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Luteolin-induced coronary arterial relaxation involves activation of the myocyte voltage-gated K^+ channels and inward rectifier K^+ channels

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ABSTRACT

Aims: Luteolin has been shown to be beneficial to cardiovascular tissues and organs. We aimed to study its vasospasmolytic effects against various vasoconstrictors in the isolated rat coronary arteries (RCAs) and its electrophysiological effects on K^+ currents via voltage-gated potassium (Kv) channels and inward rectifier potassium (Kir) channels in freshly isolated rat coronary arterial smooth muscle cells (RCASMCs).

Main methods: The vascular tone of the endothelium-denuded RCAs was recorded by a wire myograph. Kv currents and Kir currents in RCASMCs were assessed using whole-cell patch clamp.

Key findings: Preincubation with luteolin depressed the contractions elicited by KCl, thromboxane A_2 analog U46619, vasopressin, Kir blocker $BaCl_2$, Kv blocker 4-aminopyridine and elevation of extracellular calcium ($[Ca^{2+}]_o$) in high K^+ depolarizing solution. Instant application of luteolin produced concentration-dependent relaxations in the endothelium-denuded RCAs precontracted with KCl or U46619. Both 4-aminopyridine and $BaCl_2$ attenuated luteolin-induced relaxation in U46619-precontracted RCAs, while neither nitric oxide synthetase inhibitor NG-nitro-L-arginine methyl ester nor cyclooxygenase inhibitor indomethacin affected the relaxation. Luteolin augmented both Kv currents and Kir currents in RCASMCs and the augmentations were antagonized by 4-aminopyridine and $BaCl_2$, respectively.

Significance: The present results demonstrated that luteolin antagonizes various vasoconstrictors in RCAs and augments both Kv currents and Kir currents in RCASMCs, suggesting that the direct action of luteolin on Kv channels and Kir channels is contributory to its vasospasmolytic effect. These findings indicate that luteolin may be a promising food additive with the aim of preventing coronary arterial spasm.

1. Introduction

In the last several decades, many flavonoid compounds have been reported to be beneficial to the prevention and treatment of cardiovascular diseases [1,2]. Luteolin (3, 4, 5, 7-tetrahydroxy flavone, Fig.1) is a flavonoid naturally present in many vegetables and fruits such as parsley, sweet peppers, celery, as well as many medicinal plants [3–5]. Luteolin has been reported to possess a variety of biological and pharmacological activities, including anti-tumor [6,7], anti-inflammation [8,9], anti-oxidative stress and anti-apoptosis [10,11]. Cardiovascular studies showed that luteolin depressed hypertensive aorta remodeling in spontaneous hypertensive rat [12], relaxed porcine coronary [13] and aortic [14] arteries and improved cardiac function after myocardium ischemia/reperfusion injury. Luteolin is also anti-angiogenesis [15]. Epidemiological study showed that high dietary intake of luteolin is related to a decreased risk of acute myocardial infarction [16,17].

Cardiomyocyte blood supply depends on the coronary arterial resistance that is determined by the tone of coronary arterial smooth muscle cells, which is regulated by a variety of ion channels including K^+ channels. Concentration of K^+ inside vascular myocyte is much higher than outside. K^+ channel activation results in K^+ efflux increase, which leads to a chain of events including the cell membrane hyperpolarization, $[Ca^{2+}]_o$ influx depression and prevention of the myocyte contraction [18]. Thus, K^+ channels play an important role in the regulation of vascular tone in resistance arteries and arterioles [19–22]. K^+ conductance across vascular myocyte membrane is mainly determined by voltage-dependent K^+ channels (Kv), inwardly rectifier K^+ channels (Kir), Ca^{2+}-activated K^+ channels (K_{Ca}) and ATP-activated K^+ channels (K_{ATP}). Kv channels [19,23–25] and Kir channels [26–28]

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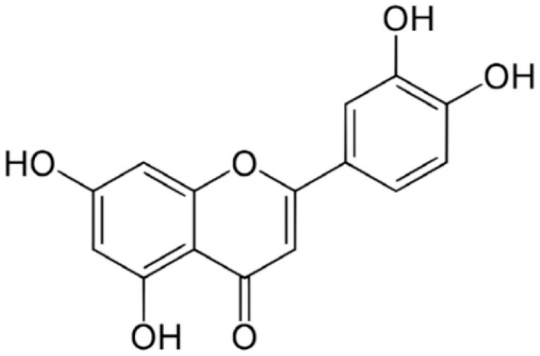

Fig. 1. Luteolin (3, 4, 5, 7-tetrahydroxy flavone).

are of great significance for the arterial tone regulation of coronary arterioles.

The effects of luteolin on K^+ channels in coronary arterial smooth muscle cells and their contribution to its vasorelaxation have not been well elucidated. The present studies aimed to study the effect of luteolin on the contractions induced by various vasoconstrictors in isolated rat coronary arteries (RCAs) and on the K^+ currents via Kv channels and Kir channels in rat coronary arterial smooth muscle cells (RCASMCs).

2. Materials and methods

2.1. Drugs and chemicals

Luteolin (HPLC > 98%) was purchased from PERFEMIKER (Shanghai Canspec Scientific Instruments Co). It was dissolved in dimethyl sulfoxide (DMSO) to make stock solutions and was kept at −20 °C. The stock solution was diluted with proper saline solution. The final concentration of DMSO in the bath was < 0.1%. 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), 9, 11-dideoxy-9α, 11α-methanoepoxy prostaglandin $F_{2\alpha}$ (U46619), $BaCl_2$, KCl, 4-aminopyridine (4-AP), indomethacin (Indo), NG-nitro-L-arginine methyl ester (L-NAME), vasopressin (VP), acetylcholine, tetraethylammonium (TEA), ethylene glycol-bis (2-aminoethylether)-N, N, N′, N′-tetraacetic acid (EGTA), bovine serum albumin, papain, collagenase F, collagenase H, dithiothreitol, D-glucose, Mg-ATP, K_2-ATP were purchased from Sigma-Aldrich (St. Louis, MO, USA). Indo was dissolved in DMSO and other reagents were all dissolved in distilled water.

2.2. Animals

Adult healthy Male Wistar rats (270–320 g) were provided by the Animal Facility Center of Shanxi Medical University, China. Animals were anesthetized with intraperitoneal administration of sodium pentobarbital (40 mg/kg) before exsanguination. After sacrifice, RCAs (250–350 μm in inner diameter) were isolated for myograph study. The single RCASMCs were dispersed enzymatically for patch clamp study.

2.3. Measurement of arterial tension and tissue bath solutions

After sacrifice, the rat heart was removed and RCAs were gently isolated in 4 °C HEPES solution containing (in mM): 144 NaCl, 5.8 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 5 HEPES, 11 D-glucose, pH = 7.4 [29]. RCAs were cut into cylindrical rings of 2 mm-long. To observe the direct effects of luteolin on RCASMCs, the endothelium was denuded mechanically by carefully penetrating a fine stainless-steel wire through the vessel lumen and rubbing gently for several times. The arterial rings were threaded on two stainless-steel wires (25 μm- diameter) and mounted in a 5 ml chamber of a wire myograph system (Model 610 M, Danish Myo Technology A/S, Denmark). The tissue chambers were filled with K−H solution containing (mM): 118 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgCl_2$, 20 $NaHCO_3$, 10 HEPES, 2.5 $CaCl_2$. K−H solution was continuously bubbled with 95% O_2 and 5% CO_2, 37 °C. The arterial tension was normalized to a transmural pressure equivalent to 80 mmHg according to the standard procedures. After equilibration for 1 h in the bath, the vessels were contracted repeatedly with 60 mM KCl to make sure that the responses of the vessels were repeatable. In the experiments with K^+-induced contraction, KCl substituted for NaCl on an equimolar basis. In the vasorelaxation experiments, the effects of vasodilators were observed only when the precontraction was relatively sustained. When 1 μM acetylcholine-induced relaxation on 60 mM KCl-induced contraction was < 20%, the endothelium was considered denuded [29]. After each contraction, the vessels were washed three times with fresh preheated (37 °C) K−H solution until it restored to the basal tension. The bath solution in the myograph chamber was changed every 15 min with fresh preheated K−H solution or corresponding solution throughout the experiment.

2.4. Effects of luteolin on contractions induced by KCl, U46619, 4-AP, $BaCl_2$ and VP

Contractive responses to cumulatively increasing concentrations of KCl (20–108 mM), U46619 (0.01–10 μM), $BaCl_2$ (0.05–30 mM), 4-AP (0.05–30 mM), VP (0.1–10 μM) were recorded. The responses to each concentration of a stimulator were allowed to develop until a relatively stable plateau was reached. When the successive concentration-contraction curves were repeatable, the vessels were washed with drug-free preheated K−H solution several times and allowed to recover to the basal tension. The vessels were incubated with luteolin (10, 30 or 100 μM) for 15 min before the concentration-contraction curves were reconstructed again in the presence of luteolin. At the end of the experiment, the curves were reconstructed in the absence of luteolin to verify luteolin effects, the reversibility of luteolin effects and the vessel vitality. The contractions induced by 108 mM KCl, 10 μM U46619, 30 mM $BaCl_2$, 30 mM 4-AP, 10 μM VP in the absence of luteolin were taken as 100%, respectively. Contractive responses to each concentration of a vasoconstrictor were compared between before and after treatment with luteolin.

2.5. Effects of luteolin on the contraction induced by $[Ca^{2+}]_o$ elevation in high K^+ depolarizing solution

When the contraction induced by 60 mM KCl was repeatable, the rings were rinsed with and incubated for 20 min in Ca^{2+}-free K−H solution containing 1 mM EGTA. Afterwards, the bath solution was changed to Ca^{2+}-free high K^+ (60 mM KCl) K−H solution without EGTA. 10 min later, $CaCl_2$ (0.03, 0.1, 0.3, 1, 3, 10 and 30 mM) was cumulatively added to the bath to construct the concentration- contraction curves. When $CaCl_2$ curves were repeatable, the vessels were incubated with luteolin (10, 30 or 100 μM) for 20 min before the curves were reconstructed again in the presence of luteolin.

2.6. Effects of luteolin on the precontractions induced by KCl or U46619

When the precontractions induced by 60 mM KCl or 0.3 μM U46619 were repeatable, luteolin was cumulatively added into the tissue chamber. The end concentrations of luteolin in the chamber were 1, 3, 10, 30 and 100 μM. The vascular responses to each concentration of luteolin were allowed to develop to a relatively stable tone plateau (usually 10 min). The vascular tension changes were expressed as percentages of the respective precontractions.

2.7. Effects of inhibitors on luteolin-induced RCA relaxation

To explore the possible mechanisms, effects of inhibitors on luteolin-induced RCA relaxation were determined. The following inhibitors were used: cyclooxygenase inhibitor Indo (10 μM) [30], nitric

oxide synthesis inhibitor L-NAME (0.1 mM) [31], Kv blocker 4-AP (10 mM) [32], Kir blocker $BaCl_2$ (0.3 mM) [33]. When 0.3 μM U46619-induced precontraction was sustained, luteolin-induced RCA relaxations were observed in the absence and presence of an inhibitor. The relaxations were presented as percentages of the precontraction.

2.8. Cell isolation

The denuded RCAs were transferred into a physiological saline solution (PSS) containing 0.5 mg/ml papain, 1 mg/ml bovine serum albumin and 1 mg/ml dithioerythritol. The PSS was composed of (mM) 140 NaCl, 5.4 KCl, 1 $MgCl_2$, 5 HEPES, 0.33 NaH_2PO_4, 10 Glucose, 0.1 $CaCl_2$, pH = 7.4 [34]. After incubation for 15–20 min at 37 °C, the arteries were transferred into the PSS including 1 mg/ml bovine serum albumin, 0.5 mg/ml collagenase F and 0.5 mg/ml collagenase H. After 4–6 min incubation at 37 °C, 4 °C PSS was added to the test tube to terminate the digestion. Cell suspension solution was centrifuged at 800 rpm for 6 min twice, the supernatant was discarded and single RCASMCs were obtained. The cells were used immediately for electrophysiological recording. Excess cells were stored at 4 °C PSS for use within 8 h.

2.9. Patch clamp measurements

The cell suspension was placed in the recording chamber on the stage of inverted microscope. Traditional whole cell patch-clamp was performed with Axopatch 200B driven by Clampex 10.4 (32-bit data acquisition system, Axon Instruments, Foster City, CA, USA). Micropipettes were pulled from glass capillaries (1.5 mm OD, 0.84 mm ID, Vltalsense Scientific Instruments Co., Ltd., Wuhan, China) with a micropipette puller (PP-830, NARISHIGE, Japan) and polished with a micro-forge (MF-830, NARISHIGE, Japan). Micropipette resistance was 3–5 MΩ when filled with pipette solution. Analog-digital conversion was AXON-200. The pipette was controlled by micromanipulator. Data were collected at 10 kHz, and filtered at 1 kHz. Currents were analyzed with Clampfit 10.4. All experiments were carried out at 22–25 °C. Cell capacitance was 7 to 15 pF. The stability of leak currents and series resistance were determined and ensured by compensated electronically.

To record Kv currents, cells were held at a holding potential of −60 mV. Whole-cell K^+ currents were recorded in response to a depolarizing voltage from −60 mV to +60 mV for 500 ms by 10 mV increment. The micropipette was filled with a solution composed of (mM) 110 KCl, 1.2 $MgCl_2$, 5 Na_2ATP, 10 EGTA, 10 HEPES, adjusted to pH 7.2 with KOH. This pipette solution minimizes ATP-sensitive K^+ currents and Ca^{2+}-activated K^+ currents. The remainder outward K^+ currents, which were markedly attenuated by 4-AP (10 mM), were supposed to efflux mainly through Kv channels [35]. Bath solution was composed of (mM) 135 NaCl, 5 KCl, 2 $MgCl_2$, 5 HEPES, 10 D-glucose, and pH = 7.4. The current I−V relationship was established both before (control) and after application of luteolin (10, 30 or 100 μM), 4-AP or 4-AP + luteolin. The Kv current density was calculated by a ratio of the average plateau phase Kv currents to the cell capacitance (pA/pF).

To record Kir current, cells were held at a holding potential of −60 mV and subjected to step depolarizations of 500 ms from −140 mV to +20 mV in 20 mV increment. The bath solution was composed of (mM) 12 NaCl, 130 KCl, 0.4 KH_2PO_4, 0.3 NaH_2PO_4, 2 $NaHCO_3$, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 HEPES, 5.5 glucose, adjusted to pH = 7.4 with NaOH [34]. Pipette-filling solution was composed of (mM) 100 potassium-D-gluconate, 30 KCl, 1 $MgCl_2$, 1 EGTA, 15 HEPES, 1 Na_2-ATP. KCl was substituted with potassium-D-gluconate on an equimolar basis to minimize chloride currents [28,36]. Whole-cell currents were recorded in the absence or presence of luteolin (10, 30 and 100 μM), 300 μM $BaCl_2$, or 300 μM $BaCl_2$ + 100 μM luteolin. The current density of Kir channels was calculated by a ratio of the average plateau phase Kir currents to the cell capacitance (pA/pF).

2.10. Data analysis

Statistical differences between recordings before (control) and after a treatment were compared using Student's paired *t*-test. Comparison was carried out with one-way repeated measures ANOVA followed by Bonferroni's post hoc test among groups of > 2. Differences were considered statistically significant when $P < 0.05$. *n* represents the number of vessels or cells with each vessel or cell isolated from a separate rat. IC_{50} (antagonist concentration depressing 50% of the maximal contraction) and RC_{50} (vasodilator concentration inducing 50% relaxation on the precontraction) were calculated by non-linear regression using GraphPad Prism ®, version 6.00 (GraphPad Software, San Diego, CA, USA).

3. Result

3.1. Luteolin depressed RCA contractions induced by various vasoconstrictors

To obtain a better understanding of vasospasmolytic characteristics of luteolin, the effects of preincubation with luteolin on the contractions induced by various vasoconstrictors were evaluated. KCl, U46619, $BaCl_2$, 4-AP, VP and $[Ca^{2+}]_o$ contracted the isolated RCAs concentration-dependently. The maximal contractions were 4.97 ± 1.68–11.50 ± 2.37 mN. Luteolin (10–100 μM) shifted all of the concentration-contraction curves non-parallel to right and decreased the maximal contraction ($P < 0.05$, Fig.2). The values of IC_{50} were 14.82 μM, 10.99 μM, 11.07 μM, 9.40 μM, 14.02 μM and 13.45 μM for KCl, U46619, $BaCl_2$, 4-AP, VP and $[Ca^{2+}]_o$, respectively.

3.2. Luteolin relaxed RCAs precontracted by KCl or U46619

In the endothelium- denuded RCAs precontracted with 60 mM KCl (Fig.3A) or 0.3 μM U46619 (Fig.3B), cumulative addition of luteolin (1–100 μM) produced concentration-dependent relaxations. The RC_{50} values of luteolin were 16.89 μM for 60 mM KCl and 8.71 μM for 0.3 μM U46619 (Fig.3C and D). The vehicle DMSO, up to 0.1%, did not significantly affect the precontractions.

3.3. Luteolin-induced RCA relaxation was attenuated by 4-AP and $BaCl_2$

To explore the mechanisms underlying the luteolin-induced relaxation, we observed the effects of L-NAME (0.1 mM, a nitric oxide synthase inhibitor), Indo (0.01 mM, a cyclooxygenase inhibitor), 4-AP (10 mM, a Kv channel blocker) and $BaCl_2$ (0.3 mM, a Kir channel blocker) on the relaxation. In RCAs precontracted with 0.3 μM U46619, instant addition of 30 μM luteolin induced a fast-plateaued tone decline. 4-AP and $BaCl_2$ significantly decreased luteolin-induced relaxation by 37.05% and 15.40% ($P < 0.05$, Fig.4), while L-NAME and Indo did not significantly affect the relaxation ($P > 0.05$).

3.4. Luteolin increased Kv currents in RCASMCs

In the present experimental condition, K_{ATP} and K_{Ca} current were minimized by high concentrations of ATP (5 mM) and EGTA (10 mM) in the micropipette solution [36]. As a result, neither K_{ATP} blocker glibenclamide (10 μM) nor K_{Ca} blocker TEA (1 mM) affected the remainder outward K^+ currents, while Kv channel inhibitor 4-AP (3 mM and 10 mM) reduced the currents by 61.14 ± 14.53% and 81.16 ± 10.42%, respectively. This remainder 4-AP sensitive K^+ currents were supposed to be mainly through Kv channels [35]. At a testing potential of +60 mV, the stable peak current was 865.97 ± 211.17 pA and the current density was 35.50 ± 6.06 pA/pF. Exposure of the cells to luteolin (10, 30 and 100 μM) increased the currents in a concentration-dependent manner (Fig.5A) and shifted Kv I−V curves upwards. At a testing potential of +60 mV, luteolin (10, 30, and 100 μM) augmented

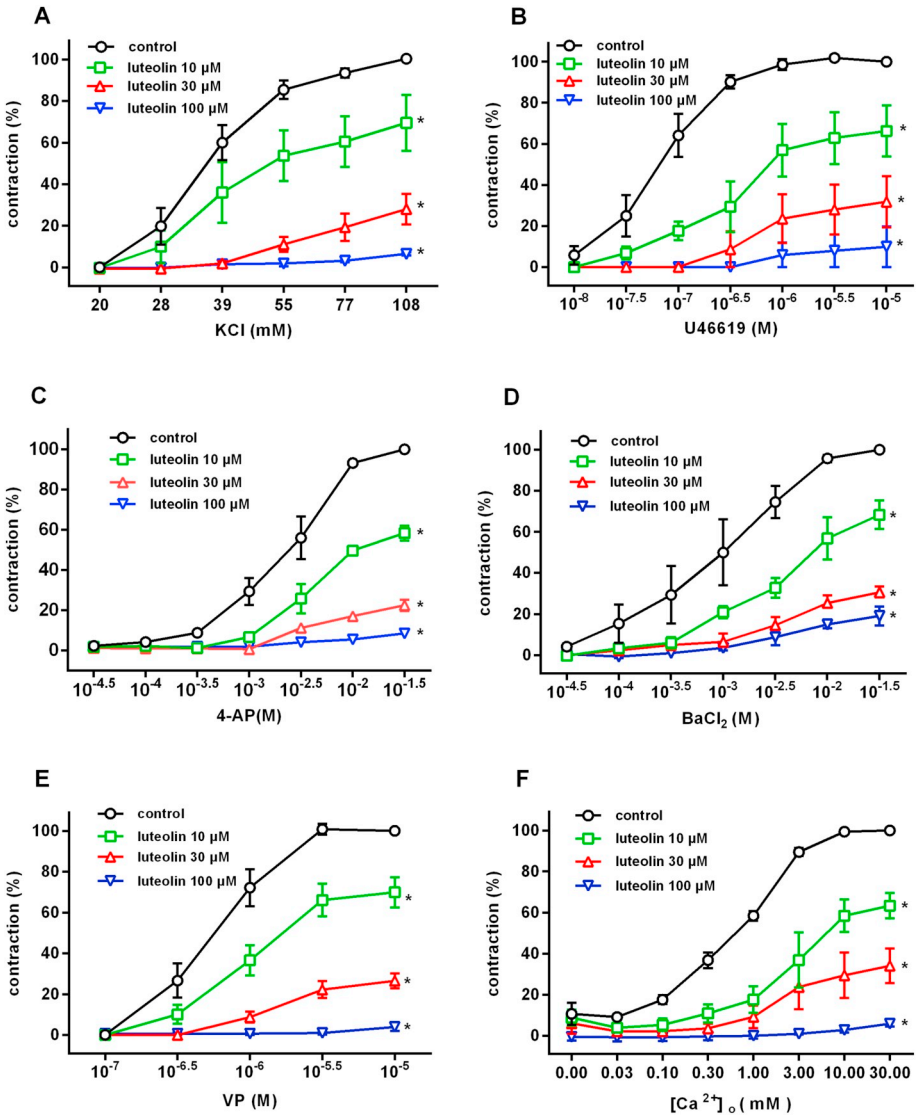

Fig. 2. Luteolin depressed RCA contractions. The contractive responses of RCAs to the cumulative increment of KCl (A), U46619 (B), 4-AP (C), $BaCl_2$ (D), VP (E) and $[Ca^{2+}]_o$ (F) were recorded before (control) and after treatment with luteolin (10, 30 or 100 μM) in the isolated RCAs. Luteolin was added into the bath 15 min before the concentration-contraction curves were reconstructed again. The maximal contractions induced by KCl, U46619, 4-AP, $BaCl_2$, VP and $[Ca^{2+}]_o$ in the absence of luteolin (control) were 11.50 ± 2.37 mN, 4.97 ± 1.68 mN, 6.25 ± 2.95 mN, 7.92 ± 2.63 mN, 6.72 ± 3.03 mN and 6.74 ± 3.24 mN, respectively. The results (means ± SD) were expressed as percentages of the respective maximal contractions in the absence of luteolin. n = 6–8, $^*P < 0.05$ vs control.

the Kv current densities by 36.64 ± 11.45%, 75.86 ± 8.45% and 105.6 ± 7.23%, respectively (Fig.5B). Luteolin-induced net Kv current increases were obtained by subtracting the control Kv currents from the Kv currents recorded after application of luteolin. 4-AP (3 mM and 10 mM) reduced 100 μM luteolin-induced net Kv current increases by 23.6 ± 4.07% and 47.76 ± 6.19%, respectively (Fig.5 C).

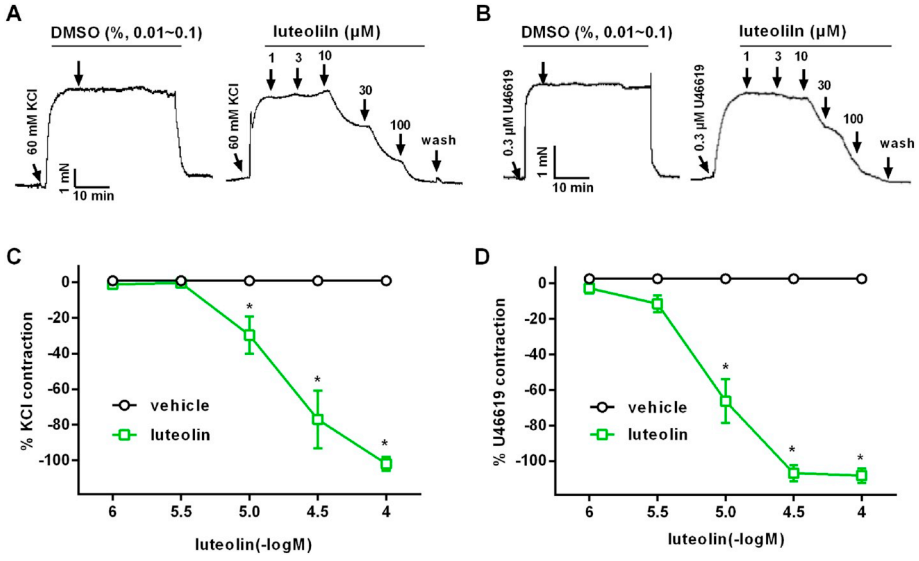

Fig. 3. Luteolin relaxed RCAs precontracted with 60 mM KCl or 0.3 μM U46619. A and B: The original traces of vasorelaxation induced by cumulative addition of luteolin in RCAs precontracted with KCl (A) or U46619 (B). C and D: Pooled data of A and B. Vasorelaxant responses (means ± SD) were expressed as percentages of the respective precontractions, n = 7. *P < 0.05 vs vehicle.

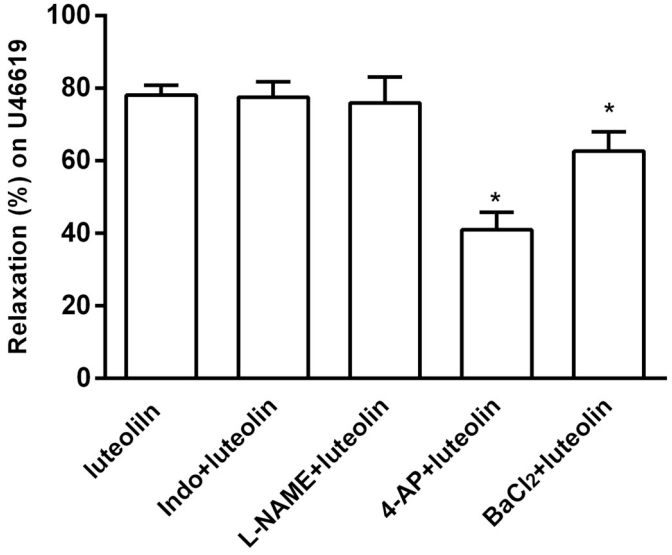

Fig. 4. Effects of various inhibitors on luteolin-induced RCA vasorelaxation. The relaxations induced by luteolin (30 μM) were measured in the absence or presence of Indo (0.01 mM), L-NAME (0.1 mM), 4-AP (10 mM) or $BaCl_2$ (0.3 mM) in the same RCAs precontracted with 0.3 μM U46619. Vasorelaxant responses (means ± SD) were expressed as vascular tone decline percentage on the precontraction induced by 0.3 μM U46619, $n = 6$. *P < 0.05 vs luteolin.

3.5. Luteolin augmented Kir currents in RCASMCs

The Kir currents elicited by a series of depolarization voltage from −140 mV to +20 mV were recorded before and after treatment with luteolin (10, 30 and 100 μM), 300 μM $BaCl_2$, or 300 μM $BaCl_2$ + 100 μM luteolin using step protocol (Fig.6A). At a test potential of −140 mV, luteolin (30 and 100 μM) increased Kir current densities by 66.73 ± 9.41% and 125.92 ± 11.36%, respectively (Fig. 6A and B). 300 μM Ba^{2+} depressed the control Kir currents by 80.21 ± 10.44% (Fig. 6A) and reduced 100 μM luteolin-induced net Kir current increase by 42.14 ± 5.28% (Fig. 6A and C).

4. Discussion

Since the 1950s to date, increasing researches have focused on the cardiovascular effects of luteolin [37]. Accumulated data show that luteolin exhibits various beneficial activities on cardiovascular system via complex signal transduction pathways and targets [38–41]. Luteolin-induced vasorelaxations were demonstrated in porcine coronary and splenic arteries precontracted with U46619 [12] and rat aorta precontracted with phenylephrine and KCl [17,42,43]. To get a clearer understanding about the effect of luteolin on coronary artery, we studied its vasomotor effect against various vasoconstrictors and its electrophysiological effect on RCASMCs. The main findings are: (1) Luteolin non-competitively inhibited RCA vasoconstrictions induced by high K^+ depolarization, U46619, 4-AP, $BaCl_2$, VP and $[Ca^{2+}]_o$ elevation. (2) In the precontracted endothelium-denuded RCAs, luteolin produced striking relaxations, which was attenuated by $BaCl_2$ and 4-AP, while was not significantly affected by L-NAME and Indo. (3) Luteolin augmented Kv currents and Kir currents in freshly isolated RCASMCs.

Non-competitivity and broad antagonistic spectrum of luteolin against all vasoconstrictors used in the present study indicate that luteolin has multiple acting targets and/or affects common mechanisms shared by or related with these agonists. Cell membrane depolarization, $[Ca^{2+}]_o$ influx and K^+ channel modulation are common events in the

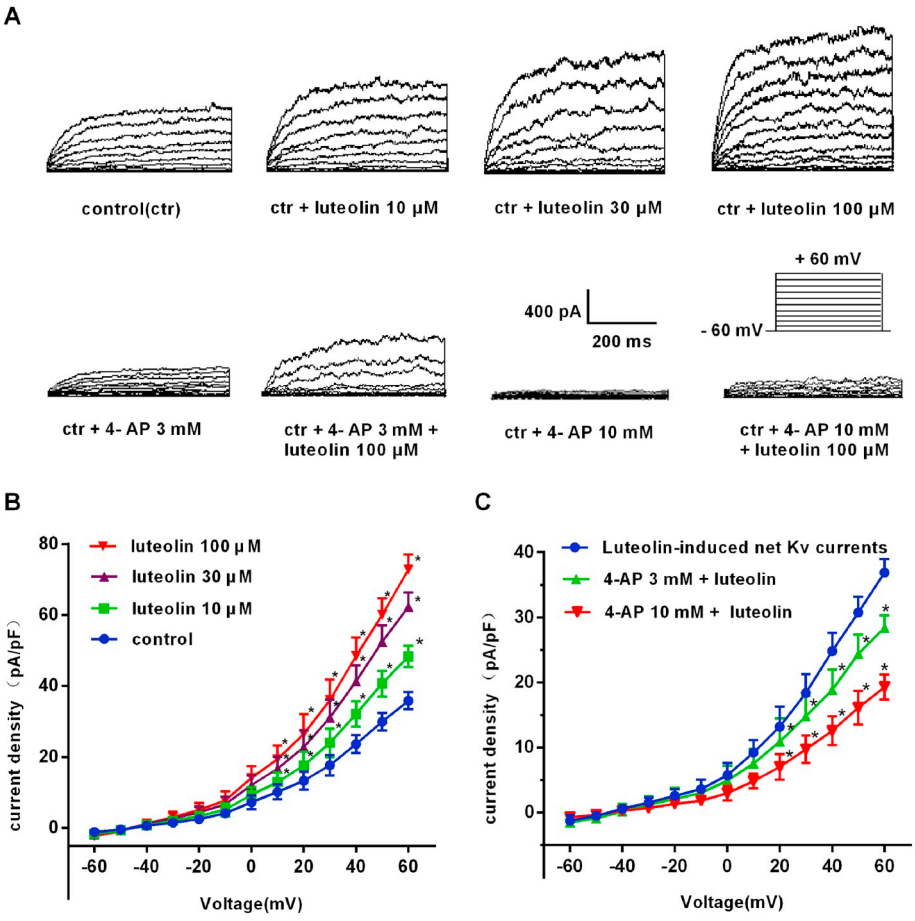

Fig. 5. Luteolin augmented Kv currents in freshly isolated single RCASMCs. A. The original recording traces of voltage-gated K^+ currents elicited by a series of depolarization voltage from −60 mV to +60 mV in 10 mV increments for 500 ms each step. Kv currents were recorded before (control, ctr) and after different treatments with luteolin, 4-AP or 4-AP + luteolin. B. I-V curves of Kv currents before and after application of luteolin. Means ± SD, n = 6, *P < 0.05 vs control. C. I-V curves of luteolin (100 μM)-induced net Kv currents in the absence or presence of 4-AP. Means ± SD, n = 6, *P < 0.05 vs luteolin-induced net Kv currents.

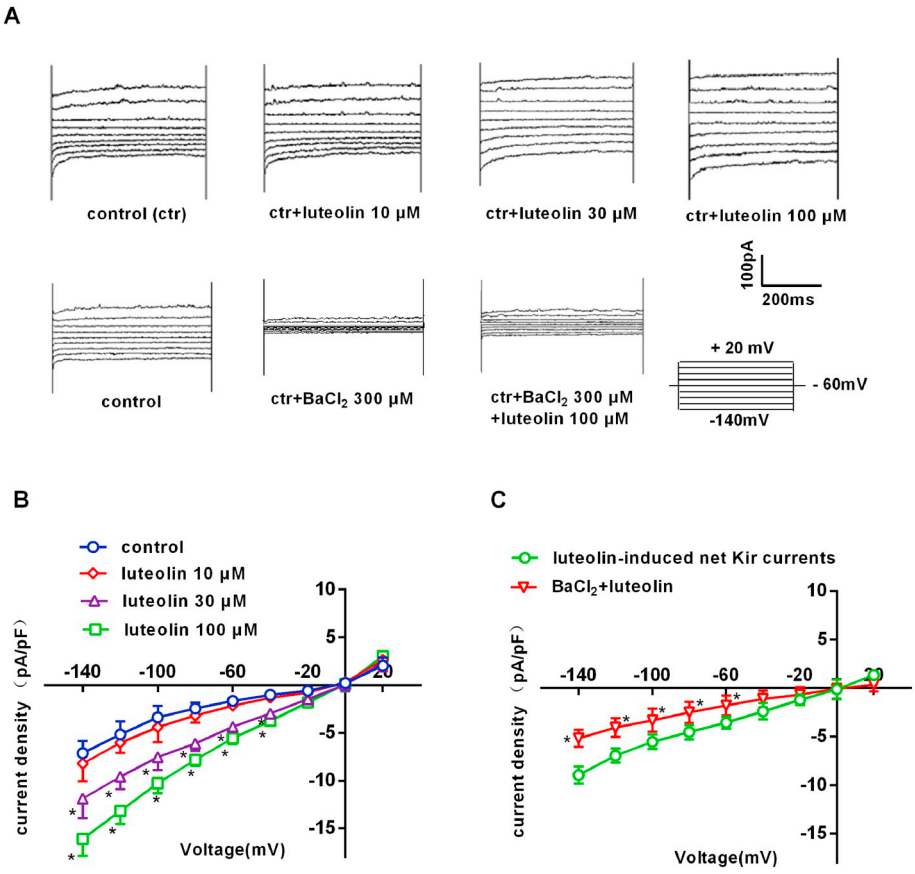

Fig. 6. Luteolin augmented Kir currents in RCASMCs. A. The representation of Kir currents in RCASMCs by depolarizing from −140 mV to +20 mV with 20 mV increments for 500 ms. Kir currents were recorded before (control, ctr) and after different treatments with luteolin, $BaCl_2$ (300 μM) or $BaCl_2$ (300 μM) + luteolin (100 μM). B. I-V curves of Kir currents before and after application of luteolin. Means ± SD, n = 6, *P < 0.05 vs control. C: I-V curves of luteolin (100 μM)-induced net Kir currents in the absence or presence of $BaCl_2$ (300 μM). Means ± SD, n = 6, *P < 0.05 vs luteolin-induced net Kir currents.

contractions induced by these vasoconstrictors. Elevation of extracellular K^+ concentrations decrease the K^+ gradient across the cell membrane, reduces the efficacy of K^+ channels and depolarizes the cell membrane. 4-AP and $BaCl_2$ depolarize the cell membrane by blocking Kv and Kir channels, respectively [44]. Depolarization facilitates $[Ca^{2+}]_o$ influx and eventually induces contraction. Modulation of K^+ channels, cell membrane potential or/and Ca^{2+} channels is also involved in contractions induced by U46619 [45,46] and VP [47]. The present results showed that luteolin markedly inhibited the contraction induced by $[Ca^{2+}]_o$ influx in high K^+- depolarizing solution. This result is consistent with the previous report that luteolin relaxed porcine coronary artery through inhibition of $[Ca^{2+}]_o$ influx across the cell membrane of the myocyte [12]. Based on the present results, it was speculated that K^+ channel activation may be involved in the vascular effect of luteolin. The speculation was consistent with the previous observations that chemical blockers of K^+ channels attenuated luteolin-induced vasorelaxation [48,49]. The speculation was also supported by our previous studies that natural flavonoids hesperetin [29] and quercetin [50] augment Kv currents in RCASMCs.

It was reported that vasorelaxation induced by luteolin in rat aorta was endothelium-dependent and was inhibited by nitric oxide synthase inhibitor L-NAME [17,42]. To illuminate the direct effect of luteolin on RCASMCs and the underlying mechanisms, we observed the effects of various inhibitors on luteolin-induced relaxation in the endothelium-denuded RCAs. In the present experimental conditions, luteolin elicited fast-plateaued relaxations in the endothelium-denuded RCAs precontracted by KCl or U46619. Neither L-NAME nor Indo affected the relaxation, suggesting the direct relaxant effect of luteolin on RCASMCs. The findings that 4-AP and $BaCl_2$ significantly attenuated luteolin-induced relaxation in U46619-precontracted RCAs suggest the involvement of activation of Kv channels and Kir channels in the relaxation.

As far as we know, the direct evidence that luteolin acts on the myocyte K^+ channels is lacking. We studied the effect of luteolin on Kv currents and Kir currents in RCASMCs with patch clamp technique to further explore the possible involvement of K^+ channels in luteolin-induced RCA relaxation. The present results showed that luteolin augmented K^+ currents in a concentration-dependent manner. Most (~80%) of luteolin-induced net K^+ current increase was sensitive to blockade of 10 mM 4-AP, suggesting that luteolin augmented K^+ currents mainly through 4-AP sensitive Kv channels. About 20% of luteolin-induced net K^+ current increase was insensitive to 4-AP, suggesting that luteolin may also affect other subtypes of Kv channels. Luteolin also augmented $BaCl_2$-sensitive K^+ currents, suggesting that Kir may be also involved in luteolin-induced RCA relaxation. Based on these electrophysiological results, it may be supposed that enhancement of Kv channels and Kir channels underlies luteolin-induced RCA vasorelaxation. However, comprehensive consideration is needed in evaluating the contribution of activation of Kv channels and Kir channels to luteolin-induced RCA relaxation. In RCAs precontracted by 60 mM KCl, which almost eliminates the electrochemical gradient for diffusion of K^+ through opened K^+ channels, luteolin still elicited a profound relaxation. This means that other mechanisms independent of K^+ channel activation are involved in luteolin-induced RCA relaxation. This notion is supported by the findings that luteolin-induced relaxation was only partially attenuated by 4-AP and $BaCl_2$ and that 60 mM KCl-induced contraction was more resistant to luteolin-induced relaxation than U46619-induced contraction (RC_{50}: 16.89 μM vs 8.71 μM). Taken together, the present results suggested that activation of Kv channels and Kir channels is involved in luteolin-induced RCA relaxation and a considerable proportion of the relaxation may be independent of K^+ channels.

5. Conclusions

In conclusion, the present study demonstrates that luteolin is vasospasmolytic against a variety of vasoconstrictors in RCAs, and augments both Kv currents and Kir currents in RCASMCs, suggesting that

the enhancement of Kv and Kir channel activity is, at least in part, contributory to its vasospasmolytic effects. Luteolin is naturally present in our daily foods. Therefore, these findings indicate that luteolin may be a promising food additive with the aim of preventing coronary arterial spasm.

Conflict of interest policy form and author contribution to study form

Author contribution statement: WL and MZ designed the experiments, analyzed data and wrote the manuscript; WL, RC, MD, PG, YL and YJ carried out wire myograph experiments. WL, RC, PG and YJ did patch clamp study. All authors read and approved the manuscript. The authors declare no conflict of interest.

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Compliance with ethical standards

Ethical standards

The authors declare that all protocols and procedures described in this animal study were approved by the Animal Ethics Committee of Shanxi Medical University (Taiyuan, China) and the studies were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. No human body materials and data were used in the present study.

Conflict of interest

The authors declare no conflict of interest.

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