrhiza* plant roots, we have isolated isoliquiritigenin (Sato et al 2007) and glycycoumarin (Sato et al 2006), a species-specific ingredient of *G. uralensis* root, as potent muscle relaxants. This study was undertaken to clarify the relaxant activity of a hot-water extract of *G. inflata* root and to identify the active components in the extract. *G. inflata* roots are used as the raw materials for making food additives and glycyrrhizin preparations in Japan (Yamamoto & Tani 2006).

The *G. inflata* root extract showed a relaxant effect on carbachol-induced contraction of isolated mouse jejunum. The EC50 value of the extract (96.3 μg mL^{-1}) was somewhat higher than the reported value (134 μg mL^{-1}) for *G. uralensis* root extract. To improve understanding of the activity of the extract, further analysis led to isolation and identification of an active component, licochalcone A, the EC50 value (1.91 μg mL^{-1}) of which was approximately 50-times higher than that of the original boiled water extract (96.3 μg mL^{-1}). The EC50 value of the extract (96.3 μg mL^{-1}) could be converted into the EC50 value (0.20 μg mL^{-1}) of licochalcone A from its content (0.21 ± 0.06%). This was only approximately 10% of the observed EC50 value (5.64 μM; 1.91 μg mL^{-1}) of licochalcone A, therefore other constituents such as isoliquiritigenin may have participated in the relaxant activity of the extract. As shown in Figure 3, the EC50 of licochalcone A (5.64 ± 1.61 μM) for carbachol-induced contraction was somewhat weaker than that reported for glycycoumarin (2.93 ± 0.94 μM) (Sato et al 2006), but similar to that of isoliquiritigenin (4.96 ± 1.97 μM) (Sato et al 2007). Licochalcone A was originally isolated as one of the retrocalchones from *G. inflata* root (Saitoh & Shibata 1975) and is known to be a species-specific component of *G. inflata* root (Hatano et al 1991). Recently it was also isolated from *Pogostemon cablin* (Labiatae) as a cytotoxic component (Park et al 1998). Although licochalcone A has been demonstrated to have

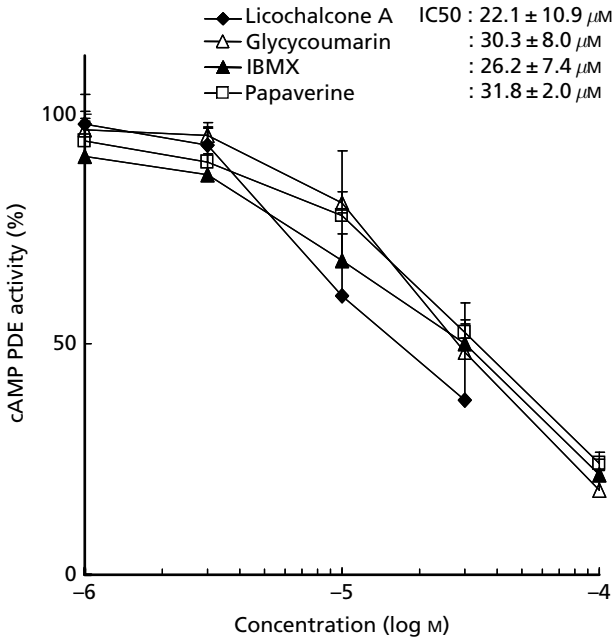

Figure 6 Inhibitory effects of licochalcone A, glycycoumarin, IBMX and papaverine on cAMP phosphodiesterase. Each point represents mean ± s.d. (n = 3–4). PDE inhibitory activity was determined using a cyclic nucleotide phosphodiesterase assay kit (BIOMOL International, LP) and measured as the absorbance at 620 nm using a microplate reader (NJ-2100; Intermed, Japan). The IC50 values of the sample compounds were determined from the concentration–cAMP PDE activity curve for each compound.

various pharmacological activity, such as immunomodulatory effects (Barfod et al 2002), anti-inflammatory activity (Shibata et al 1991) and anti-*Helicobacter pylori* activity (Fukai et al 2002), this is the first report on the in-vitro muscle relaxant activity of licochalcone A. As *G. inflata* roots contain numerous phenolic components including licochalcone A and its analogues with different side chains (Shibata 2000), further systematic comparison is required.

The EC50 of licochalcone A (2.63 ± 2.05 μM) for A23187-induced contraction was similar to that of papaverine (2.97 ± 0.75 μM). The spasmodic action of A23187, a calcium III ionophore, against smooth muscle was considered to be related to intracellular Ca^{2+} accumulation due to its Ca^{2+}influx effect (Ito et al 1985). Therefore, licochalcone A might have acted by modulating the intracellular Ca^{2+} concentration, causing a papaverine-like myotropic action.

To investigate the mechanisms of the relaxant activity of licochalcone A, its relaxant effects on phasic and tonic phase contraction of isolated mouse jejunum after acetylcholine or KCl stimulation were determined. It is accepted that the tonic phase contraction occurred due to intracellular Ca^{2+} influx (Ohmura 1976), and therefore the relaxant activity of licochalcone A on tonic phase contraction seemed to be due to inhibition of the contractile action after influx of extracellular Ca^{2+}. Moreover, the relaxant effect of forskolin, an adenylyl cyclase activator, on carbachol-induced contraction tended to be increased by pretreatment with licochalcone A (Figure 5). Since the mode of the increased relaxant effect induced by licochalcone A was similar to that of the nonspecific cyclic nucleotide PDE inhibitor, IBMX, it seemed probable that licochalcone A could have inhibited PDE. As was expected, licochalcone A was effectively able to inhibit cAMP PDE in the same manner as IBMX and glycycoumarin, which has been reported to be a potent inhibitor of PDE (Kusano et al 1991). These results provide a mechanistic explanation for the relaxant effect of licochalcone A on contraction of isolated mouse jejunum induced by various stimulants.

The presence of isoliquiritigenin, one of the relaxant components of *G. uralensis* root, in this *G. inflata* extract was confirmed by HPLC analysis (data not shown). However, its purification and identification were not possible due to the very small quantity obtained. Glycyrrhizin, a well known constituent of *G. inflata* root (Hatano et al 1991), had no influence on carbachol-induced contraction at a concentration of more than 30 μM. Therefore, licochalcone A was responsible for the relaxant activity of *G. inflata* root as well as isoliquiritigenin.

Conclusion

G. inflata root extract had relaxant effects on the contraction of jejunum, similar to the medicinal effects of *G. uralensis* root. Licochalcone A was isolated as one of the potent relaxant components by relaxant activity-guided fractionation from the extract and identified by comparison with the reported ^{1}H and ^{13}C NMR spectral data. It was possible that licochalcone A exerted its relaxant effect on smooth muscle contraction through inhibition of cAMP PDE.

References

Barfod, L., Kemp, K., Hansen, M., Kharazmi, A. (2002) Chalcones from Chinese liquorice inhibit proliferation of T cells and production of cytokines. *Int. Immunopharmacol*. **2**: 545–555

Fukai, T., Marumo, A., Kaitou, K., Kanda, T., Terada, S., Nomura, T. (2002) Anti-*Helicobacter pylori* flavonoids from licorice extract. *Life. Sci*. **71**: 1449–1463

Hatano, T., Fukuda, T., Liu, T.-Z., Noro, T., Okuda, T. (1991) Phenolic constituents of licorice. IV. Correlation of phenolic constituents of licorice specimens from various sources, and inhibitory effects of licorice extracts on xanthine oxidase and monoamine oxidase. *Yakugaku Zasshi* **111**: 311–321

Itoh, T., Kanmura, Y., Kuriyama, H. (1985) A23187 increases calcium permeability of store sites more than of surface membranes in the rabbit mesenteric artery. *J. Physiol*. **359**: 467–484

Katsura, T. (1995) The remarkable effect of Kanzo-to and Shakuyakukanzo-to in the treatment of acute abdominal pain. *Jpn. J. Orient. Med*. **46**: 293–299

Kusano, A., Nikaido, T., Kuge, T., Ohmoto, T., Monache, G. D., Botta, B., Botta, M., Saitoh, T. (1991) Inhibition of adenosine 3′,5′-cyclic monophosphate phosphodiesterase by flavonoids from licorice roots and 4-arylcoumarins. *Chem. Pharm. Bull*. **39**: 930–933

Nagai, H., Yamamoto, Y., Sato, Y., Akao, T., Tani, T. (2006) Pharmaceutical evaluation of cultivated *Glycyrrhiza uralensis* roots in comparison of their antispasmodic activity and glycycoumarin contents with those of licorice. *Biol. Pharm. Bull*. **29**: 2442–2445

Ohmura, I. (1976) Action mechanisms of the contracting drugs, K, acetylcholine, histamine and Ba and of the antispasmodics, isoproterenol

and papaverine in the isolated guinea pig ileum, particularly in relation to Ca. *Nippon Yakurigaku Zasshi* **72**: 201–210

Olukoga, A., Donaldson, D. (2000) Liquorice and its health implications. *J. R. Soc. Health*. **120**: 83–89

Park, E. J., Park, H. R., Lee, J. S., Kim, J. (1998) Licochalcone A: an inducer of cell differentiation and cytotoxic agent from *Pogostemon cablin. Planta Med*. **64**: 464–466

Saitoh, T., Shibata, S. (1975) New type chalcones from licorice root. *Tetrahedron Lett*. **50**: 4461–4462

Sato, Y., Akao, T., He, J. X., Nojima, H., Kuraishi, Y., Morota, T., Asano, T., Tani, T. (2006) Glycycoumarin from Glycyrrhizae Radix acts as a potent antispasmodic through inhibition of phosphodiesterase 3. *J. Ethnopharmacol*. **105**: 409–414

Sato, Y., He, J. X., Nagai, H., Tani, T., Akao, T. (2007) Isoliquiritigenin, one of the antispasmodic principles of *Glycyrrhiza uralensis* roots, acts in the lower part of intestine. *Biol. Pharm. Bull*. **30**: 145–149

Shibata, S. (2000) A drug over the millennia: pharmacognosy, chemistry, and pharmacology of licorice. *Yakugaku Zasshi* **120**: 849–862

Shibata, S., Inoue, H., Iwata, S., Ma, R., Yu, L., Ueyama, H., Takayasu, J., Hasegawa T., Tokuda, H., Nishino, A., Nishino, H., Iwashima, A. (1991) Inhibitory effects of licochalcone A isolated from *Glycyrrhiza inflata* root on inflammatory ear edema and tumor promotion in mice. *Planta Med*. **57**: 221–224

Wang, Q. E., Lee, F. S., Wang, X. (2004) Isolation and purification of inflacoumarin A and licochalcone A from licorice by high-speed counter-current chromatography. *J. Chromatogr. A*. **1048**: 51–57

Yamamoto, Y., Tani, T. (2006) Brief survey on *Glycyrrhiza* plant resources in Xinjiang, China. *J. Trad. Med*. **23**: 27–35