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Cardiac effects of isoliquiritigenin

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Abstract

The effects of isoliquiritigenin on force of contraction (F_c), L-type Ca^{2+} current (I_{Ca}) and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were investigated in rat ventricular heart muscle. Isoliquiritigenin increased F_c and I_{Ca} and, after longer exposure times, resting tension and $[Ca^{2+}]_i$. The effect of isoliquiritigenin (100 μM) on I_{Ca} was diminished by Rp-cAMPS (30 μM). 1*H*-[1,2,4]oxa- diazolo[4,3-*a*]quinoxalin-1-one (50 μM) did not influence the effects of isoliquiritigenin on F_c and I_{Ca}. The positive inotropic effects of isoprenaline and forskolin, but not of 3-isobutyl-1-methylxanthine, were potentiated by isoliquiritigenin (100 μM). In the presence of milrinone (10 μM), no further effects of isoliquiritigenin (100 μM) on F_c and I_{Ca} were observed. It is suggested that the increase in F_c and I_{Ca} by isoliquiritigenin is due to an accumulation of cyclic AMP. These effects are probably unrelated to an effect of the drug on soluble guanylyl cyclase, as reported for smooth muscle, but rather due to a direct inhibition of phosphodiesterase III activity.

Keywords: Heart; Guanylyl cyclase; Phosphodiesterase; Ca^{2+} current, L-type; Force of contraction; Fura-2

1. Introduction

The second messengers cyclic AMP and cyclic GMP may play opposing roles in their influence on myocardial contractility (Nawrath, 1976). It is well established that the cyclic AMP pathway accounts for the myocardial effects of several neurotransmitters and hormones (Reuter, 1983). The molecular mechanism of action involves receptor-mediated activation of adenylyl cyclase by stimulatory GTP-binding proteins which increases intracellular cyclic AMP levels. Cyclic AMP, in turn, activates cyclic AMP-dependent protein kinases leading to phosphorylation of L-type Ca^{2+} channels resulting in an increase in macroscopic L-type Ca^{2+} current (I_{Ca}) and cardiac contraction (Trautwein and Osterrieder, 1986). The turn-off of the cyclic AMP signal is achieved by breakdown of cyclic AMP through the activity of cyclic AMP-phosphodiesterases (Beavo, 1988).

On the other hand, the physiological role of the cyclic GMP pathway is less well characterised. Muscarinic agonists have been shown to enhance cyclic GMP levels as well as the activity of cyclic GMP-dependent protein kinases in heart muscle (Goldberg et al., 1975; Lincoln and Keely, 1981). However, inhibitors of cyclic GMP-dependent protein kinase have been shown to antagonize the effect of a cyclic GMP analogue (8-Br-cyclic GMP) but not of acetylcholine in rat atria (Bäumner and Nawrath, 1995) and the idea that cyclic GMP may serve as the second messenger for the acetylcholine-mediated regulation of cardiac contractility has continually been challenged (Brooker, 1977; Hartzell, 1988).

The combination of patch-clamp techniques with intracellular perfusion techniques has helped to elucidate the molecular mechanisms possibly involved in the cardiac actions of cyclic GMP. Three main pathways have been demonstrated: (1) activation of cyclic GMP-dependent protein kinase which induces inhibition of myocardial I_{Ca} in several species (Levi et al., 1989; Méry et al., 1991; Tohse and Sperelakis, 1991); (2) activation of cyclic GMP-stimulated phosphodiesterase which accounts for the inhibition of I_{Ca} stimulated by cyclic AMP and isoprenaline in cardiomyocytes from frogs (Fischmeister and Hartzell, 1987); and (3) blockade of the cyclic GMP-inhibited phosphodiesterase which results in an increase in I_{Ca} in mammalian cardiomyocytes if the concentration of cyclic AMP is not saturated (Ono and Trautwein, 1991). These obser-

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vations clearly suggest a role of cyclic GMP as a second messenger for regulating cardiac contractility.

Cyclic GMP levels are thought to be increased by the activity of guanylyl cyclase in response to various stimuli (MacFarland, 1995). Two forms of guanylyl cyclase are present in the heart. One is a membrane-bound receptor molecule elevating cyclic GMP levels when stimulated with atrial natriuretic factor (Rugg et al., 1989; McCall and Fried, 1990). The second form is a soluble enzyme which is activated by nitric oxide (NO) and NO-related compounds such as glycerol trinitrate, sodium nitroprusside or 3-morpholino-sydnonimine (SIN-1) (Ignarro, 1990; Stamler et al., 1992; Ishibashi et al., 1993). Studies using NO donors have led to conflicting results with respect to the regulation of cardiac contractility (reviewed by Hare and Colucci, 1995). The variable results may be also due to the fact that NO produces various effects independent of guanylyl cyclase stimulation (Bolotina et al., 1994; Koh et al., 1995).

Recently, isoliquiritigenin, a chalcone derivative isolated from the plant *Dalbergia odorifera T.*, has been described to exert vasorelaxant effects by activating soluble guanylyl cyclase and thereby increasing cyclic GMP levels in rat aorta (Yu and Kuo, 1995). It would be interesting to know whether isoliquiritigenin may generally serve as a tool to activate soluble guanylyl cyclase in various tissues, independent from NO. In this study, we investigated the effects of isoliquiritigenin on myocardial contractility, I_{Ca}, and $[Ca^{2+}]_i$ in the rat heart.

2. Materials and methods

2.1. Preparations

Sprague-Dawley rats (200–300 g) of either sex were anaesthetised with ether and bled from the carotid arteries. The hearts were quickly removed and immersed into warmed and oxygenated Tyrode's solution. Ventricular strips were cut off from the right ventricle and supplied at two ends with silk ligatures. Single ventricular myocytes were isolated as described previously (Wegener and Nawrath, 1995). Briefly, the hearts were enzymatically digested by perfusion with a collagenase-containing buffer solution via the aorta using a Langendorff setup. Single myocytes were obtained from ventricular tissue pieces by mechanical dispersion.

2.2. Experimental procedures

Ventricular strips were mounted next to two platinum electrodes built in a muscle holder. They were then placed in organ baths (5 ml) containing oxygenated Tyrode's solution at 37 ± 1°C and connected via stainless-steel wires to an inductive force-displacement transducer whose output was fed to a frequency preamplifier (Carrier amplifier/TA2000, Gould, Cleveland, OH, USA). Resting tension was set at 10 mN. The muscle preparations were electrically stimulated by square-wave voltage pulses (Grass S4, 1 ms duration, voltage 20% above threshold) at 1 or 3 Hz. The results obtained at 1 or 3 Hz were not significantly different. Drugs were added from stock solutions to the organ bath as single or repeatedly applied doses to achieve the final concentrations as indicated.

Electrophysiological experiments were performed on rod-shaped myocytes with clear cross-striations using the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981). The bath solution contained (in mM) NaCl 137, CsCl 5.4, $MgCl_2$ 0.5, $CaCl_2$ 1.8, glucose 5, HEPES 10; pH was adjusted to 7.4 with NaOH. Membrane currents of single myocytes were recorded with an Axopatch amplifier (200A, Axon, Foster City, CA, USA), if the seal resistance was > 5 GΩ. The recording patch pipettes were built from borosilicate glass (Science Products, Frankfurt, Germany). The resistances of the pipettes ranged from 1.3 to 1.5 MΩ, when filled with pipette solution (composition in mM: CsCl 125, $MgCl_2$ 6, $CaCl_2$ 0.15, Na_2ATP 5, Na_2GTP 0.1, EGTA 5, HEPES 10; pH was adjusted to 7.4 with CsOH). Before the formation of the membrane seals, junction potentials between the pipette and bath solution were corrected. The current signal was filtered at 1 kHz, digitized at 3 kHz using an A/D-D/A converter (DigiData 1200 system, Axon) and stored on an IBM-compatible computer running pClamp 6 software (Axon) which was additionally used for the generation of voltage pulses and data analysis. During the experiments, the myocytes were voltage-clamped at a holding potential of -80 mV. To inactivate the fast sodium current, a 15 ms prepulse to -40 mV was set before activating the Ca^{2+} current. L-type Ca^{2+} currents (I_{Ca}) were elicited by 180 ms depolarising voltage pulses to 0 mV at 0.2 Hz. The experiments were performed at 36 ± 1°C. Solutions in the bath compartment could be changed within 1 min.

Fluorescence measurements were performed on single cardiomyocytes using an inverted microscope (Olympus, Hamburg, Germany) equipped for epifluorescence (Photon Technology International, Brunswick, NJ, USA). The myocytes were loaded with the acetoxymethylester form of the fluorescent Ca^{2+} indicator fura-2 (fura-2-AM; 3 μM) for 30 min at room temperature. After loading, the cells were washed and centrifuged twice at $50 \times g$ to remove extracellular dye. The cells were resuspended and stored in bath solution for 45 min to allow complete conversion of fura-2-AM to fura-2-free acid. Single cells were sequentially excited at 340 and 380 nm wavelength light using two monochromators at a switching frequency of 100 Hz controlled by an optical chopper. The emission light signals at 510 nm wavelength were focused on a photon counting device, digitized and stored on an IBM-compatible computer running Felix software (Photon Technology International) which was additionally used for data analy-

sis. The experiments were performed at 36°C. Solutions in the bath compartment could be changed within 1 min.

2.3. Chemicals

All salts and solvents used were at least p.a. grade and purchased from Sigma (St. Louis, MO, USA), unless otherwise indicated. Na_2GTP was obtained from Boehringer-Mannheim (Mannheim, Germany), HEPES and 3-isobutyl-1-methylxanthine (IBMX) from Serva (Heidelberg, Germany), isoliquiritigenin from Sigma, fura-2-AM from Calbiochem (Bad Soden, Germany), Rp-cAMPS from Biomol (Hamburg, Germany), and 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ) from Tocris Cookson (Bristol, UK). Stock solutions of fura-2-AM, forskolin, isoliquiritigenin, and ODQ were prepared in dimethyl sulfoxide (DMSO) and further diluted to achieve the final bath concentration. The final amount of DMSO did not exceed 0.2% (vol.).

2.4. Evaluation of results

Data are presented as original recordings or expressed as means ± S.E.M. (in % of control values or in % of maximal effects). F_c was measured as the difference between resting and peak tension. Changes in resting tension were expressed in % of F_c under control conditions. Peak I_{Ca} was measured as the difference of peak inward current and steady-state current value at the end of the voltage pulse. $[Ca^{2+}]_i$ was expressed as the ratio of fura-2 emission spectra at 510 nm obtained at 340 and 380 nm excitation wavelengths which provides a relative measure of $[Ca^{2+}]_i$ (Grynkiewicz et al., 1985). Concentration–response curves were fitted by sigmoidal functions (correlation coefficient > 0.99) using GraphPad Prism 2.0 (GraphPad Software, San Diego, CA, USA). Statistical analysis was performed using either paired or unpaired Student's *t*-test. *P*-values < 0.05 were considered as significant.

3. Results

3.1. Effects of isoliquiritigenin on F_c and I_{Ca}

After the addition of isoliquiritigenin (100 μM), force of contraction (F_c) transiently declined and then increased to maximal values within 2 min (Fig. 1a). The initial decline of F_c was exclusively seen when the solvent DMSO (0.2 vol.%) was added without isoliquiritigenin (not shown). The positive inotropic effect of isoliquiritigenin was accompanied by an increase in L-type Ca^{2+} current (I_{Ca}; Fig. 1b). On an average, isoliquiritigenin (100 μM) increased F_c by 51 ± 7% within 2 ± 0.4 min ($n = 15$; means ± S.E.M.) and peak I_{Ca} by 66 ± 6% within 1 ± 0.1 min ($n = 14$; means ± S.E.M.; Fig. 3).

3.2. Effects of Rp-cAMPS on the increase in I_{Ca} induced by isoliquiritigenin

Rp-cAMPS, an inhibitor of cyclic AMP-dependent protein kinase (Schaap et al., 1993), was used to investigate whether the effects of isoliquiritigenin were mediated by intracellular cyclic AMP. In control experiments, the increase by isoprenaline (30 nM) of peak I_{Ca} was reduced from 62 ± 8% ($n = 5$; means ± S.E.M.) to 10 ± 3% ($n = 3$; means ± S.E.M.) by intracellular Rp-cAMPS (30 μM) (data not shown). Rp-cAMPS (30 μM) also inhibited the effect of isoliquiritigenin on I_{Ca} (Fig. 2). Isoliquiritigenin (100 μM) increased peak I_{Ca} by 66 ± 6% ($n = 14$; means ± S.E.M.) under control conditions and by 18 ± 4% ($n = 5$; means ± S.E.M.) in the presence of intracellular Rp-cAMPS (30 μM).

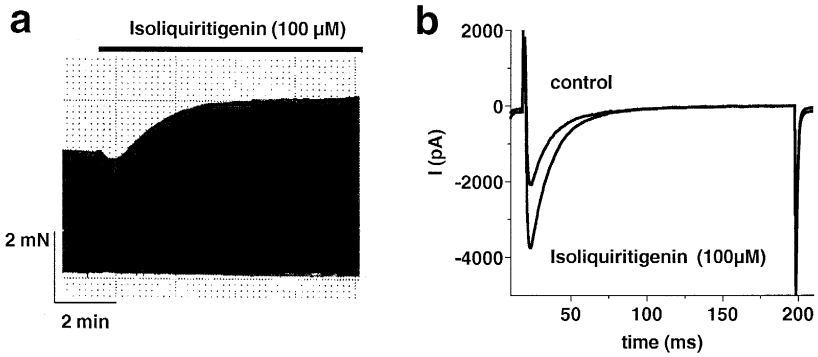

Fig. 1. Effects of isoliquiritigenin on F_c in ventricular strips and I_{Ca} in single ventricular myocytes from rat heart. (a) Time course of F_c. The bar indicates the presence of isoliquiritigenin (100 μM). (b) Original recordings of I_{Ca}. Current traces under control conditions and in the presence of isoliquiritigenin (100 μM) are superimposed.

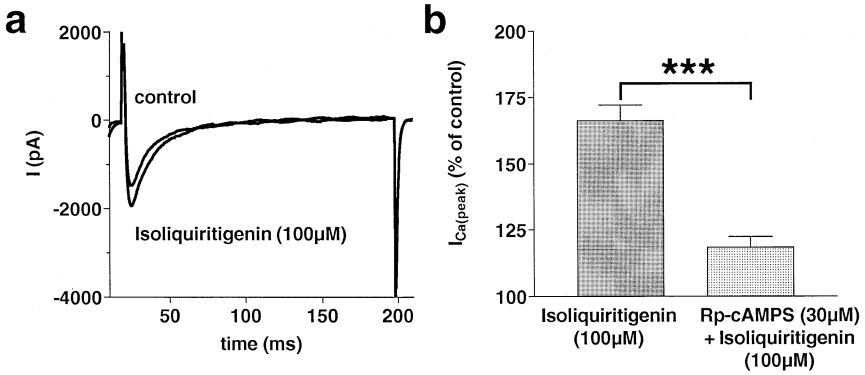

Fig. 2. Effect of Rp-cAMPS on the increase in I_{Ca} induced by isoliquiritigenin. (a) Original recordings of I_{Ca} after intracellular application of 30 μM Rp-cAMPS. Current traces under control conditions and in the presence of isoliquiritigenin (100 μM) are superimposed. (b) Effects of isoliquiritigenin (100 μM) on the peak current value of I_{Ca} ($I_{Ca(peak)}$) under control conditions and in the presence of intracellular Rp-cAMPS (30 μM). Columns represent means ± S.E.M. ($n = 5–14$). The asterisks denote a statistically significant difference ($P < 0.001$) between the columns, evaluated by unpaired Student's *t*-test.

3.3. Effects of ODQ on the increase in F_c and I_{Ca} induced by isoliquiritigenin

ODQ, an inhibitor of soluble guanylyl cyclase (Garthwaite et al., 1995), was used to clarify whether activation of soluble guanylyl cyclase is involved in the positive inotropic effect of isoliquiritigenin. The concentration-dependent effects of isoliquiritigenin on F_c were not influenced by ODQ (50 μM; Fig. 3a). The EC_{50} values of the concentration–response curves of isoliquiritigenin

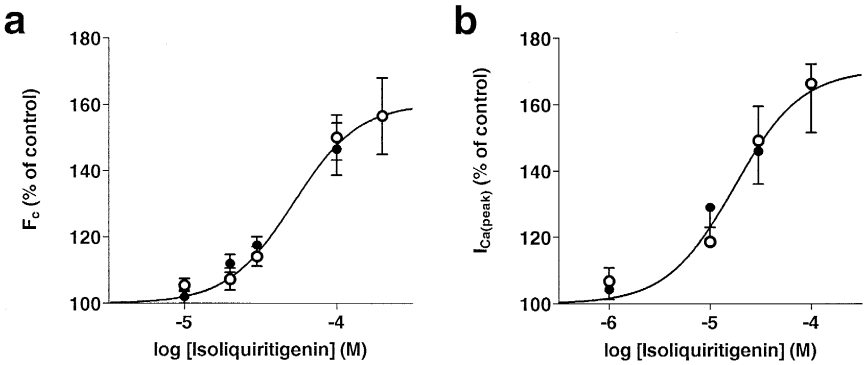

Fig. 3. Effects of 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ) on the concentration-dependent increase of F_c and I_{Ca} induced by isoliquiritigenin. Isoliquiritigenin was added cumulatively at 3 min intervals using three concentrations in each experiment. (a) Concentration–response (F_c) curves of isoliquiritigenin under control conditions (○) and in the presence of 50 μM ODQ (●). The EC_{50} values of the curves amounted to 45 μM and to 47 μM, respectively. Data represent means ± S.E.M. ($n \geq 5$ each). (b) Concentration–response ($I_{Ca(peak)}$) curves of isoliquiritigenin under control conditions (○) and in the presence of 50 μM ODQ (●). The EC_{50} values amounted to 18 μM in the absence and to 17 μM in the presence of 50 μM ODQ. Data represent means ± S.E.M. ($n \geq 6$ each).

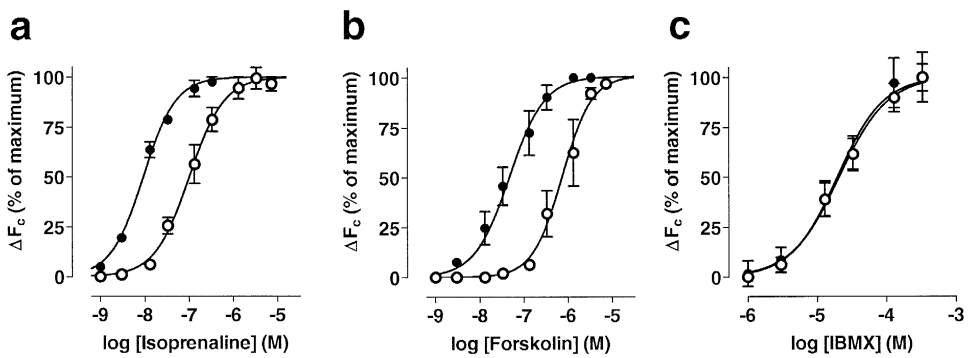

Fig. 4. Effects of isoliquiritigenin on the positive inotropic response to isoprenaline, forskolin and IBMX. Cumulative concentration–response (F_c) curves of isoprenaline (a), forskolin (b), and IBMX (c) are shown under control conditions (○) and in the presence of isoliquiritigenin (●). The EC_{50} values amounted to 100 nM and 9.3 nM for isoprenaline, to 750 nM and 47 nM for forskolin, and to 21 μM and 20 μM for IBMX in the absence and presence of isoliquiritigenin (100 μM), respectively. Data represent means ± S.E.M. ($n \geq 5$ each).

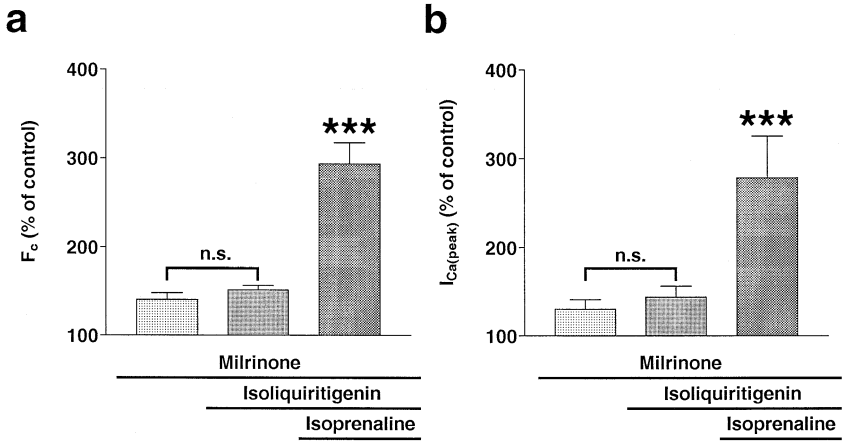

Fig. 5. Effects of isoliquiritigenin on F_c and I_{Ca} in the presence of milrinone. The columns represent F_c (a) and the peak current value of I_{Ca} ($I_{Ca(peak)}$) (b) after the addition of (1) milrinone (10 μM), (2)isoliquiritigenin (100 μM), and (3) isoprenaline (100 nM) as indicated by the bars. Drugs were added sequentially at 5-min intervals. Steady-state effects of drugs were evaluated after 3 min each. Columns represent means ± S.E.M. ($n \geq 4$ each). The asterisks indicate a statistically significant difference ($P < 0.001$), whereas no statistically significant difference is marked by n.s., evaluated by paired Student's *t*-test.

amounted to 45 μM under control conditions and to 47 μM in the presence of ODQ, respectively. Likewise, ODQ (50 μM) did not influence the effect of isoliquiritigenin on I_{Ca} (Fig. 3b). The concentration–response curves revealed an EC_{50} value of 18 μM under control conditions and of 17 μM in the presence of ODQ (50 μM). ODQ itself had no significant effect either on F_c or on I_{Ca}.

3.4. Effects of isoliquiritigenin on the potency of isoprenaline, forskolin, and IBMX

Since the positive inotropic effect of isoliquiritigenin was dependent on intracellular cyclic AMP, but not on soluble guanylyl cyclase activity, the interaction of isoliquiritigenin with other substances which induce an increase in intracellular cyclic AMP levels was studied. Isoliquiritigenin potentiated the effects of isoprenaline and forskolin, but not of IBMX, on F_c (Fig. 4) suggesting that the effects of isoliquiritigenin are related to an inhibition of phosphodiesterase activity. The EC_{50} of isoprenaline amounted to 100 nM under control conditions and to 9.3 nM in the presence of isoliquiritigenin (100 μM). Likewise, the EC_{50} of forskolin was changed by isoliquiritigenin (100 μM) from 750 nM to 47 nM. In contrast, the EC_{50} of IBMX remained unchanged by isoliquiritigenin and amounted to 21 μM under control conditions and to 20 μM in the presence of isoliquiritigenin (100 μM).

3.5. Effects of isoliquiritigenin on F_c and I_{Ca} in the presence of milrinone

The positive inotropic effects of phosphodiesterase inhibitors are generally ascribed to inhibition of the phospho-

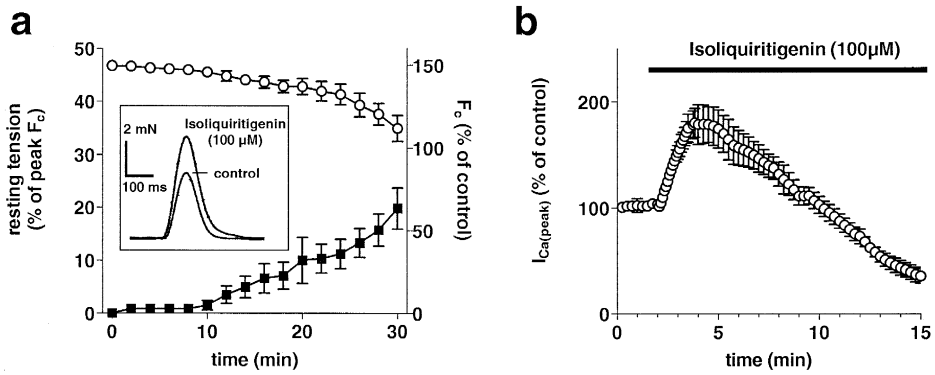

Fig. 6. Effects of isoliquiritigenin on resting tension, F_c, single contractions and I_{Ca} after longer exposure times. (a) Time-course of resting tension (■) and force of contraction (○) after isoliquiritigenin (100 μM) had reached its maximal effect on F_c ($t = 0$). Data represent means ± S.E.M. ($n = 3$). The inset shows recordings of superimposed single contractions under control conditions and 7 min after application of isoliquiritigenin (100 μM). (b) Time-course of the peak current values of I_{Ca} ($I_{Ca(peak)}$) during application of isoliquiritigenin (100 μM). The bar indicates the presence of isoliquiritigenin (100 μM). Data represent means ± S.E.M. ($n = 3$).

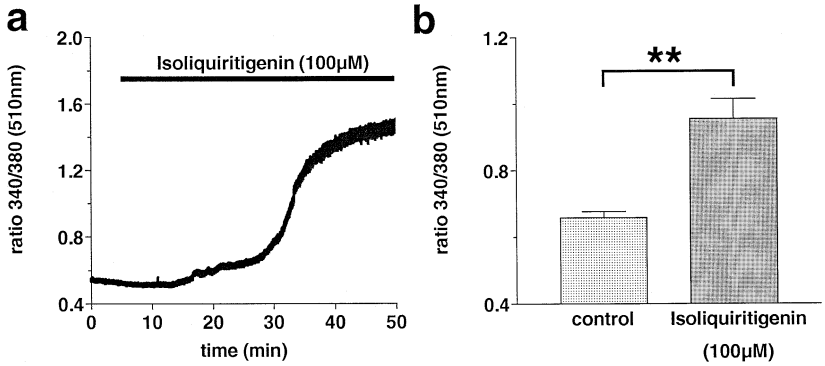

Fig. 7. Effect of isoliquiritigenin on $[Ca^{2+}]_i$ in resting cardiomyocytes. $[Ca^{2+}]_i$ is expressed as the 510 nm emission wavelength ratio at 340 and 380 nm excitation wavelengths. (a) Original recording demonstrating the time course of $[Ca^{2+}]_i$ during application of isoliquiritigenin (100 μM). The bar indicates the presence of isoliquiritigenin (100 μM). (b) $[Ca^{2+}]_i$ under control conditions and 30 min after the addition of isoliquiritigenin (100 μM). The asterisks denote a statistically significant difference ($P < 0.01$) between the columns revealed by paired Student's *t*-test. Data represent means ± S.E.M. ($n = 6$).

diesterase III subtype (Reeves and England, 1990). Milrinone, a selective inhibitor of phosphodiesterase III (K_i = 0.26 μM; Reeves and England, 1990), was used to investigate whether this type of phosphodiesterase is also involved in the effects of isoliquiritigenin on F_c and I_{Ca}. Milrinone (10 μM) increased F_c and I_{Ca} within 5 min to 140 ± 8% ($n = 10$) and to 130 ± 11% ($n = 4$), respectively (Fig. 5). Further addition of isoliquiritigenin (100 μM) did not significantly increase F_c and I_{Ca}. In the presence of both drugs, F_c and I_{Ca} amounted to 151 ± 5% ($n = 10$) and to 144 ± 12% ($n = 4$), respectively. However, further addition of isoprenaline (100 nM) increased F_c and I_{Ca} to 294 ± 24% ($n = 10$) and to 278 ± 47% ($n = 4$), respectively.

3.6. Delayed effects of isoliquiritigenin on F_c and I_{Ca}

The immediate enhancing effects of isoliquiritigenin on F_c and I_{Ca} were followed by a decline in the magnitude of both parameters (Fig. 6). In ventricular strips, peak F_c returned virtually to control values within 30 min; in addition, resting tension increased (Fig. 6a). The inset in Fig. 6a shows that the time-course of single contractions was slightly prolonged by isoliquiritigenin (100 μM). Peak I_{Ca} declined to values far below the control values within 15 min (Fig. 6b); under control conditions, peak I_{Ca} decreased to 94 ± 3% within the same time interval ($n = 5$; means ± S.E.M.), reflecting a slight run-down of I_{Ca} also without drug (data not shown). Additionally, a shortening of the myocytes was observed after longer exposure times (8–12 min) to isoliquiritigenin (100 μM).

3.7. Effect of isoliquiritigenin on $[Ca^{2+}]_i$

Isoliquiritigenin (100 μM) increased $[Ca^{2+}]_i$ in resting cardiomyocytes (Fig. 7). Fig. 7a shows the time-course of the 510 nm emission ratio of fluorescent signals at 340 and 380 nm excitation wavelengths in response to isoliquiritigenin (100 μM). In all experiments, the effects developed within 10 min; steady-state values were reached within 30–40 min. On an average, ratios amounted to 0.66 ± 0.22 ($n = 6$; means ± S.E.M.) under control conditions and to 0.96 ± 0.06 ($n = 6$; means ± S.E.M.) in the presence of isoliquiritigenin (100 μM) indicating that $[Ca^{2+}]_i$ was increased to 145% (Fig. 7b).

4. Discussion

The present study has demonstrated that isoliquiritigenin increases F_c and I_{Ca} in ventricular heart muscle preparations from rats. These effects of isoliquiritigenin are probably mediated by cyclic AMP since Rp-cAMPS, an inhibitory cyclic AMP analogue (Schaap et al., 1993), antagonized the effects of the drug on I_{Ca}. These results, in connection with those of Yu and Kuo (1995), led us to assume that isoliquiritigenin may increase cyclic GMP levels in the heart by activating soluble guanylyl cyclase, thereby inhibiting a cyclic GMP-dependent phosphodiesterase (type III). This mechanism of action was also suggested for the potentiation of β-adrenergic stimulation of I_{Ca} by cyclic GMP in guinea pig ventricular cells (Ono and Trautwein, 1991). However, the cardiac effects of isoliquiritigenin were not influenced by ODQ which has been shown to be a potent inhibitor of soluble guanylyl cyclase (IC_{50} = 10–60 nM) inhibiting the NO-dependent cyclic GMP response to glutamate receptor agonists in rat neuronal tissue (Garthwaite et al., 1995) and inhibiting the relaxation in rat vascular smooth muscle induced by NO donors (Moro et al., 1996). Therefore, it seems unlikely that the stimulatory effects of isoliquiritigenin on F_c and I_{Ca} are indeed mediated by activation of soluble guanylyl cyclase and a subsequent increase in cyclic GMP levels in the rat heart.

How can the cardiac effects of isoliquiritigenin on F_c and I_{Ca} which are obviously dependent on cyclic AMP then be explained? If the guanylyl cyclase/cyclic GMP pathway is not involved, a direct inhibition of cyclic

AMP-hydrolysis may be accomplished by isoliquiritigenin. This view is supported by the findings that isoliquiritigenin enhances the effects of isoprenaline and forskolin, but not of the phosphodiesterase inhibitor IBMX, on F_c. Indeed, it has been shown that isoliquiritigenin acts as an inhibitor of bovine cardiac phosphodiesterase activity (type I and III) under cell-free conditions (Kusano et al., 1991). The IC_{50} value (18 μM) for inhibition of phosphodiesterase by isoliquiritigenin found in this study correlates well with the EC_{50} values for the stimulating effects of isoliquiritigenin on F_c and I_{Ca} obtained in our study in multicellular preparations and single cells (46 and 18 μM, respectively). Milrinone (10 μM), a selective inhibitor of phosphodiesterase III (Reeves and England, 1990), increased F_c and I_{Ca}. In the presence of this drug, isoliquiritigenin exerted no significant further effects, unlike isoprenaline, whose effects were potentiated in the presence of milrinone. These findings suggest, in connection with the lack of ODQ to influence the action of isoliquiritigenin, that isoliquiritigenin inhibits phosphodiesterase III activity in the rat heart also under physiological conditions. Thereby, the stimulatory effects on the adenylyl cyclase/cyclic AMP pathway are strongly enhanced by isoliquiritigenin.

Isoliquiritigenin induced a delayed decrease in F_c and I_{Ca} following the immediate stimulatory effects on these parameters. Simultaneously, resting tension increased in multicellular preparations. In addition, an increase in $[Ca^{2+}]_i$ was observed after longer exposure times (30 min) of resting cardiomyocytes to isoliquiritigenin. These secondary effects were not influenced by ODQ or Rp-cAMPS indicating that cyclic nucleotides are not involved in these effects. It remains speculative whether these effects are due to accumulation of the lipophilic drug within the cell membrane or reflect an inhibition of other enzymes. For example, aldose reductase is also inhibited by isoliquiritigenin (Aida et al., 1990).

In summary, it is concluded that the increase in F_c and I_{Ca} in myocardial preparations, induced by isoliquiritigenin, is related to a direct inhibition of phosphodiesterase III activity and the subsequent accumulation of cyclic AMP. In contrast to the action of isoliquiritigenin in rat aorta (Yu and Kuo, 1995), activation of soluble guanylyl cyclase is probably not involved in the effects of isoliquiritigenin in rat heart.

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