J Pharmacol Sci **108**, 439 – 445 (2008)

Journal of Pharmacological Sciences

Full Paper

Inhibitory Effects of Isoliquiritigenin and Licorice Extract on Voltage-Dependent K^+ Currents in H9c2 Cells

Chisato Noguchi[1], Jing Yang[2], Kazuho Sakamoto[3], Ryo Maeda[3], Kenji Takahashi[3], Hiroshi Takasugi[4], Tomoyuki Ono[3], Masahiro Murakawa[1], and Junko Kimura[3,*]

Departments of [1]Anesthesiology and [3]Pharmacology, Fukushima Medical University, School of Medicine, Fukushima 960-1295, Japan
[2]Department of Pharmacology, School of Medicine, Wuhan University, Wuhan, China
[4]Drug Research Section, Tokyo Research Laboratories, TOA EIYO Ltd., Saitama 330-0834, Japan

Received August 29, 2009; Accepted October 1, 2008

Abstract. The effect of isoliquiritigenin (ISL), a component of licorice, on the voltage-dependent, ultra-rapidly activating delayed-rectifier K^+ current (IKur) was examined in H9c2 cells, a cell-line derived from rat cardiac myoblasts. IKur was recorded using the whole-cell patch clamp method with a pipette solution containing 140 mM K^+. Depolarizing voltage pulses of 200-ms duration were given with 10-mV steps every 10 s from −40 mV holding potential. ISL inhibited IKur in a concentration-dependent manner. The median inhibitory concentration (IC_{50}) of ISL was approximately 0.11 μM and the Hill coefficient was 0.71. Using CHO cells expressing Kv1.5 IKur channels, ISL also inhibited Kv1.5 IKur, but less potently than the IKur current in H9c2 cells. Furthermore, in H9c2 cells, the licorice extract itself inhibited IKur in a manner similar to ISL. We conclude that ISL, one component of licorice, is a potent inhibitor of K^+ channels, which specifically in H9c2 cells could be Kv2.1, and that this inhibition may be involved in various pharmacological effects of licorice.

Keywords: isoliquiritigenin, ultra-rapidly activating delayed-rectifier K^+ current, H9c2 cell, Kv2.1, Kv1.5

Introduction

K^+ channels represent the most diverse class of ion channels in the heart and are the targets of several anti-arrhythmic drugs (1). In the human heart, K^+ channels are classified as voltage-gated and non-voltage-gated. Voltage-gated K^+ channels include rapidly activating and inactivating transient outward current (Ito), delayed rectifer currents with ultra-rapid (IKur), rapid (IKr), and slow (IKs) activation components. Non-voltage-gated K^+ channels include inwardly rectifying K^+ currents such as the inward-rectifier K^+ current (IK_1), ATP-sensitive K^+ current (IK_{ATP}), and acetylcholine-activated K^+ current (IK_{ACh}) (2). Inward rectifier K^+ currents regulate the resting membrane potential, whereas voltage-activated currents control action potential duration (1). There are expression pattern variations of K^+ channels in different myocardial cell types. Each K^+ current could be a target of therapeutic drugs in various cardiac disorders. Therefore, pursuing selective K^+-channel inhibitors has been continued both for clinical reasons and basic scientific interests in clarifying characteristics of each K^+ channel.

In the human heart, IKur is present in the atrium but not the ventricle (3). Therefore, IKur is the predominant current responsible for human atrial repolarization (4, 5). Drugs that specially inhibit IKur may provide means of preventing supra-ventricular arrhythmia without the risk of ventricular pro-arrhythmia (6). Atrial fibrillation is a highly prevalent arrhythmia. Reduction in refractory period and action potential duration are associated with onset and maintenance of chronic atrial fibrillation. Blocking IKur and subsequent prolongation of the action potential duration are expected to be beneficial in chronic atrial fibrillation (3). The concept of blocking

*Corresponding author. jkimura@fmu.ac.jp
Published online in J-STAGE
doi: 10.1254/jphs.08227FP

IKur as a therapeutic target in atrial fibrillation is widely accepted (7). Wettwer et al. demonstrated that 4-aminopyridine, which inhibits Ito and IKur in human atrial myocytes with IC_{50} values of 1 mM and 8 μM, respectively, prolonged the action potential duration of human atrial trabeculae in atrial fibrillation and increased the force of contraction in a concentration-dependent manner in sinus rhythm and atrial fibrillation (3).

Licorice is one of the oldest and most important natural drugs. It is included in 70% – 80% of Chinese traditional herbal medicines, which have been used in both Eastern and Western cultures for thousands of years (8, 9). Commercial licorice products are derived from extracts of the plant root system (8). In addition to medicine, licorice is also widely used as a food additive and a cosmetic throughout the world (8).

Licorice contains major bioactive triterpenoid glycosides such as glycyrrhizin and glycyrrhetic acid and many phenolic compounds such as glabridin, glycycoumarin, licochalcone, liquiritigenin, liquiritin, liquiritin apioside, isoliquiritin, isoliquiritigenin, and their corresponding aglycones. Licorice possesses many therapeutic properties, including effects against gastric ulcer, cough, sputa, convulsion, inflammation, and allergy, and hepatoprotective effects (8, 9). Adverse effects of licorice include hypertension, hypokalaemia, myopathy, muscle weakness, edema, cardiac dysrhythmias, and loss of consciousness (10, 11).

Isoliquiritigenin (ISL) is a flavonoid contained in licorice with a simple chalcone structure 4,2',4'-trihydroxychalcone (Fig. 1). ISL has been shown to exert various actions including vasorelaxant, antioxidant, anti-platelet, anti-tumor, anti-allergic, and antispasmodic (on intestine) effects and has estrogenic properties (12 – 18). In addition, ISL also has protective effects on the heart and the brain from ischemia-reperfusion injury (19, 20). In cardiac myocytes, it has been reported that ISL induces a positive inotropic effect by increasing the L-type Ca^{2+} current (21). However, the effect of ISL on K^+ currents is unknown. Therefore, we examined the effect of ISL and licorice on a class of voltage-dependent K^+ channels, IKur, which is probably mediated by Kv2.1, in H9c2 cells derived from rat cardiac myoblasts. We also compared the effect of ISL on Kv1.5 IKur channels expressed in CHO cells.

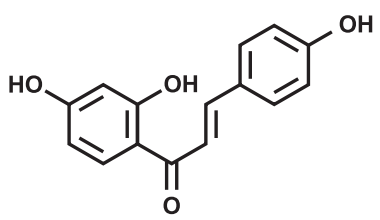

Fig. 1. The chemical structure of Isoliquiritigenin (ISL).

Materials and Methods

Cell culture

H9c2 cells (Dai-Nihon Seiyaku, Osaka) were maintained in Dulbecco's modified Eagle's medium (Wako, Osaka) containing penicillin G (Banyu Pharmaceutical, Tokyo), and streptomycin (Meiji Seika, Tokyo) supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere of 10% CO_2 and 90% air at 37°C.

CHO (Chinese Hamster Ovary) cells were stably expressed with human Kv1.5 and were kindly provided by Toa-Eiyo Co., Ltd. (Fukushima). CHO cells were maintained in Hams' F-12 medium (Sigma-Aldrich, St. Louis, MO, USA) containing 10% FBS, penicillin (Invitrogen, Carlsbad, CA, USA), and geneticin 200 μg /ml (Sigma-Aldrich). Cells were incubated in 37°C with 95% air and 5% CO_2.

For electrophysiological recordings, cells were separated from the culture dish using a Ca^{2+}-free solution containing 0.02% trypsin and then placed in the recording chamber.

Patch-clamp recording

Membrane currents were recorded by the whole-cell patch clamp method. The recording chamber was attached to the stage of an inverted microscope (Model 80121; Nikon, Tokyo). Cells were superfused with Tyrode solution at a rate of 1 ml/min. The temperature of the bath solution was maintained at 37°C with a water jacket. Patch pipettes were forged from glass capillaries with 1.5-mm internal diameter and 2.1-mm external diameter (Nihon Rikagaku Kikai, Tokyo) using a micro-electrode puller (pp-83; Narishige, Tokyo). Pipette resistance was 2 – 4 MΩ when the pipette was filled with pipette solution. The pipette solution consisted of 120 mM KOH, 20 mM KCl, 3 mM $MgCl_2$, 5 mM MgATP, 5 mM HEPES (4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid), 5 mM BAPTA (1,2-bis (2-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid), and 50 mM aspartic acid (pH 7.2 with aspartic acid). Tyrode solution contained 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 0.33 mM NaH_2PO_4, 5 mM HEPES, and 5.5 mM glucose (pH 7.4 with 2.4 mM NaOH). The electrode was connected to a patch-clamp amplifier (TM-1000; Act ME, Tokyo). Recording signals were filtered at 2.5-kHz bandwidth, and the series resistance was compensated. Current signals were stored online and analyzed using pClamp Version 9

(Axon Instruments, Union City, CA, USA). Depolarizing voltage pulses of 200-ms duration were given with 10-mV step increments every 10 s from the holding potential (HP) of −40, −50, or −60 mV over the voltage range between −50 and 100 mV. The current magnitude was measured at 60 mV for analyzing the concentration-response relationship.

Drugs

ISL used in this study is from the same origin as in the experiments described by Zhan and Yang (20). Briefly, *G. glabra* roots were collected in Yili, Xinjiang Province, China and were identified by Dr. Mingxi Jiang of Wuhan Institute of Botany, Chinese Academy of Sciences, Wuhan, China. The air-dried roots of *G. glabra* were crushed into powder and then extracted with ethylacetate (EtOAc) three times for 12 h each at room temperature. The EtOAc extract was subjected to silica gel column chromatography and further separated to obtain ISL (a yellow amorphous solid) (Fig. 1) as described previously (22).

ISL was dissolved in dimethylsulphoxide (DMSO) as a stock solution of 100 mM and added to the extracellular solutions to obtain a desired concentration. The final concentration of DMSO was <0.1%, which did not affect the K^+ current.

Licorice powder was purchased from Tsumura & Co. (Tokyo). Licorice powder was dissolved in Tyrode solution at 3 g/l. Then, the solution was spun at 3000 rpm for 10 min. The resulting supernatant was diluted with the Tyrode extracellular solution to obtain a desired licorice concentration.

Data analyses

All values are presented as mean ± S.E.M. The percent inhibition of the current magnitude was measured at 60 mV for various ISL concentrations. The median inhibitory concentration (IC_{50}) and the Hill coefficient values were obtained by using Origin version 6.1 (Microcal, CA, USA) on a Windows computer. The concentration-response curve was fitted using the following logistic equation:

$$I = 14.2 + (100 - 14.2) / \{1 + ([ISL] / IC_{50})^{nH}\}$$

where [ISL] indicates the ISL concentration, IC_{50} is the median inhibitory concentration of ISL, and nH is an empirical parameter describing the steepness of the fit, which has the same meaning as the Hill coefficient.

Results

Effects of ISL on outward K^+ current

In H9c2 cells, an ultra-rapidly activating, slowly inactivating outward current was elicited by a series of depolarizing voltage pulses with 200-ms duration from −40 mV to a voltage range between −30 and 100 mV (Fig. 2A). ISL at 0.3 μM suppressed this K^+ outward current (Fig. 2B). Figure 2C illustrates the ISL-sensitive currents, which were obtained by subtraction of the family of currents in the presence of ISL (Fig. 2B) from the control currents in the absence of ISL (Fig. 2A). The averaged current-voltage (I-V) relationships were obtained in the control and in the presence of 0.3 μM ISL from four cells (Fig. 2D). The average I-V curve of the net ISL-sensitive current is shown in Fig. 2E.

To obtain a concentration-response curve of ISL,

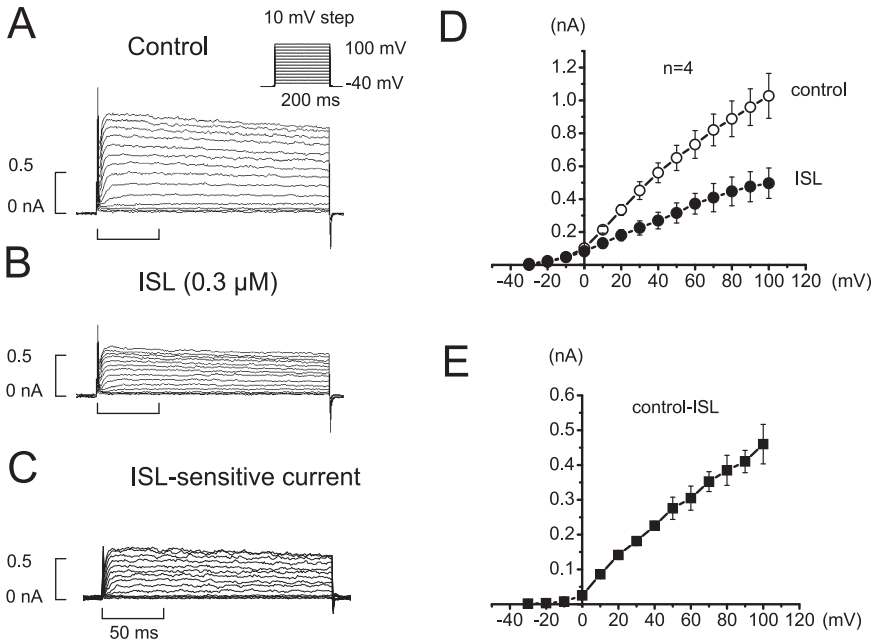

Fig. 2. Effect of ISL on IKur in H9c2 cells. A: Representative IKur recorded under control conditions. The voltage steps are shown in the inset. B: IKur in the presence of 0.3 μM ISL. IKur was substantially suppressed by ISL. Voltage pulses were the same as in panel A. C: Net ISL-sensitive current obtained by subtracting B from A. D: Averaged isochronal I-V curves plotted from the control (open circles) and in the presence of 0.3 μM ISL (closed circles). The current magnitude was measured at the end of 200-ms pulses from four different cells and averaged. E: Averaged I-V curve of ISL-sensitive current. The current was measured at the end of 200-ms pulses from four different cells and averaged.

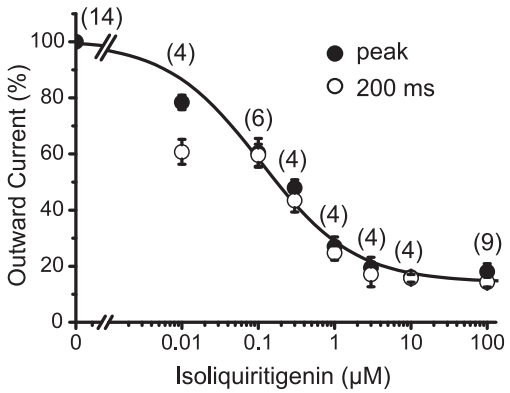

Fig. 3. Concentration–response relationships between outward current and ISL. The current amplitude was measured at 60 mV at the peak current (closed circles) and at the end of the 200-ms depolarizing pulses (open circles). The mean ± S.E.M. values are indicated. Numbers in parenthesis indicate the number of cells. The IC_{50} value was 0.11 μM and the Hill coefficient was 0.71.

various ISL concentrations between 10 nM and 100 μM were applied (Fig. 3). The current amplitude was measured at the peak current and at the end of the 200-ms depolarizing pulses. The percent inhibition of the K^+ currents was calculated at 60 mV for each ISL concentration. There was no time dependence in the inhibitory effect of ISL between the peak and at the end of the 200-ms pulse. A sigmoidal fitting of the curve yielded an IC_{50} value of 0.11 μM with a Hill coefficient of 0.71 (Fig. 3).

Effect of ISL on Kv1.5 IKur expressed in CHO cells

Kv1.5 is another IKur type of voltage-dependent K^+ channels in cardiac myocytes (23). We tested whether ISL inhibited Kv1.5 by using CHO cells that stably expressed Kv1.5 K^+ channels. As shown in a representative record in Fig. 4, Kv1.5 channel current developed as an ultra-rapidly activating, slowly inactivating outward current. Series of depolarizing square voltage pulses with 200-ms duration were given from −50 mV to a range between −40 and 50 mV. ISL at 100 μM suppressed this K^+ outward current by about 60% in this cell (Fig. 4B). The averaged current-voltage (I-V) relationships were obtained in the control and in the presence of 100 μM ISL from three cells (Fig. 4C). ISL at 100 μM inhibited the Kv1.5 current to 38.3 ± 3.3% at 40 mV. This result indicates that ISL inhibits Kv1.5 less potently than IKur in H9c2 cells.

Effect of licorice extract on IKur in H9c2 cells

Since ISL is a constituent of licorice and licorice is often used as an ingredient in Chinese herbal medicines, we investigated whether licorice extract itself affected

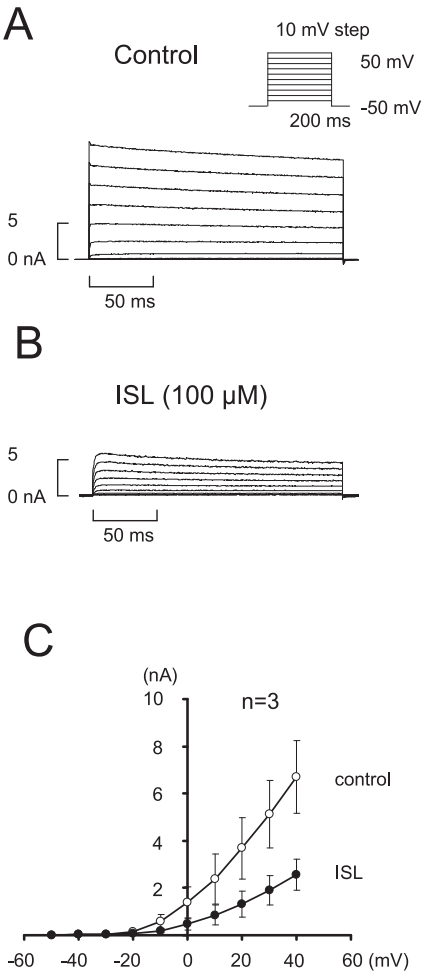

Fig. 4. Effect of ISL on Kv1.5 in CHO cells. A: Representative Kv1.5 current recorded under control conditions. The voltage steps are shown in the inset. B: Kv1.5 current in the presence of 100 μM ISL. Voltage pulses were the same as in panel A. C: Averaged isochronal I-V curves plotted from the control (open circles) and in the presence of 100 μM ISL (closed circles). The current magnitude was measured at the end of 200-ms pulses from three different cells and averaged.

IKur. In H9c2 cells, licorice extract inhibited IKur in a manner similar to ISL (Fig. 5: A and B). The averaged I-V relationships were obtained in the control and in the presence of 10 μg/ml licorice from four cells (Fig. 5C). The concentration–response curve of licorice was obtained with various licorice concentrations (1 – 300 μg/ml) (Fig. 5D). Percent inhibition of the K^+ currents was calculated by measuring the current amplitudes at +60 mV at the end of the 200-ms depolarizing pulses in the control and in the presence of licorice (Fig. 5D). Licorice inhibited IKur dose-dependently up to 10 μg /ml, which achieved a maximum IKur inhibition of about 50%, with no further increase in inhibition even at higher licorice concentrations.

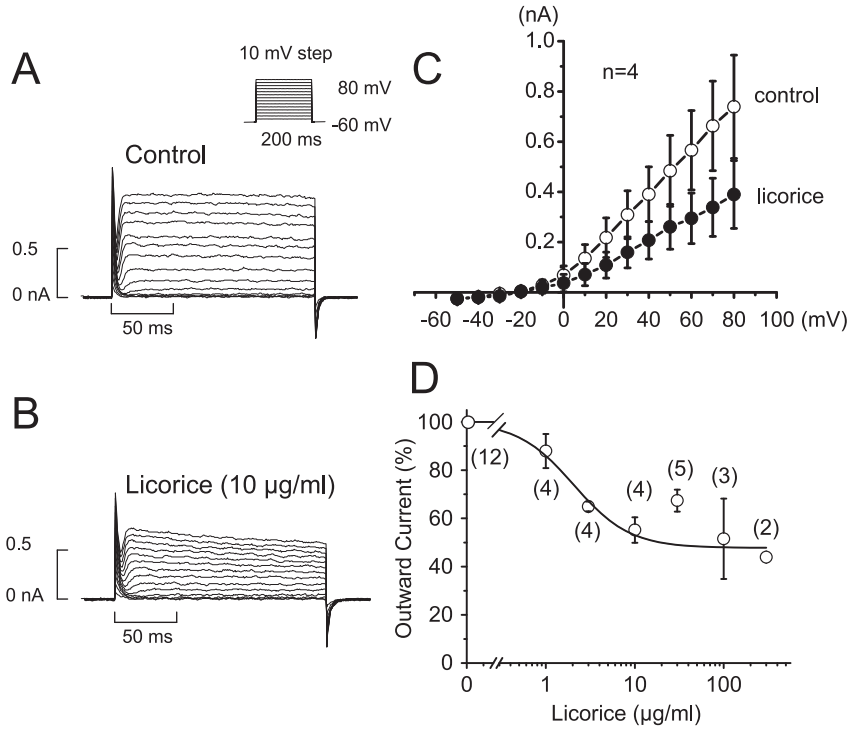

Fig. 5. Effect of licorice extract on IKur in H9c2 cells. A: IKur recorded under control conditions. The voltage steps are shown in the inset. B: IKur in the presence of 10 μg/ml licorice. IKur was substantially suppressed by licorice. Voltage pulses are the same as in (A). C: Averaged isochronal I-V curves plotted from the control (open circles) and in the presence of 10 μg/ml licorice (closed circles). The current magnitude was measured at the end of 200-ms pulses from four different cells and averaged. D: Concentration–response curve between outward current and licorice. The current magnitude was measured at 60 mV. The mean ± S.E.M. values are indicated. Numbers in parenthesis indicate the number of cells.

Discussion

The present study demonstrated for the first time that ISL inhibits IKur-type K^+ outward current in a concentration-dependent manner in H9c2 cells. There are two types of voltage-dependent K^+ channel genes known to be responsible for cardiac IKur, namely, Kv1.5 and Kv2.1 (23 – 25). Suzuki et al. previously examined the expression of voltage-dependent K^+ channel subunits by the reverse transcriptase polymerase chain reaction (RT-PCR) in H9c2 cells and demonstrated H9c2 cells abundantly expressed Kv2.1 mRNA, but did not express Kv1.5 mRNA (26). Wang et al. also reported that H9c2 cells expressed Kv2.1 mRNA predominantly, while no Kv1.5 mRNA was detected (27). Therefore, the ISL-sensitive IKur in this study in H9c2 cells was most likely mediated by Kv2.1 channels.

To date, there have been only a few inhibitors of Kv2.1 channels reported. In H9c2 cells, thiopental and nifedipine inhibited Kv2.1 IKur with IC_{50} values of 97 and 9.1 μM, respectively (26). Celecoxib, an inhibitor of cyclooxygenase-2, inhibited rat Kv2.1 channels expressed in HEK-293 cells with an IC_{50} of 10.3 μM (28). More recently, Kitamura et al. found in our laboratory that a plant alkaloid anti-cancer agent, paclitaxel, inhibited IKur in H9c2 cells in a time-dependent manner with an IC_{50} of about 11 μM (29). In the present study on H9c2 cells, we found that ISL inhibited IKur with an IC_{50} of 0.11 μM. Apart from hanatoxin, a spider venom peptide, which binds and inhibits Kv2.1 with a K_d value of 42 nM (30), ISL seems to have the lowest IC_{50} value among the Kv2.1-channel inhibitors so far reported. Therefore, assuming that IKur in H9c2 cells is Kv2.1, the blocking effect of ISL on IKur in H9c2 cells is more potent than any other synthetic blocker reported to date.

In our hands, ISL at 100 μM inhibited Kv1.5 IKur by only 60%, while it could almost fully inhibit IKur in H9c2 cells. Therefore, ISL is a selective inhibitor of Kv2.1 over the Kv1.5 channels. In human atrial myocytes, IKur, which was probably mediated by Kv1.5, was inhibited by nifedipine, diltiazem, and verapamil with IC_{50} values of 8.2, 11.2, and 3.2 μM, respectively (31, 32). Since the IKur in H9c2 cells was inhibited by nifedipine with an IC_{50} value of 9.1 μM (26), unlike ISL, nifedipine seems to inhibit both Kv2.1 and Kv1.5 channels with similar potency.

In the heart, both Kv2.1 and Kv1.5 are expressed in the human atrium (3). It has been reported that Kv1.5 underlies the major component of the delayed-rectifier K^+ current in human atrial myocytes (2). Van Wagoner et al. suggested the presence of Kv2.1 protein in human atrial tissue (25). However, Bertaso et al. examined the relative expression of mRNAs for the voltage-gated K^+ channel subunits in samples of human atrial appendage and could not detect Kv2.1 expression (33). The reason for this discrepancy is unclear. Xu et al. showed that the expression patterns of Kv α-subunits vary during development of the heart (34). Therefore, there is a

possibility that Kv2.1 expression levels may vary depending on the developmental stages and also during the course of physiological or pathological conditions of the heart.

It has been well established that expression levels of ion channels in the cardiac myocytes change during the course of various heart diseases; these phenomena are called ion-channel–remodeling and as a result, cardiac action potential shapes are altered (35). Atrial fibrillation induces shortening of action potential duration (APD) and effective refractory period, known as electrical remodeling, which is associated with changes in expression and activity of the involved ion channels (36). On one hand, human IKur was found to be reduced by 50% in chronic atrial fibrillation, whereas others reported no change in IKur (25, 37). Van Wagoner et al. reported that Kv1.5 protein expression levels were reduced in chronic human atrial fibrillation, while there was no significant difference in the expression levels of Kv2.1 protein (25). In such a case where Kv1.5 was reduced and as a result the relative importance of Kv2.1 was increased, the blocking effect of drugs such as ISL on Kv2.1 might become relatively more effective.

We found that licorice also suppressed IKur in H9c2 cells in a concentration-dependent manner (Fig. 5). Nakamura et al. reported that the amount of ISL contained in aqueous licorice extracts is 0.03% – 0.30% (38). If the ISL content in the licorice extract is 0.3%, 10 μg/ml of licorice contains 0.03 μg/ml of ISL. Since the molecular weight of ISL is 256.23, ISL at 0.03 μg/ml is equivalent to 0.12 μM. Thus, 10 μg/ml licorice contains about 0.1 μM of ISL. According to Fig. 5D, IKur was suppressed to about 50% of the control by 10 μg/ml licorice. In Fig. 3, IKur was suppressed by 0.1 μM ISL to about 60% of the control. Therefore, the inhibitory effect of ISL itself and that of licorice, which is supposed to contain an equivalent amount of ISL, are surprisingly similar. However, as shown in Fig. 5D, inhibition of IKur by licorice at concentrations higher than 10 μg/ml plateaued at approximately 50%. In contrast, ISL inhibited IKur in a dose-dependent manner and 100 μM ISL inhibited IKur completely. The reason for this difference may be that licorice extract contains many components, and therefore the inhibition of IKur by ISL might be masked by other components, which have an effect opposite to that of ISL.

In summary, the present study demonstrated for the first time that ISL is a potent inhibitor of IKur, which in H9c2 cells is most likely Kv2.1, with an IC_{50} of 0.11 μM. This effect of ISL may be involved in various pharmacological actions of licorice extract or ISL itself in different organs and tissues.

Acknowledgments

This work was supported in part by Grants-in-Aid from the Japan Foundation for Promotion of Science to JK (No. 17590223) and the Smoking Research Foundation (KI18003) to JK. The authors thank Ms. Sanae Sato for her technical help.

References

1 Deal KM, England SK, Tamkun M. Molecular physiology of cardiac potassium channels. Physiol Rev. 1996;76:49–67.
2 Tamargo J, Caballero R, Gomez R, Valenzuela C, Delpon E. Pharmacology of cardiac potassium channels. Cardiovasc Res. 2004;62:9–33.
3 Wettwer E, Hala O, Christ T, Heubach JF, Dobrev D, Knaut M, et al. Role of IKur in controlling action potential shape and contractility in the human atrium. Circulation. 2004;110:2299–2306.
4 Nattel S, Yue L, Wang Z. Cardiac ultrarapid delayed rectifiers. A novel potassium current family of functional similarity and molecular diversity. Cell Physiol Biochem. 1999;9:217–226.
5 Wang Z, Fermini B, Nattel S. Sustained depolarization-induced outward current in human atrial myocytes. Evidence for a novel delayed rectifier K^+ current similar to Kv1.5 cloned channel currents. Circ Res. 1993;73:1061–1076.
6 Brendel J, Peukert S. Blockers of the Kv1.5 channels for treatment of atrial arrhythmias. Exp Opin Ther Pat. 2002;12:1589–1598.
7 Nattel S. New ideas about atrial fibrillation 50 years on. Nature.2002;415:219–226.
8 Isbrucker RA, Burdock GA. Risk and safety assessment on the consumption of Licorice root (Glycyrrhiza sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. Regul Toxicol Pharmacol. 2006;46:167–192.
9 Armanini D, Fiore C, Mattarello MJ, Bielenberg J, Palermo M. History of the endocrine effects of licorice. Exp Clin Endocrinol Diabetes. 2002;110:257–261.
10 Eriksson JW, Carlberg B, Hillorn V. Life-threatening ventricular tachycardia due to liquorice-induced hypokalaemia. J Int Med. 1999;245:307–310.
11 Yasue H, Itoh T, Mizuno Y, Harada E. Severe hypokalemia, rhabdomyolysis, muscle paralysis, and respiratory impairment in hypertensive patient taking herbal medicines containing licorice. Intern Med. 2007;46:575–578.
12 Yu SM, Kuo SC. Vasorelaxant effect of isoliquiritigenin,a novel soluble guanylate cyclase activator, in rat aorta. Br J Pharmacol. 1995;114:1587–1594.
13 Vaya J, Belinky PA, Avira M. Antioxidant constituents from licorice root : isolation, structure elucidation and antioxidative capacity toward LDL oxidation. Free Radic Biol Med. 1997;23:302–313.
14 Tawata M, Aida K, Noguchi T, Ozaki Y, Kume S, Sasaki H, et al. Anti-platelet action of isoliquiritigenin, an aldose reductase inhibitor in licorice. Eur J Pharmacol. 1992;212:87–92.
15 Kanazawa M, Satomi Y, Mizutani Y, Ukimura O, Kawauchi A, Sakai T, et al. Isoliquiritigenin inhibits the growth of prostate cancer. Eur Urol. 2003;43:580–586.

16 Kakegawa H, Matsumoto H, Satoh T. Inhibitory effects of some natural products on the activation of hyaluronidase and their anti-allergic actions. Chem Pharm Bull. 1992;40:1439–1442.
17 Sato Y, He J, Nagai H, Tani T, Akao T. Isoliquiritigenin, one of the antispasmodic principles of *Glycyrrhiza Ularensis* Roots, acts in the lower part of intestine. Biol Pharm Bull. 2007;30:145–149.
18 Tamir S, Eizenberg M, Somjen D, Izrael S, Vaya J. Estrogen-like activity of glabrene and other constituents isolated from licorice root. J Steroid Biochem Mol Biol. 2001;78:291–298.
19 An W, Yang J, Ao Y. Metallothionein mediates cardioprotection of isoliquiritigenin against ischemia-reperfusion through JAK2/STAT3 activation. Acta Pharmacol Sin. 2006;27:1431–1437.
20 Zhan C, Yang J. Protective effect of isoliquiritigenin in transient middle cerebral artery occlusion-induced focal cerebral ischemia in rats. Pharmacol Res. 2006;53:303–309.
21 Wegener JW, Nawrath H. Cardiac effects of isoliquiritigenin. Eur J of Pharmacol. 1997;326:37–44.
22 Baba M, Ohmura M, Kishi N, Okada Y, Shibata S, Peng J, et al. Saponins isolated from Allium chinese G. Don and antitumor-promoting activities of isoliquiritigenin and laxogenin from the same drug. Biol Pharm Bull. 2000;23:660–662.
23 Fedida D, Wible B, Wang Z, Fermini B, Faust F, Nattel S, et al. Identity of a novel delayed rectifier current from human heart with a cloned K^+ channel current. Circ Res. 1993;73:210–216.
24 Ordog B, Brutyo E, Puskas LG, Papp JG, Varro A, Szabad J, et al. Gene expression profiling of human cardiac potassium and sodium channels. Int J Cardiol. 2006;111:386–393.
25 Van Wagoner DR, Pond AL, McCarthy PM, Trimmer JS, Nerbonne JM. Outward K^+ current densities and Kv1.5 expression are reduced in chronic human atrial fibrillation. Circ Res. 1997;80:772–781.
26 Suzuki H, Momoi N, Ono T, Maeda S, Shikama Y, Matsuoka I, et al. Inhibitory effect of thiopental on ultra-rapid delayed rectifier K^+ current in H9c2 cells. J Pharmacol Sci. 2005;99:177–184.
27 Wang W, Hino N, Yamasaki H, Aoki T, Ochi R. Kv2.1 K^+ channels underlie major voltage-gated K^+ outward current in H9c2 myoblasts. Jpn J Physiol. 2002;52:507–514.
28 Frolov RV, Berim IG, Singh S. Inhibition of delayed rectifier potassium channels and induction of arrhythmia. J Biol Chem. 2008;283:1518–1524.
29 Kitamura N, Sakamoto K, Ono T, Kimura J. [Inhibitory effect of paclitaxel on K^+ current (Kv2.1) in H9c2 Cells.] Folia Pharmacol Jpn (Nippon Yakurigaku Zasshi). 2008;131:6P. (in Japanese)
30 Swartz KJ, Mackinnon R. An inhibitor of the Kv2.1 potassium channel isolated from the venom of a Chilean tarantula. Neuron. 1995;15:941–949.
31 Gao Z, Sun H, Chiu SW, Lau CP, Li GR. Effects of diltiazem and nifedipine on transient outward and ultrarapid delayed rectifier potassium currents in human atrial myocytes. Br J Pharmacol. 2005;144:595–604.
32 Gao Z, Lau CP, Chiu SW, Li GR. Inhibition of ultra-rapid delayed rectifier K^+ current by verapamil in human atrial myocytes. J Mol Cell Cardiol. 2004;36:257–263.
33 Bertaso F, Sharpe CC, Hendry BM, James AF. Expression of voltage-gated K^+ channels in human atrium. Basic Res Cardiol. 2002;97:424–433.
34 Xu H, Dixon JE, Barry DM, Trimmer JS, Merlie JP, McKinnon D, et al. Developmental analysis reveals mismatches in the expression of K^+ channel α subunits and voltage-gated K^+ channel currents in rat ventricular myocytes. J Gen Physiol. 1996;108:405–419.
35 Nattel S, Maguy A, Bouter SL, Yen YH. Arrhythmogenic ion-channel remodeling in the heart: heart failure, myocardial infarction, and atrial fibrillation. Physiol Rev. 2007;87:425–456.
36 Dobromir D, Ravens U. Remodeling of cardiomyocyte ion channels in human atrial fibrillation. Basic Res Cardiol. 2003; 98:137–148.
37 Brandt MC, Priebe L, Bohle T, Sudkamp M, Beuckelmann DJ. The ultrarapid and the transient outward K^+ current in human atrial fibrillation. Their possible role in postoperative atrial fibrillation. J Mol Cell Cardiol. 2000;32:1885–1896.
38 Nakamura R, Kase Y, Hashimoto K, Sakakibara I, Amagaya S, Aburada M. Elucidation of anti-gastric ulcer constituents of Glycyrrhizae Radix. Nat Med. 2003;57:172–177.