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Original Article

Vasomotor tone regulation by Rhodiola in rat thoracic aorta

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Abstract: Objective: This study was designed to investigate the effects of Rhodiola and one of its known active components, salidroside, on vasomotor tone regulation in rat isolated thoracic aorta and to explore the possible mechanisms. Materials and methods: Isometric tension was recorded from aortic rings in an organ bath for controlled drug application. To evaluate the influences of Rhodiola and salidroside on endothelial function, aortic rings with intact or denuded endothelium were always tested in parallel. Results: Salidroside (0.1-10.0 g/L) had no influence on the vasomotor tone of aortic rings, while Rhodiola evoked mechanistically distinct all-or-nothing responses at low and high doses. At a relatively low dose (0.1 g/L), Rhodiola induced endothelium-independent contraction of aortic rings at resting tension ($P<0.01$), a response dependent on extracellular Ca^{2+} and blocked by an L-type Ca^{2+} channel antagonist ($P<0.01$). At a higher dose (0.5 g/L), Rhodiola produced vasoconstriction at resting tension in Ca^{2+}-free medium ($P<0.01$), a response inhibited by a protein kinase C (PKC) antagonist ($P<0.05$). Conversely, Rhodiola at the higher dose dilated aortic rings precontracted with phenylephrine (PE) ($P<0.01$), likely by inhibiting extracellular Ca^{2+} influx through PE-activated receptor-operated calcium channels (ROCCs) ($P<0.01$). Rhodiola had no significant influence on K^+ channels of vascular smooth muscle cells or on endothelial vascular tension regulation. Conclusions: Rhodiola has mechanistically distinct dose-dependent effects on vascular tension in rat isolated thoracic aorta. However, these responses are produced by active ingredients other than salidroside.

Keywords: Rhodiola, salidroside, aorta, vasoconstriction, vasorelaxation, vasomotor tone

Introduction

Rhodiola rosea (Rho) is a flowing plant distributed at altitudes >2000 m in arctic, mountainous, chilly, hypoxic, and radioactive regions throughout Asia and Europe. As a traditional folk medicine, Rho has been widely used since ancient times to promote mental and physical endurance [1], improve sleep quality, and enhance resistance to high altitude sickness, stress, depression [2], microwave radiation [4], hypoxia [3], fatigue [5], and oxidative stress [1] in many countries [6], including Russia, India, Sweden, and China.

A systematic review and meta-analysis of randomized controlled trials [7] concluded that Rho formulations are cardioprotective against ischemic heart disease. However, there are no experimental demonstrations of specific effects of Rho on vascular tension, which would help clarify the pharmacologic actions of Rho. The aim of the present study was to evaluate the effects of Rho on vascular tension *in vitro* and to explore the possible mechanisms.

Materials and methods

Chemicals

Rho extracts, a gift from Tibet Rhodiola Pharmaceutical Holding Co., Ltd. (Chengdu, China), were separated by supercritical CO_2 fluid extraction technology. Salidroside (batch number: 110818-200404) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

Acetylcholine (Ach), phenylephrine (PE), 2-aminoethyl diphenyl borate (2-APB), staurosporine (SP), procaine hydrochloride, nifedipine (NRDP), and mibefradil (MBFD) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

2-APB and NFDP were dissolved in dimethyl sulfoxide (DMSO), while other drugs were dissolved in distilled water and diluted with Kreb-Henseleit (K-H) saline solution before use. Preliminary experiments showed that a DMSO concentration <0.2% (v/v) had no effect on vascular tension of isolated aortic rings. The modified K-H solution (pH 7.4) was of the following composition (in mM) [8]: 4.7 KCl, 118.3 NaCl, 1.2 $MgSO_4$, 25.0 $NaHCO_3$, 1.2 KH_2PO_4, 2.5 $CaCl_2$, and 11.0 glucose. The Ca^{2+}-free medium contained (in mM) 4.7 KCl, 118.3 NaCl, 1.2 $MgSO_4$, 25.0 $NaHCO_3$, 1.2 KH_2PO_4, 11.0 glucose, and 0.05 EGTA.

Experimental animals

All animal procedures followed the guidelines of the Animal Care and Use Committee of Zhejiang University. Healthy male Sprague-Dawley rats weighing 200-250 g (clean grade, purchased from Laboratory Animal Center of the Chinese Academy of Science, Shanghai, China) were housed in conventional cages at controlled temperature (21-24°C) and humidity (40-60%) under a 12:12 h light/dark cycle with free access to standard pellet chow and water.

Rat aortic ring preparation

The thoracic aorta was immediately isolated and placed in oxygenated K-H solution pre-chilled to 4°C. Fat and connective tissues were cleaned carefully and the aorta was cut into 3 to 4 mm rings. Six to eight rings per animal were prepared. The endothelium was mechanically removed from some rings by gently rubbing the internal luminal surface with a metal rod. The rings were suspended horizontally between two stainless steel wires in organ baths containing 5 ml K-H solution aerated with 95% O_2 and 5% CO_2 and maintained at 37±0.5°C. The isometric tension was recorded via a force-displacement transducer (JZ101, metrical range: 0-5.0 g) connected to a MedLab 6.0 polygraph (Nanjing Medease, China).

Rings were equilibrated in the organ bath for 60 min at 2.0 g resting tension and then challenged with KCl (6.0×10^{-2} M) at least 3 times until a reproducible maximal contractile response was obtained. The presence of functional endothelium was confirmed by at least 80% relaxation in response to Ach (1.0×10^{-5} M) in rings precontracted with PE (1.0×10^{-6} M). The endothelium was considered fully denuded when there was <10% relaxation in response to Ach.

Before drug treatment experiments (described in the following section), the rings were washed at least 3 times and equilibrated in the organ bath for 20 min.

Drug treatments at isometric tension

Effects of salidroside and Rho on vascular tension: When the tension was at resting state or reached a plateau induced by PE (1.0×10^{-6} M) or KCl (6.0×10^{-2} M), either salidroside (0.1-10.0 g/L) or Rho (0.05-5.0 g/L) was added into the organ bath at 15 min intervals. Rings with intact or denuded endothelium were always tested in parallel.

Mechanisms for Rho-induced contraction of endothelium-denuded rings: Endothelium-denuded rings were incubated in Ca^{2+}-free medium and Rho (0.1-5.0 g/L) added at 15 min intervals (control group). To identify possible mechanisms for Rho-induced contraction in Ca^{2+}-free medium, some rings were first pre-incubated for 20 min in the inositol-1,4,5-trisphosphate receptor (IP_3R) blocker 2-APB (100 μM) [9], the protein kinase (PKC) inhibitor SP (0.1 μM) (Bai *et al*. 2007), or the ryanodine receptor (RYR) blocker procaine (10 mM) [9]. A time control was always run in parallel. To refill the intracellular Ca^{2+} stores of rings pre-incubated with 2-APB, SP, or procaine under external Ca^{2+}-free conditions, contraction was induced in K-H solution by addition of 6.0×10^{-2} M KCl before the second test application of Rho.

To investigate the effect of Rho on extracellular Ca^{2+} influx, 2.5 mM $CaCl_2$ was added after Rho application. A control pre-incubated with the same volume of Ca^{2+}-free solution before Rho application was always run in parallel. To identify possible mechanisms for Rho-induced contraction (0.5 g/L) in the presence of extracellular Ca^{2+}, rings were pre-incubated for 10 min with the L-type Ca^{2+} channel blocker NFDP (20 μM) (Triggle 2006) or T-type Ca^{2+} channel blocker MBFD (5 μM) before $CaCl_2$ was added to Ca^{2+}-free medium.

Mechanisms for Rho-induced relaxation of endothelium-denuded rings precontracted with PE (1.0×10^{-6} M): To explore the probable influence of Rho on intracellular Ca^{2+} mobilization, rings were pre-incubated with Rho (0.1-5.0 g/L) in Ca^{2+}-free medium before addition of PE (1.0×10^{-6} M). A control pre-incubated with Ca^{2+}-free solution alone prior to PE stimulation in the same volume was always run in parallel.

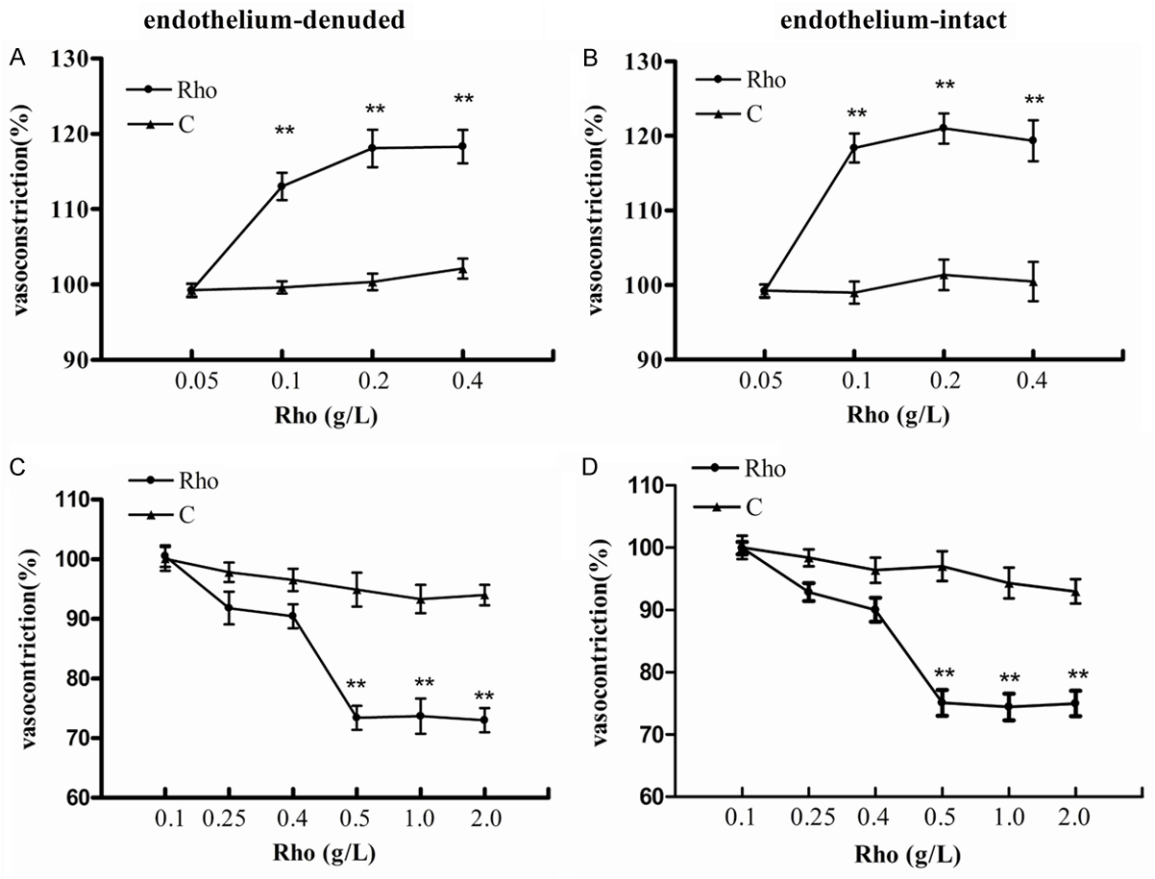

Figure 1. Cumulative Rho dose-response on aortic rings. Rho (0.05-2.0 g/L) was cumulatively added at 15-min intervals into a organ bath containing both a endothelium-denuded aortic ring (A, C) and an endothelium-intact aortic ring (B, D). The curves show the cumulative dose-response of Rho on aortic rings at resting tension (A, B) and precontracted by 1.0×10^{-6} M PE (C, D). Values are expressed as mean ± SEM of 6-8 observations. **P<0.01 compared to corresponding control.

To investigate the possible effect of extracellular Ca^{2+} influx, 2.5 mM $CaCl_2$ was added to the Ca^{2+}-free medium after PE stimulation of rings pre-incubated with Rho (0.5 g/L) for 20 min. A control pre-incubated with the same volume of Ca^{2+}-free solution plus Rho was always run in parallel.

To explore the possible effects of Rho on K^+ channel activity, endothelium-denuded rings were pre-incubated with the K^+ channel blocker $BaCl_2$ (1 mM) [10] in standard K-H solution for 20 min before PE was added. Rho (0.1-5.0 g/L) was added when the contractile plateau was attained.

Data presentation and statistical analyses

Change in vasoconstriction is expressed as the ratio of the response following Rho application to that before Rho application. Vasodilation is expressed as the percentage of the contractile response to PE or KCl (= 100%).

All group data are expressed as mean ± SEM. Unpaired or paired t-test followed by Newman-Keuls test were used to compare responses between control and treated groups or pre-treated and post-treated groups. Multiple groups were compared by one-way ANOVA followed by Bonferroni post-hoc tests. A P<0.05 indicated a significant difference.

Results

Effects of salidroside and Rho on vascular tension

Salidroside (0.1-10.0 g/L) had no influence on the vascular tension of aortic rings with or without endothelium (denuded). No clear graded response to Rho was observed in dose-dependence experiments. Rather, vasomotor res-

Table 1. Vasocontractile effect of Rho on endothelium-denuded aortic rings

In Ca^{2+}-free K-H solution	*n*	Vasoconstriction (%)
Control	6	99.14±1.35
Rho (0.1 g/L)	8	104.36±2.76
Rho (0.5 g/L)	8	109.53±1.76*
Control + $CaCl_2$	6	100.23±0.68
Rho (0.1 g/L) + $CaCl_2$	8	112.23±2.89**
Rho (0.1 g/L) + NFDP + $CaCl_2$	8	106.38±2.24***
Rho (0.1 g/L) + MBFD + $CaCl_2$	8	113.99±2.75

Rho, Rhodiola; NFDP, nifedipine; MBFD, mibefradil. The concentration of $CaCl_2$ is 2.5 mM. The concentration of NFDP is 20 μM, and the concentration of MBFD is 5 μM. Data expressed as mean ± SEM of *n* observations. *P<0.01 compared with control. ***P*<0.01 compared with corresponding values in the absence of $CaCl_2$. ****P*<0.05 compared with corresponding values in the absence of NFDP.

ponse to Rho appeared to be all-or-nothing. In the current study, therefore, the lowest concentrations producing specific vasomotor responses were used.

As depicted in **Figure 1**, a low Rho concentration (0.1 g/L) contracted aortic rings at resting tension, whether with endothelium-intact (t = 4.619, P = 0.001, control versus treatment) or endothelium-denuded (t = 6.380, P = 0.000, control versus treatment). The contractile effect of Rho did not differ significantly between endothelium-intact and endothelium-denuded aortic rings (t = 2.117, P = 0.166, intact versus denuded).

A higher Rho dose (0.5 g/L) produced relaxation of aortic rings at plateau tension induced by PE (1.0×10^{-6} M) (endothelium-intact rings: t = -6.052, P = 0.001, control versus treatment; endothelium-denuded rings: t = -6.683, P = 0.001, control versus treatment). The vasorelaxant response to Rho was slightly higher in endothelium-intact rings but the difference was not significant (t-test, t = 0.692, P = 0.519, intact versus denuded).

Rho over a broad range of concentrations (0.1-5.0 g/L) had no vasoactive effect on aortic rings stimulated by KCl (6.0×10^{-2} M) whether endothelium-intact or endothelium-denuded (each P>0.05).

Mechanisms for Rho-induced contraction of endothelium-denuded rings

Contraction induced by the lower dose of Rho (0.1 g/L) was dependent on extracellular Ca^{2+} as addition of 2.5 mM $CaCl_2$ significantly enhanced the magnitude of contraction compared to the Rho response in the absence of extracellular Ca^{2+} (t = 4.607, P = 0.006, control versus treatment). The enhanced contraction by addition of extracellular Ca^{2+} was significantly attenuated in aortic rings pre-incubated for 10 min with the L-type Ca^{2+} channel blocker NFDP (20 μM) [11], while the T-type Ca^{2+} channel blocker MBFD (5 μM) (Tanaka & Shigenobu 2005) did not influence the enhancement by extracellular Ca^{2+} (**Table 1**).

The higher Rho dose (0.5 g/L) contracted endothelium-denuded rings in Ca^{2+}-free medium (t-test, t = -3.887, P = 0.001, control versus treatment) and this response was inhibited by the PKC inhibitor SP [12] (paired t-test, t = 4.425, P = 0.021) but not by the IP_3R blocker 2-APB [9] (P = 0.751) or the RYR blocker procaine [9] (P = 0.425, **Figure 2**).

Mechanism for Rho-induced relaxation of endothelium-denuded rings precontracted with PE

Rho (0.1-5.0 g/L) did not influence the contraction induced by PE in Ca^{2+}-free medium (ANOVA, F = 1.880, P = 0.187, control versus treatment). However, the enhancement of contraction induced by addition of extracellular Ca^{2+} in the presence of PE was blocked by Rho (0.5 g/L) (P = 0.008, control versus treatment). Thus, the higher dose of Rho appeared to block PE-dependent calcium influx (**Figure 3**).

The K channel antagonist $BaCl_2$ (1 mM) did not influence the vasorelaxant response to Rho in endothelium-denuded rings precontracted with PE (t-test, t = 1.857, P = 0.093, control versus $BaCl_2$ treatment).

Discussion

This *in vitro* study on isolated aortic rings suggests that the medicinal herb Rhodiola bidirectionally regulates vascular tone through distinct mechanisms. Vasoconstriction in the relaxed state is mediated by enhanced Ca^{2+} influx through L-type Ca channels and by PKC signaling, while vasorelaxation in the contracted state results from inhibition of calcium influx through ROCCs.

Dose-response experiments showed that Rho induced all-or-nothing rather than graded responses **Figure 1**, akin to the responses of

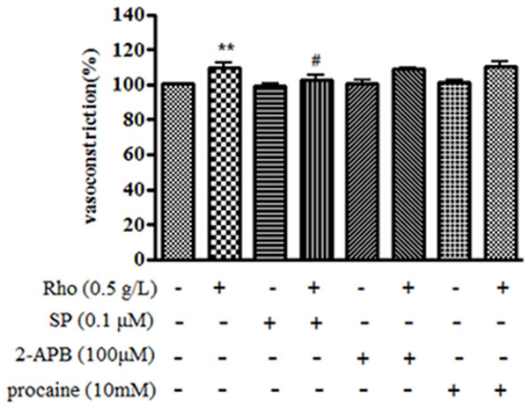

Figure 2. Vasocontractile effect of Rho (0.5 g/L) on endothelium-denuded aortic rings in Ca^{2+}-free medium. Values are expressed as mean ± SEM of 6-8 observations. **P<0.01 compared to control. #P<0.05 compared to corresponding values in the absence of SP. Rho, Rhodiola; SP, staurosporine; 2-APB, 2-aminoethyl diphenyl borate.

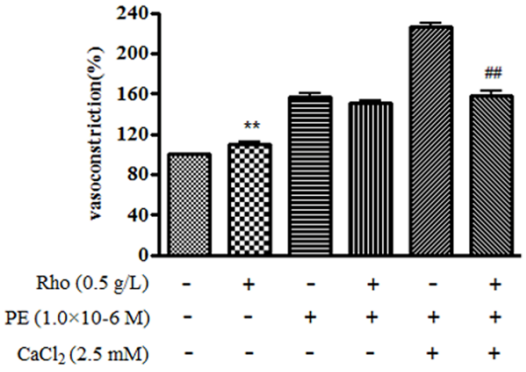

Figure 3. Bidirectional effects of Rho (0.5 g/L) on the vascular tension of endothelium-denuded aortic rings in Ca^{2+}-free medium. Values are expressed as mean ± SEM of 6-8 observations. **P<0.01 compared to control. ##P<0.01 compared to corresponding values in the absence of Rho.

sodium aescinate and stevioside [13] on aortic tension. At a relatively low dose (0.1 g/L), Rho enhanced the resting tension of aortic rings. This vasocontractile response was independent of endothelial function, as response magnitude did not differ between endothelium-intact rings and endothelium-denuded rings. Such direct vasoconstriction by Rho may contribute to the therapeutic effects against hypoxia and altitude sickness [2, 3].

This endothelium-independent vasoconstriction indicates that vascular smooth muscle is the main target of Rho. In principle, this contractile response could involve several possible mechanisms [8, 14]: (1) mobilization of Ca^{2+} from intracellular stores via receptor-operated Ca^{2+} channels, including IP_3R channels and RYR channels; (2) stimulation of extracellular Ca^{2+} influx through transmembrane Ca^{2+} channels, including voltage-operated Ca^{2+} channels (VOCCs), receptor-operated Ca^{2+} channels (ROCCs), and store-operated Ca^{2+} channel (SOCCs); (3) activation of PKC, inducing myosin light-chain phosphorylation and eliciting Ca^{2+}-independent vasoconstriction; (4) direct stimulation of contractile apparatus induced by increasing the intracellular Ca^{2+} concentration.

Rho at a relatively low dose (0.1 g/L) did not induce significant contraction in Ca^{2+}-free K-H solution, while addition of 2.5 mM $CaCl_2$ triggered significant vasoconstriction, which was inhibited by the L-type channel blocker NFDP. Thus, low-dose Rho stimulated vasoconstriction by facilitating extracellular Ca^{2+} influx through L-type VOCCs. In contrast, Rho at a relatively high dose (0.5 g/L) induced vasoconstriction in Ca^{2+}-free K-H solution, suggesting enhanced mobilization of Ca^{2+} from intracellular stores or activation of PKC and direct stimulation of the contractile apparatus. Blockade of the contractile response by a PKC inhibitor indicates that PKC is involved, although further studies are needed to confirm direct effects on the contractile apparatus. Further, modulation of the intracellular Ca^{2+} concentration cannot be excluded. In either case, Rho had no significant inhibitory effect on the contractile apparatus.

Rho at a relatively high dose (0.5 g/L) dilated aortic rings precontracted by PE but not by KCl. This vasodilation was also endothelium-independent. Such vasodilation by Rho may contribute to the prevention and treatment of cardiovascular ischemia [2]. The mechanism of vasoconstriction induced by PE differs from that of KCl [8, 15]. KCl depolarization activates VOCCs and allows influx of extracellular Ca^{2+}. This Ca^{2+} influx further triggers Ca^{2+}-induced Ca^{2+} release through RYR channels in sarcoplasmic reticulum (SR) membrane. The finding that Rho did not influence vasoconstriction induced by KCl indicates that Rho has no significant influence on RYR channels. As an α-adrenoceptor agonist, PE activates ROCCs in the membrane of vascular smooth muscle cells

and triggers Ca^{2+} influx, while as an activator of phospholipase C (PLC), PE stimulates the formation of IP_3 and diacylglycerol (DAG). The former second messenger causes transient vasoconstriction via IP_3R channel activation and ensuing transient Ca^{2+} released from the SR, while DAG-induced activation of PKC leads to myosin light-chain phosphorylation and Ca^{2+}-independent vasoconstriction [14]. Rho at a relatively high dose (0.5 g/L) did not significantly influence vasoconstriction induced by PE in Ca^{2+}-free medium but antagonized vascular contraction induced by extracellular Ca^{2+} influx in the presence of PE. As Rho had no significant inhibitory effect on the contractile apparatus, this result strongly suggests that Rho inhibits extracellular Ca^{2+} influx through PE-activated ROCCs.

The opening of K^+ channels on vascular smooth muscle cells induces membrane hyperpolarization and depresses vasoconstriction by inhibition of extracellular Ca^{2+} influx through VOCCs [16]. However, the nonspecific K^+ channel blocker $BaCl_2$ [10] had no significant influence on the vasorelaxant response to Rho in endothelium-denuded rings precontracted with PE, so it is unlikely that Rho influences vascular smooth muscle K^+ channels.

The Protocol 1 experiments showed no significant differences in Rho-induced vasoconstriction at resting tension and relaxation after PE-induced contraction between endothelium-intact rings and endothelium-denuded rings. This suggests that Rho has no significant influence on endothelial function related to regulation of vascular tension.

Salidroside is regarded as the primary active component of Rho [17]. However, salidroside had no influence on the tension of aortic rings, whether with intact endothelium or denuded endothelium, under any condition tested (resting state or vasoconstricted by PE or KCl) even at concentrations 100-fold higher than effective Rho concentrations. This result strongly suggests that the vasomotor responses to Rho are produced by other as yet unidentified active components.

This study has several limitations: Further studies are needed to directly measure the effects of Rho on membrane potential, membrane excitability, Ca^{2+} currents, intracellular Ca^{2+} concentrations, and myosin-light chain phosphorylation. Further, an *in vivo* toxicological study is necessary to assess the safe dose range.

Conclusion

Rho has mechanistically distinct dose-dependent effects on vascular tension in rat isolated thoracic aorta. Rho (0.1 g/L in K-H solution and 0.5 g/L in Ca^{2+}-free medium) induce endothelium-independent vasoconstrictions at resting tension. Rho (0.5 g/L) dilates aortic rings precontracted with PE. Vasoconstriction in the relaxed state is mediated by enhanced Ca^{2+} influx through L-type Ca channels and by PKC signaling, while vasorelaxation in the contracted state results from inhibition of calcium influx through ROCCs. However, these responses are produced by active ingredients other than salidroside.

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Disclosure of conflict of interest

None.

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