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ORIGINAL ARTICLE

The potent relaxant effect of resveratrol in rat corpus cavernosum and its underlying mechanisms

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The aim of this study was to evaluate the relaxant effect of resveratrol (RVT), one of the most commonly employed dietary polyphenols, in rat corpus cavernosum, and to further investigate the contribution of possible underlying mechanisms. Strips of corpus cavernosum were used in organ baths for isometric tension studies. RVT (10^{-6}–10^{-4} M) produced concentration-dependent relaxation responses in rat corpus cavernosum precontracted by phenylephrine. The relaxant responses to RVT partially, but significantly inhibited by removal of endothelium. Nitric oxide (NO) synthase (NOS) blocker N-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M) or soluble guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10^{-5} M) caused a significant inhibition on relaxation response to RVT, whereas cyclooxygenase inhibitor indomethacin (10^{-5} M) did not significantly alter relaxant responses of corpus cavernosum strips to RVT. Corpus cavernosum contractions induced by stepwise addition to Ca^{2+} to high KCl solution with no Ca^{2+} were significantly inhibited by RVT incubation. The treatment of corpus cavernosum tissues with non-specific potassium channel inhibitor tetraethylammonium (TEA, 10^{-2} M) did also significantly affect the relaxant activity of RVT. Otherwise, the relaxation response of corpus cavernosum induced by the phosphodiesterase-5 inhibitor sildenafil increased significantly in the group pretreated with 10^{-5} M RVT. These results demonstrated that RVT has a potent relaxant effect on rat corpus cavernosum via endothelium-dependent and -independent mechanisms. Endothelium-dependent relaxation of corpus cavernosum to RVT is thought to be mediated primarily through NO/cGMP signaling pathway, and possibly an additional mechanism, endothelium-dependent hyperpolarization factor (EDHF). The residual endothelium-independent corpus cavernosum relaxation induced by RVT is uncertain but seems to depend on the interactions of RVT with Ca^{2+} entry mechanism from the extracellular space and also other undefined direct effects in this tissue.

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INTRODUCTION

Penile erection is a neurovascular phenomenon that requires dilation of penile vasculature, relaxation of smooth muscle, increased intracavernosal blood flow and veno-occlusive function.[1] Relaxation of cavernous smooth muscle is critical for inducing and maintaining penile erection. Normal erectile function depends on a precise balance between vasoconstrictor and vasodilatory systems that determine the tone of corpora cavernosal smooth muscle of the penis.[2,3] Erectile dysfunction (ED), which has been postulated by an alteration in this balance, is defined as the permanent inability to achieve or maintain an erection sufficient for satisfactory sexual intercourse for a period of at least 6 months.[4] Most cases of ED are due to a vascular pathology, which results from impairment of endothelium-dependent or -independent smooth muscle relaxation.[5,6] ED is a condition of increasing prevalence worldwide, which has been estimated to affect ~50% of men aged 40–70 years.[7]

Currently, phosphodiesterase-5 (PDE5) inhibitors have been widely employed in the management of male ED.[8] However, PDE5 inhibitors are dependent upon the integrity of the nitric oxide (NO) generating system, thereby such drugs are less efficient in patients with diabetes.[9] The patients treated with nitrates or nitrate donors or vasodilators such as α-blockers should not take PDE5 inhibitors, otherwise, combining these drugs will result in hypotension.[9,10] In addition, these drugs have many side effects, such as headache and visual impairment,[11] which shows that novel pharmacological approaches are required for enhancing penile erection in these patients. Therefore, alternative forms of medical treatment remain clinically interesting, including the use of plant extracts, which many patients prefer as a treatment option. In this case, biologically active polyphenols may be good candidates because they are relatively safe and chronic dosing is possible.[12–15] Among them, resveratrol (trans-3,5,4′-trihydroxy-stilbene; RVT) has been used in traditional herbal medicine based on its safety and efficacy.[16–18]

RVT, one of the most commonly employed dietary polyphenol, was first isolated from the roots of white hellebore (*Veratrum grandiflorum*) in 1940, and later, in 1963, from the roots of *Polygonum cuspidatum*, a plant used in traditional Japanese medicine. It seems to be present in red wine in significant amounts, and to be partly responsible for cardiovascular benefits associated with wine consumption.[19,20] The favorable cardiovascular effects of RVT have been demonstrated by a substantial number of experimental and clinical studies. In addition, previous studies have shown that RVT inhibits KCl-, noradrenaline- and phenylephrine-induced contraction of rat aorta and mesenteric artery, human internal mammarian artery, porcine coronary artery and guinea-pig mesenteric and uterine arteries.[19–26] The relaxant effect of RVT may vary in different tissues. The RVT-induced vasorelaxation may either be

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endothelium dependent (attenuated by NO synthase (NOS) blocker N-nitro-L-arginine methyl ester, L-NAME) or endothelium independent.[19–27] The differences in the previous studies investigating the possible mechanism(s) involved the relaxant effect of RVT may be due to differences in vascular beds and/or the species tested. Recently, it has been reported that RVT has a positive effect on male reproductive function by triggering *ex vivo* penile erection, as well as enhancing blood testosterone levels, testicular sperm counts and epididymal sperm motility.[28] However, the mechanism(s) underlying the relaxant effect of RVT on corpus cavernosum tissue have been not investigated until today.

Based on the explanation above, RVT might be a therapeutically viable strategy in managing ED, however, there have not been enough studies to investigate the effect of RVT on corpus cavernosum. This study was designed to explore whether RVT produces direct relaxant effect in isolated rat corpus cavernosum and to further investigate the contribution of possible underlying mechanisms. Thus, apart from block of NO-mediated relaxation by NOS inhibitor L-NAME, role of prostacyclin and/or endothelial-derived hyperpolarizing factor (EDHF) on this response was evaluated in the corpus cavernosum to determine the contribution of vascular endothelium to the relaxation mechanism. Besides, the contribution of extracellular/intracellular Ca^{2+} to the RVT-induced dilation of corpus cavernosum was also examined using appropriate experimental protocols. Except the relaxant effect of RVT alone on isolated corpus cavernosum, we have evaluated whether RVT could interfere with NO–cGMP pathway, and thereby potentiate the effect of PDE5 inhibitors in rat corpus cavernosum.

MATERIALS AND METHODS

Experimental procedures

This study was registered by the Animal Ethics Committee of Akdeniz University Medical Faculty, Antalya, Turkey. Briefly, Male Wistar rats, weighing 250–300 g, were used in the present study. All animals were housed under the standard animal laboratory conditions (12 h lighting cycle, controlled temperature and humidity).

Isolated organ bath technique

The rats were humanly killed by cervical dislocation. Penises were surgically removed at the level of the crural attachments to the pubo-ischial bones and promptly placed in a Petri dish containing ice-cold (4 °C) Krebs solution (containing in mM: NaCl 118, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1.0, $MgSO_4$ 1.2, $CaCl_2$ 2.5 and glucose 11.2), bubbled with a mixture of 95% O_2 and 5% CO_2. The glans penis and urethra were excised and the corpus cavernosum tissue was then dissected free from the tunica albuginea. The two corpus cavernosums were separate by cutting the fibrous septum between them. The time frame from dissection of corpus cavernosum to organ bath experiments was about 20 min. The corpus cavernosum strips were carefully suspended by two stainless steel clips passed through the vessel lumen in 20 ml organ baths filled with Krebs solution maintained at 37 °C and gassed with 95% O_2 and 5% CO_2 to obtain a pH of 7.4. Isometric tension was continuously measured using an isometric force transducer (FDT10-A, Commat Ltd., Ankara, Turkey), connected to a computer-based data acquisition system (MP35, Commat Ltd.). In all, 500 mg of tension was applied to each strip and allowed to equilibrate for 60 min. During the resting periods, the organ bath solution was changed every 15 min. For the relaxation studies, corpus cavernosum strips were precontracted with Phe (10^{-5} M). This concentration was determined from the cumulative contraction–response curves to achieve 80% of the maximum contraction. In those experiments evaluating the role of endothelium, the detergent 2% CHAPS was used to remove endothelium of corpus cavernosum. The absence of functional endothelium was confirmed on each corpus cavernosum strip by the lack of relaxation response to acetylcholine (10^{-6} M).

Protocol

Experiments were done on sets of two preparations, one containing and the other denuded of endothelium. The effect of RVT (10^{-6}–10^{-4} M) on Phe-induced contractions was investigated in both endothelium-intact and -denuded corpus cavernosum strips. After further rinsing, corpus cavernosum tissues precontracted with Phe were exposed to RVT in the presence of NOS blocker L-NAME (10^{-4} M), cyclooxygenase inhibitor indomethacin (INDO, 10^{-5} M), or soluble guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10^{-5} M), and relaxation responses were recorded. ODQ, INDO and L-NAME were added to the bath 30 min before the study. In another set of experiments, to investigate the possible contribution of EDHF and K^+ channels on RVT-induced corpus cavernosum relaxation, tissues were incubated with 80 mM KCl and non-selective K^+ channel blocker tetraethylammonium (TEA, 10^{-2} M for 30 min) to inhibit the production of EDHF and K^+ channels, respectively, and RVT-induced relaxation responses were obtained. To test the effect of the solvent, ethanol alone was added cumulatively in the same volumes as those used in the experiments with RVT.

In another set of experiments, high KCl solution (Ca^{2+} free 80 mM) was used to activate the Ca^{2+} entry mechanism from the extracellular space. Following depolarization, various concentrations of Ca^{2+} were cumulatively applied in a stepwise manner to a final concentration of 2 mM. In calcium-free high KCl solution, $CaCl_2$ was replaced with $MgCl_2$, iso-osmotically and 2 mM EGTA (ethyleneglycol-bis-(b-amino-ethyl ether)-N,N,N′,N′-tetra acetic acid) was added. After the control experiments, the same protocol was repeated after the 30 min of RVT incubation (10^{-5} M) and responses were recorded. In addition, Phe was used to stimulate IP3-stimulated Ca^{2+} release from the intracellular Ca^{2+} stores in the absence of extracellular calcium (Ca^{2+}-free Krebs solution). In Ca^{2+}-free studies, the tissues were allowed to equilibrate for a further 45 min. Following this equilibration period, the bathing solution was replaced with Ca^{2+}-free Krebs solution of the following composition: NaCl 118, KCl 4.7, $NaHCO_3$ 25, KH_2PO4 1.3, $MgSO_4$ 0.6, EGTA 10 and glucose 11.1. After 5 min, tissues were constricted with Phe (10^{-5} M). Once constriction responses had reached a maximum, the tissues were washed with Krebs solution until the baseline tension had been re-established. The tissues were again allowed to equilibrate for 45 min after which time the tissues were bathed in Ca^{2+}-free Krebs for 5 min, and then the constriction to Phe was repeated. In some experiments, RVT (10^{-5} M) was added 30 min before the second Ca^{2+}-free constriction. Amplitude of the Phe- or Ca^{2+}-induced contraction was expressed in mg per mg of tissue (mg tension/mg tissue).

To investigate the possible synergistic interaction between RVT and sildenafil citrate, concentration–response curves for sildenafil citrate (10^{-9}–10^{-6} M) were constructed in precontracted preparations. Thereafter, the tissues were washed and similar curves were performed 30 min after the addition of RVT (10^{-5} M). Doses of L-NAME, ODQ, INDO, TEA and sildenafil were chosen on the basis of previously reported data.[29–33]

Materials

RVT, acetylcholine, phenylephrine, L-NAME, INDO, TEA, ODQ, sildenafil citrate and the salts for the Krebs solution were purchased from Sigma Chemical (St Louis, MO, USA). All drugs were prepared fresh daily during experiments and were dissolved in distilled water before use, except in the case of INDO and RVT (initially dissolved in ethanol). RVT was dissolved in 70% v/v ethanol with further dilution in distilled water before use. Working concentrations of ethanol in the bath were $<0.1\%$ (v/v).

Statistical analysis

All values are expressed as mean ± s.e.m. Responses to RVT are expressed as percentages of the reversal of the tension developed in response to Phe. The logarithm of the concentration of agonists that elicited a 50% of maximal response (E_{max}) was designated as the EC_{50}. These values were determined by regression analysis of the linear portions of the log concentration–response curves. Sensitivity was expressed as pD_2 ($-\log EC_{50}$). Statistical analysis of the results was performed by Student's *t*-test. A *P*-value of lower than 0.05 was considered as significant.

RESULTS

Role of endothelium, NO, PGs and EDHF on RVT-induced relaxation of corpus cavernosum

Phe elicited a stable contraction in rat corpus cavernosum strips. In the endothelium-intact tissues, which were precontracted with Phe, addition of RVT (10^{-6}–10^{-4} M) caused a potent relaxation response in a concentration-dependent manner (Figure 1). The maximal relaxation response to 10^{-4} M RVT was 71.8 ± 3.2%.

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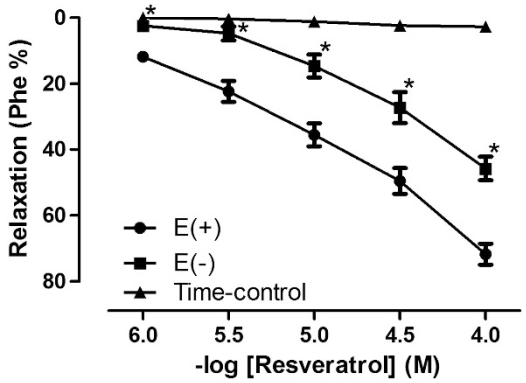


Figure 1. Effect of removal of endothelium on resveratrol-induced relaxation responses in rat corpus cavernosum. All values are expressed as mean ± s.e.m. $n = 6–8$ for all groups. E(+), endothelium-intact corpus cavernosum strips; E(−), endothelium-denuded corpus cavernosum strips. *$P < 0.05$ as compared with relaxation of E(+) corpus cavernosum strips. Phe, phenylephrine.

Time-control curve is shown in Figure 1. Our results demonstrated that the relaxant effect induced by RVT was not due to the solvent. The final concentration of solvent in the organ bath was <0.1%, which had no effect on basal tone of the corpus cavernosum strips.

The RVT-induced relaxation was partially abolished by removal of endothelium (Figure 1) ($P < 0.05$). After the endothelial injury, RVT (10^{-4} M)-induced maximal relaxation decreased from 71.8 ± 3.2% to 45.8 ± 3.6%. The relaxant response induced by RVT was also partially inhibited by either L-NAME or ODQ incubation (Figures 2a and b) ($P < 0.05$). However, the relaxant effect induced by RVT was not significantly inhibited by INDO incubation (Figure 3a) ($P > 0.05$). To investigate the role of EDHF in RVT-induced relaxation, rat corpus cavernosum strips were contracted with 80 mM KCl. RVT-induced relaxation responses in the presence of 80 mM KCl were less than those observed in Phe-contracted tissues (Figure 3b) ($P < 0.05$). Also, pretreatment of corpus cavernosum strips with non-specific K^+ channel blocker TEA caused a significant reduction in relaxation response to RVT (Figure 3c) ($P < 0.05$).

Role of extracellular/intracellular Ca^{2+} on RVT-induced relaxation of corpus cavernosum

Contraction response of corpus cavernosum induced by stepwise addition of $CaCl_2$ to high KCl solution with no Ca^{2+} was also significantly inhibited by 30 min RVT (10^{-5} M) incubation. E_{max} values for Ca^{2+}-induced contractions with and without RVT were 4.71 ± 0.82 and 1.88 ± 0.10 mg, respectively (Figure 4) ($P < 0.05$). When Ca^{2+}-free Krebs solution was used, Phe-induced contraction in rat corpus cavernosum incubated with RVT (10^{-5} M, 30 min) was similar to that observed in control corpus cavernosum strips (Figure 5) ($P > 0.05$). E_{max} values were not significantly different between control and RVT groups (1.20 ± 0.20 and 1.14 ± 0.09 mg, respectively).

Effect of RVT incubation on sildenafil-induced relaxation of rat corpus cavernosum

Cumulative addition of the PDE5 inhibitor sildenafil (10^{-9}–10^{-6} M) concentration-dependently relaxed rat corpus cavernosum with pD_2 value of 6.96 ± 0.29 (Figure 6). Relaxation response of corpus cavernosum induced by sildenafil was significantly enhanced by 30-min RVT (10^{-5} M) incubation, as shown in Figure 6. However, pD_2 and E_{max} values for sildenafil were not significantly different between control and RVT-incubated corpus cavernosum strips (Table 1).

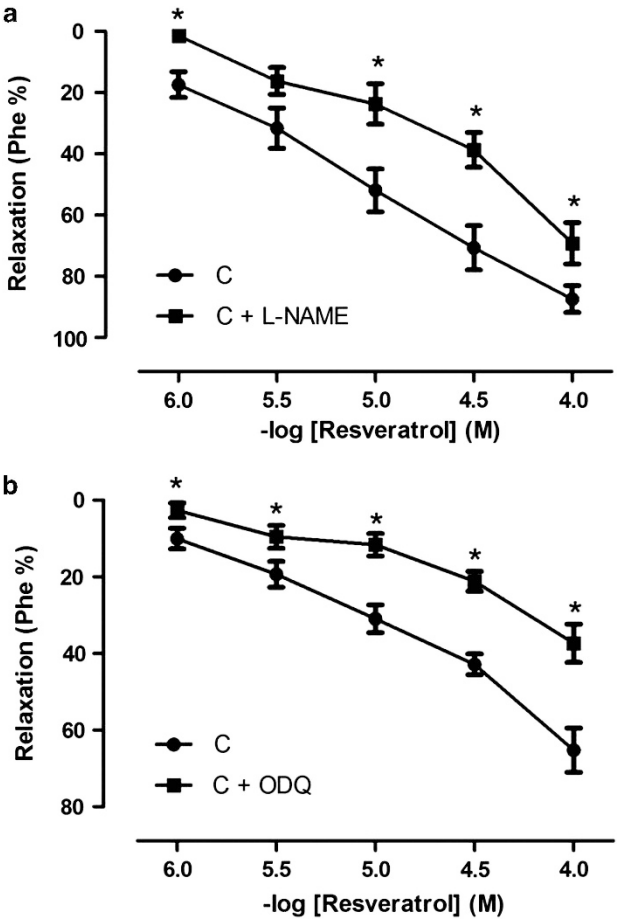


Figure 2. Effect of N-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M) (**a**) and 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10^{-5} M) (**b**) incubation on resveratrol-induced relaxation responses in rat corpus cavernosum. All values are expressed as mean ± s.e.m. $n = 6–8$ for all groups. C, control; *$P < 0.05$ as compared with control. Phe, phenylephrine.

DISCUSSION

The present study describes the potent relaxing effect of RVT in isolated rat corpus cavernosum. The results of the present study also provide, for the first time, pharmacological evidence showing that both endothelium-dependent and endothelium -independent signal pathways are involved in the relaxant effect of RVT in rat corpus cavernosum. The endothelium-dependent vasorelaxant effects of RVT are mediated via NO signaling pathway, whereas the interactions of RVT with Ca^{2+} entry mechanism from the extracellular space seem to be responsible for endothelium-independent relaxation.

A number of mechanisms have been identified for the local regulation of penile smooth muscle contractility. Therefore, it is expected that drugs interfering with these mechanisms may enhance relaxation of smooth muscle in the corpus cavernosum and thereby facilitate penile erection. The results of present study clearly showed that RVT might induce a concentration-dependent relaxation in rat corpus cavernosum. The EC_{50} value for inhibition of Phe-elicited contractions of rat corpus cavernosum calculated here was 10 μM, which is three times lower than the EC_{50} of 32 μM, which was observed with rat aortic smooth muscle.[24] These results suggest that the corpus cavernosum had higher sensitivity to RVT than aorta, which indicates its tissue selectivity. Several mechanisms may be possible to explain the relaxant effect of RVT in rat corpus cavernosum strips. In the present study, the relaxant effect of RVT was partially abolished by the removal of endothelium, suggesting that this effect is in part mediated

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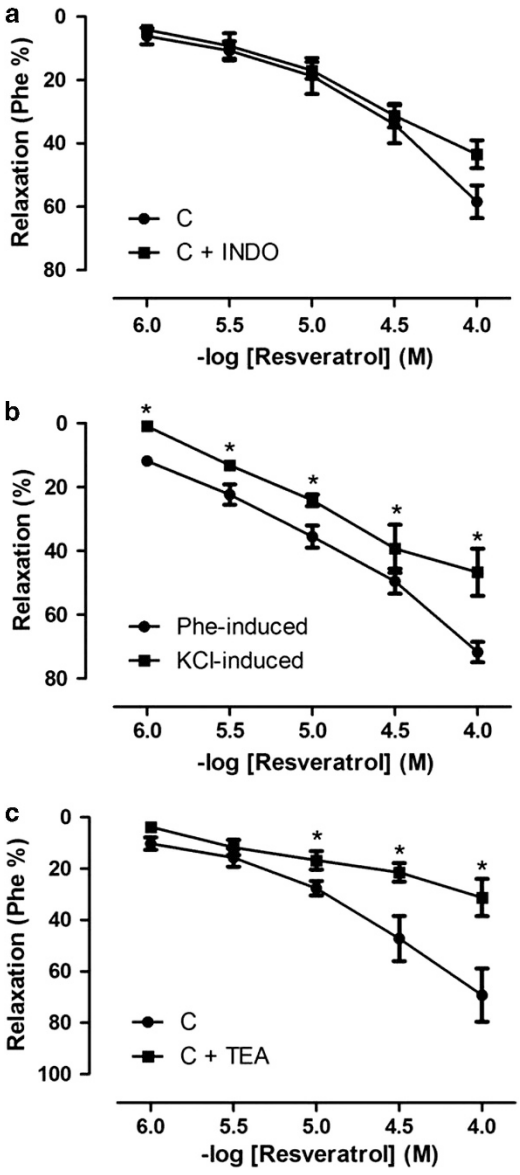


Figure 3. Effect of indomethacin (INDO) (10^{-5} M) (**a**), 80 mM KCl (**b**) and TEA (**c**) incubation on resveratrol-induced relaxation responses in rat corpus cavernosum. All values are expressed as mean ± s.e.m. $n = 6$–8 for all groups. Phe, phenylephrine; TEA, tetraethylammonium; *$P < 0.05$ as compared with relaxation of strips precontracted with Phe.

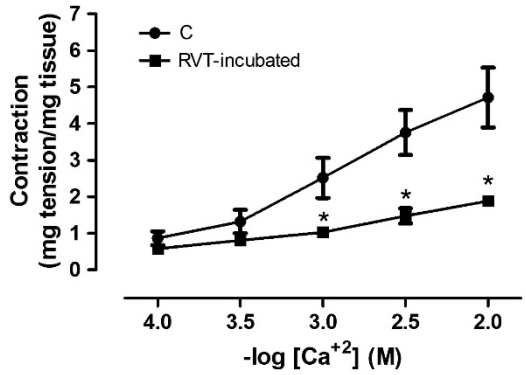


Figure 4. Effect of resveratrol incubation (10^{-5} M) on contraction induced by various concentration of $CaCl_2$ in calcium-free solution containing 80 mM KCl in rat corpus cavernosum. All values are expressed as mean ± s.e.m. $n = 8$ for all groups. C, control; RVT, resveratrol; *$P < 0.05$ as compared with control.

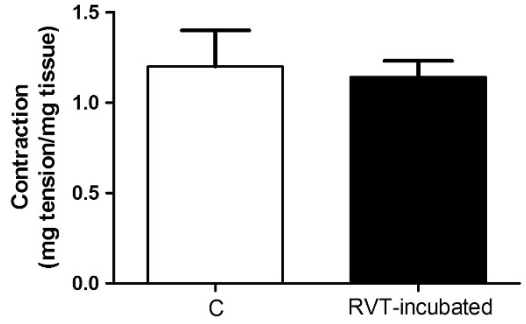


Figure 5. Effect of resveratrol incubation (10^{-5} M) on contraction induced by phenylephrine (10^{-5} M) in calcium-free solution containing 2 mM EGTA (ethyleneglycol-bis-(b-amino-ethyl ether)-N,N,N′,N′-tetra acetic acid) in rat corpus cavernosum. All values are expressed as mean ± s.e.m. $n = 6$ for all groups. C, control; RVT, resveratrol; Phe, phenylephrine; *$P < 0.05$ as compared with control.

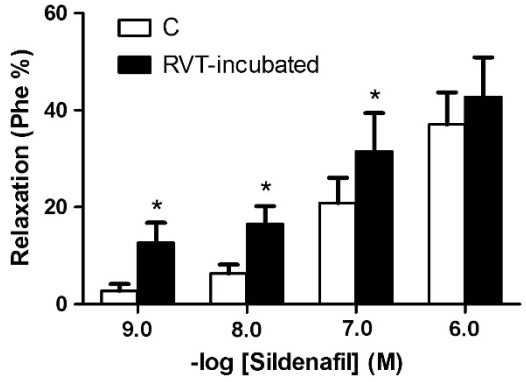


Figure 6. Effect of resveratrol incubation (10^{-5} M) on relaxation responses induced by various concentration of sildenafil citrate in rat corpus cavernosum. All values are expressed as mean ± s.e.m. $n = 8$ for all groups. C, control; RVT, resveratrol; *$P < 0.05$ as compared with control.

through endothelium in corpus cavernosum strips. It is well known fact that the activation of endothelial cells triggers the synthesis of several factors, such as NO, prostacyclin and EDHF.[34] The endothelium-dependent vascular relaxation is mainly mediated by NO and to a lesser extent by prostacyclin and EDHF.[35] In a previous study, Chen and Pace-Asciak[24] observed that trans-RVT caused an NO-mediated relaxation of precontracted endothelium-intact rat aorta. To investigate whether relaxation induced by RVT was attributable to an interaction with NO-cGMP pathway, in our study, corpus cavernosum strips were incubated with NOS inhibitor, L-NAME, and guanylyl cyclase inhibitor, ODQ. Our results have demonstrated that relaxation response induced by RVT was partially inhibited by L-NAME incubation, supporting the role of NO in RVT-induced corpus cavernosum relaxation. Additionally, RVT-induced relaxation was significantly reduced, but not completely inhibited after the ODQ incubation. These results indicate that NO-cGMP signaling may be only one of several factors mediating the relaxant response to RVT. It is well known that prostaglandins cause an increase in intracellular cAMP levels in corpus cavernosum smooth muscle, thereby promotes muscle relaxation through intracellular reduction of calcium concentration, resulting in erection.[36] Although the endothelium releases vasodilator prostaglandins such as prostacyclin, a possible role of the vasodilator prostaglandins in the RVT-induced corpus cavernosum relaxation was excluded by the preincubation

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Table 1. pD_2 ($-\log EC_{50}$) and E_{max} values for sildenafil citrate on phenylephrine-induced contractions of rat corpus cavernosum strips: effects of resveratrol (10^{-5} M) incubation

	pD_2	E_{max}	n
Sildenafil citrate	6.96 ± 0.29	37.1 ± 6.6	8
RVT + sildenafil citrate	7.16 ± 0.50	42.7 ± 8.2	8

Abbreviation: RVT, resveratrol.
All values are expressed as mean ± s.e.m.

of corpus cavernosum strips with the cyclooxygenase inhibitor INDO. In the present study, the treatment of corpus cavernosum tissues with INDO did not affect the relaxant activity of RVT. Such findings may suggest that RVT-induced relaxation of corpus cavernosum tissue is partially endothelium dependent, and rules out an involvement of prostacyclin in the relaxation response to RVT.

The relaxant effects that are resistant to inhibitors of the L-arginine–NO–cGMP pathway could be attributed to EDHF-mediated membrane hyperpolarization because it is reported that EDHF-mediated relaxation and membrane hyperpolarization are resistant to inhibitors of the L-arginine–NO pathway such as oxyhemoglobin, methylene blue or L-NAME.[37,38] EDHF has been shown to induce hyperpolarization of the cell membrane by opening K^+ channels.[39] Depolarizing artery rings *in vitro* by increasing extracellular K^+ levels prevents the relaxation response to EDHF.[40] Elevation of extracellular K^+ results in a diminished driving force for K^+ ions and abolishes hyperpolarization induced by activation of K^+ channels.[41] This common methodological approach was used in our study to confirm that different K^+ channels are involved in RVT-induced relaxation of rat corpus cavernosum. In line with these observations, we found that RVT produces less relaxation response in 80 mM KCl-contracted tissues when compared with those observed in Phe-contracted ones. Therefore, our results suggesting that the relaxant response to RVT in corpus cavernosum tissue may be partially mediated by EDHF-like mechanisms. Although nature of EDHF remains to be identified, K^+ channels, at least in part, may be involved in EDHF-induced relaxation response induced by RVT in this tissue. Using a non-selective K^+ channel inhibitor TEA, we have confirmed that K^+ channels have a part in the dilator response of corpus cavernosum to the RVT. Indeed, relaxation response of corpus cavernosum to RVT was significantly inhibited by pretreatment with TEA, suggesting that the relaxant effect may be associated with hyperpolarization due to activation of K^+ channels. The results of a previous study showed that RVT potently relaxed rat aortic rings with denuded endothelium.[42] It seems that 4-aminopiridine and margatoxin-sensitive K^+ channels located in the smooth muscle of rat aorta mediated this relaxation. Similarly, K^+ channels located in smooth muscle of human internal mammary artery seem to be responsible for the relaxant effect of RVT in this tissue.[23] The results of another previous study demonstrated that the mechanism of vasorelaxation of rat mesenteric artery induced by RVT probably involves activation of 4-AP-sensitive K^+ channels as well as K^+ channel-independent mechanism(s).[43] This discrepancy in the results obtained with different experimental models indicates tissue and species selectivity for RVT. The present experimental results are in agreement with previous studies reporting that RVT induces a marked vasodilation in different arteries by activating K^+ channels.[23,42,43] However, studies including the effects of different types of K^+ channel blockers on RVT-induced relaxation response in corpus cavernosum tissue were not performed in this study, and further studies are needed to understand the type of K^+ channels responsible for this effect.

Although essential for penile erection, NO-dependent cGMP is not the only signaling process responsible for penile smooth muscle relaxation. Endothelium of corpus cavernosum can be damaged due to several conditions and diseases, such as diabetes mellitus, hypertension, atherosclerosis, aging and smoking. Having this in mind, it is of great importance to investigate the effect of vasodilatatory substances in corpus cavernosum with denuded endothelium. Other mechanisms regulating smooth muscle tonus in the penis can be considered as targets for the development of new drugs to treat ED.[44] Our results indicated that RVT potently relaxed rat corpus cavernosum strips with denuded endothelium. It is understood that the contractile activity of corpus cavernosum smooth muscles is regulated by the concentration of free Ca^{2+} in the cytosol, which depends on voltage-dependent Ca^{2+} influx, receptor-operated Ca^{2+} influx and the release of intracellularly stored Ca^{2+}.[45] Actually, agents that decrease the concentration of $[Ca^{2+}]i$ in the cytoplasm could be expected to enhance relaxation of smooth muscle in the corpus cavernosum and thereby facilitate penile erection. Since RVT may interfere with Ca^{2+} release from intracellular stores and Ca^{2+} entry from the extracellular space through the plasma membrane Ca^{2+} channels, further studies were planned to explore this possibility in rat corpus cavernosum. The present study has indicated that contractions of corpus cavernosum strips induced by stepwise addition of Ca^{2+} to high KCl solution with no Ca^{2+} were significantly inhibited by RVT incubation. This result suggests that RVT exerted a voltage-dependent calcium channel blocking effect in corpus cavernosum smooth muscle cells, which decreased the high K^+-induced Ca^{2+} influx and relaxed corpus cavernosum smooth muscle. This might be one of the mechanisms of RVT on the relaxation of corpus cavernosum smooth muscle. Under calcium-free conditions, contractions of corpus cavernosum strips incubated with RVT (10^{-5} M, 30 min) in response to Phe were not significantly lower than those of strips from control rats. These results indicate that RVT has not a significant effect on Ca^{2+} release from intracellular stores in rat corpus cavernosum strips. Our results are consistent with the results of another previous study, which has been shown that two mechanisms participate partially to the RVT-induced relaxation of porcine coronary artery, one being the NO released from the endothelium, the other causing inhibition of Ca^{2+} influx.[46]

The combination of PDE inhibitors with K^+ channel openers resulting to further enhancement of arterial blood supply into penis has become a new feature for pharmacological treatment of ED.[47] In addition to its potent relaxant effect alone, RVT administration may cause a synergistic effect on sildenafil-induced relaxations of corpus cavernosum. Indeed, results of the present study indicate that preincubation of corpus cavernosum strips with RVT efficiently increased sildenafil citrate-induced relaxation. The relaxation activity of sildenafil citrate in rat corpus cavernosum tissue preincubated with RVT (10^{-5} M) was higher than the relaxation induced by sildenafil citrate alone. Thus, the combination of less effective doses of sildenafil and RVT has a potential advantage on erectile functions. The importance of this combination remains to be solved clinically.

Conclusion

The results of the present study have clearly shown that RVT produces endothelium-dependent relaxation of rat corpus cavernosum by a mechanism that involves NO release, and possibly an additional mechanism, EDHF. The relaxant effect of RVT is also independent from endothelium, and partially it may be related with significant changes in Ca^{2+} entry from extracellular space through the plasma membrane Ca^{2+} channels in rat corpus cavernosum. Importantly, RVT exerted an increasing effect on the sildenafil citrate-induced relaxation of corpus cavernosum. This research may provide the possibility that RVT may become an

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alternative medicine for patients who want to use natural products to improve erectile function.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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