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Pharmacological profile of evodiamine in isolated rabbit corpus cavernosum

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Abstract

This study was designed to examine the pharmacological properties of evodiamine in isolated rabbit corpus cavernosum. In phenylephrine-precontracted cavernosal strips, evodiamine (0.01–10 μM) induced a concentration-dependent relaxation. Endothelium removal, N^G-nitro-L-arginine methyl ester (L-NAME), or 1-*H*-[1,2,4]oxadiazolo [4,3-α] quinoxalin-1-one (ODQ) treatment did not affect this effect. In endothelium-denuded preparations, evodiamine-evoked response was significantly reduced in 60 mM KCl-precontracted strips and by charybdotoxin treatment, but not by glibenclamide. Higher-concentration evodiamine (≥10 μM)-induced relaxation was also accompanied by an increase in cAMP and cGMP levels, but this effect was not affected by *cis*-*N*-(2-phenylcyclopentyl)-azacyclotridec-1-en-2-amine mono-hydrochloride (MDL-12,330A, an adenylyl cyclase inhibitor) or ODQ (a guanylyl cyclase inhibitor), respectively. Evodiamine significantly augmented both the corporal relaxation and the accumulation of cyclic nucleotides to sodium nitroprusside and forskolin, respectively. Evodiamine also enhanced electrical field stimulation-evoked relaxation, and this additive effect was significantly counteracted by zaprinast. In preparations obtained from aged rabbits, evodiamine still elicited complete relaxation; in contrast, acetylcholine- and sodium nitroprusside-evoked maximal response was significantly blunted. In summary, evodiamine possesses a potent corporal relaxing effect which is attributable to endothelium-independent properties probably linked to charybdotoxin-sensitive K^+ channel activation in the cavernosal vasculature and by nonselective interfering phosphodiesterase to prevent cyclic nucleotide degradation. Furthermore, the physiological effects of evodiamine on the aged animals may implicate a potential for the treatment of erectile dysfunction.

Keywords: Evodiamine; Corpus cavernosum; K^+ channel; Cyclic nucleotide

1. Introduction

For erection to take place, the penile arteries and erectile tissue (corpus cavernosum) have to dilate, thereby increasing the blood flow into the penis (Andersson and Wagner, 1995). In the corpus cavernosum, nitric oxide (NO) releases from non-adrenergic non-cholinergic (NANC) neurons and endothelium has been reported to play an important role in mediating penile erection (Cartledge et al., 2000; Sciarra et al., 2000). NO elicits the relaxation of smooth muscle by activation of guanylyl cyclase and the generation of cGMP (Escrig et al., 1999; Sciarra et al., 2000). In turn, impairment of the integrity of these systems has been widely implicated in the pathophysiology of erectile dysfunction. In addition to guanylyl cyclase, adenylyl cyclase was also found to be important as an intracellular second messenger (Beavo, 1995; Kim et al., 2000). This enzyme causes an increase in the intracellular concentration of cAMP, which will act in calcium channels to decrease the intracellular level of this ion, leading to a relaxation of the corpus cavernosum. Therapeutically, there are several drug types that, through enhancing the NO-cGMP axis or cAMP signal transduction, may prove beneficial in treating erectile dysfunction (Jeremy et al., 1997; Padma-Nathan and Giuliano, 2001). One such class of drugs is the phosphodiesterase inhibitors that prevent the hydrolysis of cAMP and/or cGMP, thereby elevating levels of these cyclic nucleotides.

Cavernous smooth muscle relaxation is effected through a complex biochemical pathway. Another candidate for the treatment of erectile dysfunction is K^+ channel activator. The large conductance Ca^{2+}-sensitive K^+ channel (K_{Ca}), a prominent K^+ current present in human corporal smooth muscle, is an important modulator of corporal smooth

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muscle tone (Lee et al., 1999). Furthermore, K^+ channel activators have been shown to relax human isolated corpus cavernosum and produce erection when injected intracorporeally into animals and mans (Hedlund et al., 1994; Lee et al., 1999; Martinez-Pineiro et al., 1996; Trigo-Rocha et al., 1995).

Erectile dysfunction is also age-associated. For ages, in order to satisfy the demands of consumers, Chinese had been doing lots of researches on drugs to enhance the activity of sex and some formulations have been prescribed for patients who were diagnoses of impotence. Wu-Chu-Yu-Tan is one of most frequently prescribed formula for treatment of impotence (Li, 1596). This ancient recipe consists of *Evodia rutaecarpa*, *Panax ginseng*, *Angelica sinensis*, and *Ziziphus jujuba*, with *E. rutaecarpa* as the predominant constituent. The unripened fruit of *E. rutaecarpa* is called "Wu-Chu-Yu". Many compounds had been isolated from *E. rutaecarpa* including the major alkaloid evodiamine (Chang and But, 1987). Previously studies on the cardiovascular system revealed that evodiamine elicited relaxation of isolated mesenteric artery and thoracic aorta (Chiou et al., 1992, 1996) and induced positive inotropic and chronotropic effects (Kobayashi et al., 2001). Basing on the previous reports on vascular beds, evodiamine may be expected to exhibit relaxation of the corpus cavernosum. The aim of the present work is to investigate the pharmacological properties of evodiamine by using in vitro assay.

2. Materials and methods

This study was approved by the Animal Care Committee of National Research Institute of Chinese Medicine and all efforts were made to minimize animal suffering and to reduce the number of animals.

2.1. Tissue preparation and endothelium disruption

The corpus cavernosum was excised from adult (4–5 months) male New Zealand White rabbits (3.0–3.5 kg) under anesthesia with sodium pentobarbital and immediately placed in Kerbs solution (mM: NaCl, 118; $NaHCO_3$, 25; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 10; $CaCl_2$, 2.5; pH 7.4). In some experiment, the specimen was isolated from aged rabbit (3 years old). The corpus cavernosum was carefully dissected free from the surrounding tunica albuginea and mounted in organ baths maintained at 37 °C (aerated with 5% CO_2, 95% O_2). Cavernosal strip was stretched to a resting force of 0.6–0.8 g and was equilibrated for at least 60 min. During this period, the tissue was washed with fresh solution every 15 min, and tension was adjusted if necessary (Chen et al., 2000; Chiou et al., 1998).

The endothelium lining the lacunar spaces of corpus cavernosum was disrupted by detergent treatment (Bookstein et al., 1990; Chen et al., 2000; Jansakul and Hirunpan, 1999). Briefly, the intact, isolated penis was placed in a tray containing chilled physiological salt solution (PSS). A 21-gauge minicatheter was inserted into each (left and right) corporal body at the proximal end of the crus of the penis. A third minicatheter was inserted into the distal end, below the glans penis, where the right and left corpora communicate. While the distal and one proximal minicatheter was clamped, 3 ml of 0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate (CHAPS, wt/vol) in a solution of normal saline was infused into the remaining proximal catheter. Leakage of the CHAPS solution through venous drainage was minimal. After a short interval (~ 20 s), the clamped minicatheters were opened and the preparation was extensively washed by infusion of PSS. This procedure was repeated for the proximal catheter on the opposite side. The corpora cavernosa were then removed and tested for endothelial integrity. Of the tissues treated with CHAPS, 85% did not relax or relaxed poorly (< 10% of maximal relaxation) to acetylcholine (1 μM) and were considered to be functional in the removal of endothelium.

2.2. Isometric tension and cyclic nucleotide level in corpus cavernosum

All experiments were performed in the presence of 3 μM tetradotoxin. The cavernosal strips were contracted with either phenylephrine or KCl (60 mM). After the contractile response had stabilized, evodiamine, sodium nitroprusside or forskolin was added cumulatively to the preparation during the tonic contraction. When the influence of K^+ channel inhibitors on the evodiamine-induced relaxation was evaluated, inhibitors were added to the preparation 30 min before addition of phenylephrine. When the effect of evodiamine was added to the preparation 20 min before, the second concentration–response curve for sodium nitroprusside or forskolin was obtained.

In the study of cyclic nucleotide level (cAMP and cGMP), the cavernosal strips were suspended in the organ bath and a phenylephrine-induced contraction was obtained as described above. When evodiamine caused a maximal change in tension, tissue was immediately frozen in liquid nitrogen. The frozen tissue was homogenized with a micro-homogenizer in 1 ml of 6% trichloroacetic acid containing 1 mM EDTA. After centrifugation (3000 × *g*, for 15 min, 4 °C), the supernatant was extracted with water-saturated diethyl ether, and aliquots of the aqueous phase were lyophilized to dryness, and then reconstituted in 1 ml of 50 mM sodium acetate buffer (pH 6.2). The cyclic nucleotide levels in the solution were measured with commercially available cAMP and cGMP immunoassay kits (Amersham, UK) (Takagi et al., 2001). Protein content was determined using the Bio-Rad Protein Assay kit microtiter plate assay procedure (Bio-Rad, Hercules, CA) with bovine serum albumin as the standard. The cyclic nucleotide levels are expressed in pmol/mg protein.

2.3. Electrical field stimulation in rabbit corpus cavernosum

Guanethidine (5 μm) and atropine (1 μm), to produce adrenergic and chlonergic blockade, respectively, were routinely added to the bath in the last 30 min of equilibration in order to detect the relaxation response to the stimulation of non-adrenergic non-cholinergic (NANC) nerves (Hosogai et al., 2001). Electrical field stimulation was accomplished by means of two platinum plate electrodes, positioned on either side of the tissue, and a current amplifier in series with a S8800 stimulator (Grass Instruments, Quincy, MA). After an equilibration period, strips were contracted with phenylephrine. When the phenylephrine induced contractile responses had stabilized, the tissue was subjected to the first electrical field stimulation-induced relaxation at 10 V (0.5-ms pulse duration), using sequential frequencies of 2, 8, 16, 32 Hz delivered as 20-s trains. Twenty minutes after addition of evodiamine to the bath, the second electrical field stimulation was performed.

2.4. Materials

Evodiamine was obtained as first extracted from the dried fruit of *E. rutaecarpa* with ethanol (60 °C for 16 h, four times), then separated by column chromatography (Amberlite XAD-2). The melting point of evodiamine was 253.5–256 °C (Jeng et al., 1995). The purity, as determined by high performance liquid chromatograhpy (HPLC) with a UV detector (227 nm, Waters), was greater than 99.8%. Stock solution of evodiamine was dissolved in dimethyl sulphoxide and further diluted in Krebs solution. The amount of vehicle added did not produce significant effects on the responsiveness of the tissues. Acetylcholine hydrochloride, N^G-nitro-L-arginine methyl ester (L-NAME), atropine sulphate forskolin, glibenclamide, guanethidine, KCl, 1-*H*-[1,2,4]oxadiazolo [4,3-α] quinoxalin-1-one (ODQ), phenylephrine hydrochloride, sodium nitroprusside and tetradotoxin were purchased from Sigma (St. Louis, MO, USA). Charybdotoxin was purchased from Research Biochemicals International (Natick, MA, USA).

2.5. Statistics

All data are expressed as mean ± S.E.M. Student's *t*-test were used to determine the significance of difference for means. $P < 0.05$ was considered statistically significant. For simultaneous multiple comparisons, the statistical significance of differences between groups was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons; the statistical significance of differences between groups was analyzed by one-way analysis of variance (ANOVA) followed by Dunnetts's multiple comparisons test. EC_{50} values were computer-assisted by using nonlinear curve fitting software (PHARM/PCS v.4.2).

3. Results

3.1. Effect of evodiamine on isometric tension

Evodiamine (0.01–10 μM) caused a concentration-dependent relaxation in the isolated rabbit corpus cavernosum precontracted with phenylephrine with an EC_{50} of 0.36 ± 0.05 μM (Fig. 1). The evodiamine-induced corporal relaxation was attenuated neither by removal of endothelium, L-NAME nor ODQ treatment.

3.2. Effects of high K^+ condition and K^+ channel inhibitors on evodiamine-induced corporal relaxation

Since endothelium disruption did not affect the response to evodiamine, precluding an endothelium-dependent mechanism, the following experiments were conducted to evaluate the endothelium-resistant response in endothelium-deprived corpus cavernosum. Firstly, the role of K^+ permeability in the action of evodiamine was studied in high K^+ condition by adding 60 mM of KCl as a constrictor. The amplitude of contractile response to 60 mM KCl was not significantly different from that by phenylephrine (2.4 ± 0.5 vs. 2.6 ± 0.3 g force), however, the relaxant activity of evodiamine was greatly reduced (Fig. 2A). The relaxant percentage observed with 10 μM evodiamine was reduced from 100% to 34.6 ± 3.8%. In contrast to evodiamine, the

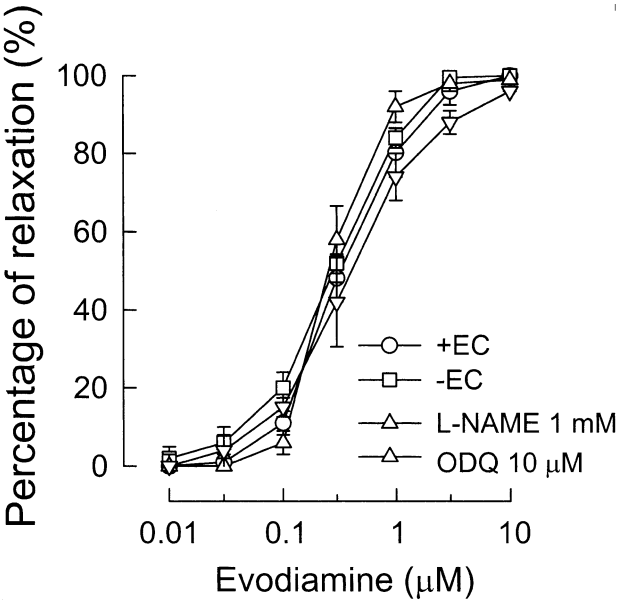

Fig. 1. Effects of N^G-nitro-L-arginine methyl ester (L-NAME), 1*H*-[1,2,4]oxadiazolo [4,3-α] quinoxalin-1-one (ODQ), and endothelium-denudation on evodiamine-induced relaxation of the isolated rabbit corpus cavernosum precontracted with phenylephrine. L-NAME or ODQ was added to endothelium-intact preparation 20 min before the addition of phenylephrine. Endothelial deprivation was described in Materials and methods. Data are shown as mean ± S.E.M. for four preparations.

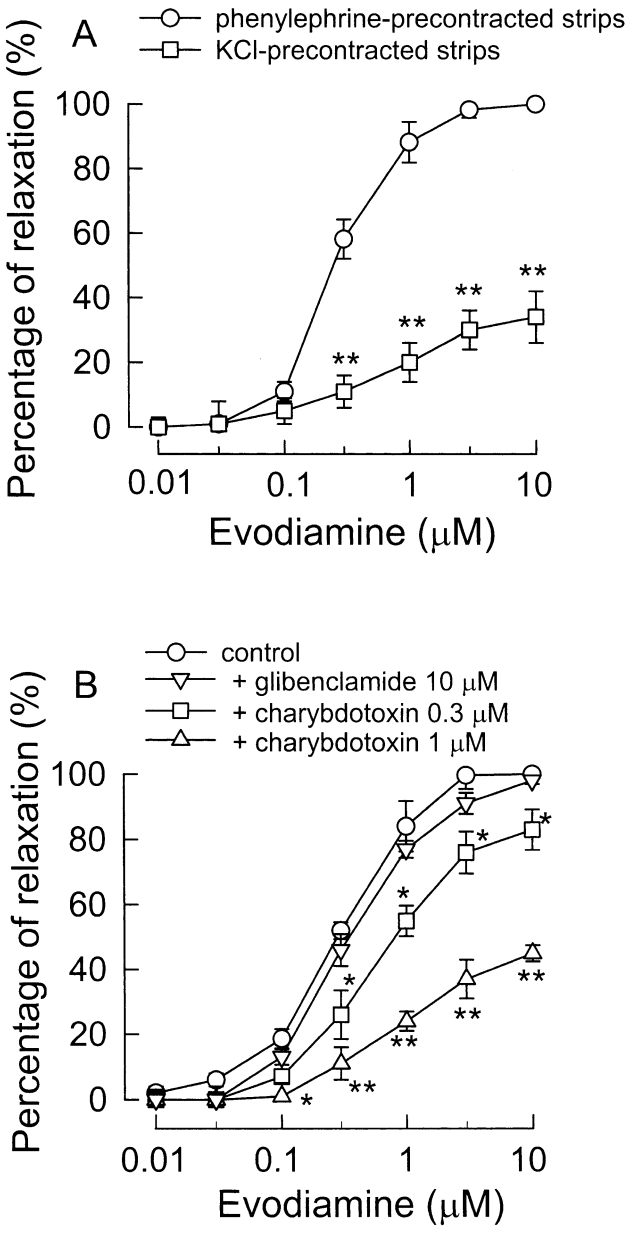

Fig. 2. Effects of high K^+ condition and K^+ channel inhibitors on the evodiamine-induced relaxation in the endothelium-denuded rabbit corpus cavernosum. (A) Relaxing effect of evodiamine in phenylephrine- and 60-mM-KCl-precontracted cavernosal strips, respectively. (B) Effect of ATP-sensitive K^+ channel inhibitor glibenclamide and large-conductance-Ca^{2+}-activated K^+ channel inhibitor charybdotoxin on the evodiamine-induced corporal relaxation. Data are shown as mean ± S.E.M. for five preparations. * $P<0.05$, ** $P<0.01$ versus control.

relaxant response to sodium nitroprusside was not affected significantly in the same condition (data not shown).

In order to ascertain further the contribution of membrane K^+ channels to evodiamine-induced relaxation, we test the effect of glibenclamide (an ATP-sensitive K^+ channel inhibitor) (Dhein et al., 2000; Ovunc, 2000) and charybdotoxin (a large conductance Ca^{2+}-activated K^+ channel inhibitor) (Hanner et al., 1997, 1998) on the relaxation to evodiamine in endothelium-denuded cavernosal strips. As shown in Fig. 2B, evodiamine-evoked relaxation was concentration-dependently inhibited by charybdotoxin (0.3 and 1 μM), with 10 μM evodiamine-evoked responses being reduced significantly to 79.2 ± 4.7% and 40.1 ± 2.5%, respectively. The attenuation by charybdotoxin (1 μM) resembled that in high K^+ condition. Preincubation of the tissues in glibenclamide (10 μM) almost abolished cromakalin (an ATP-sensitive K^+ channel activator)-induced re-

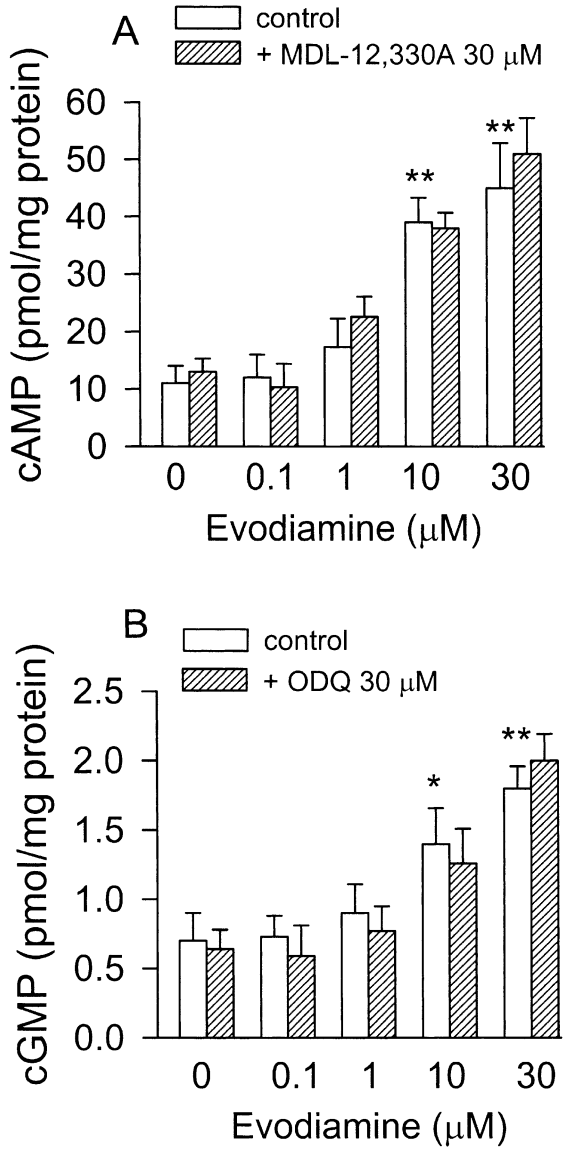

Fig. 3. Changes in cyclic nucleotide levels by evodiamine in phenylephrine-precontracted endothelium-denuded cavernosal strips before (control) and after adenylyl cyclase inhibitor MDL-12,330A or guanyl cyclase inhibitor ODQ treatment, respectively. (A) cAMP levels. (B) cGMP levels. Cyclic nucleotide (cAMP and cGMP) was measured with a commercially available kit (see Materials and methods). Data are shown as mean ± S.E.M. for five preparations. * $P<0.05$, ** $P<0.01$ versus group without evodiamine (indicated as "0"). ODQ: 1*H*-[1,2,4]oxadiazolo [4,3-α] quinoxalin-1-one.

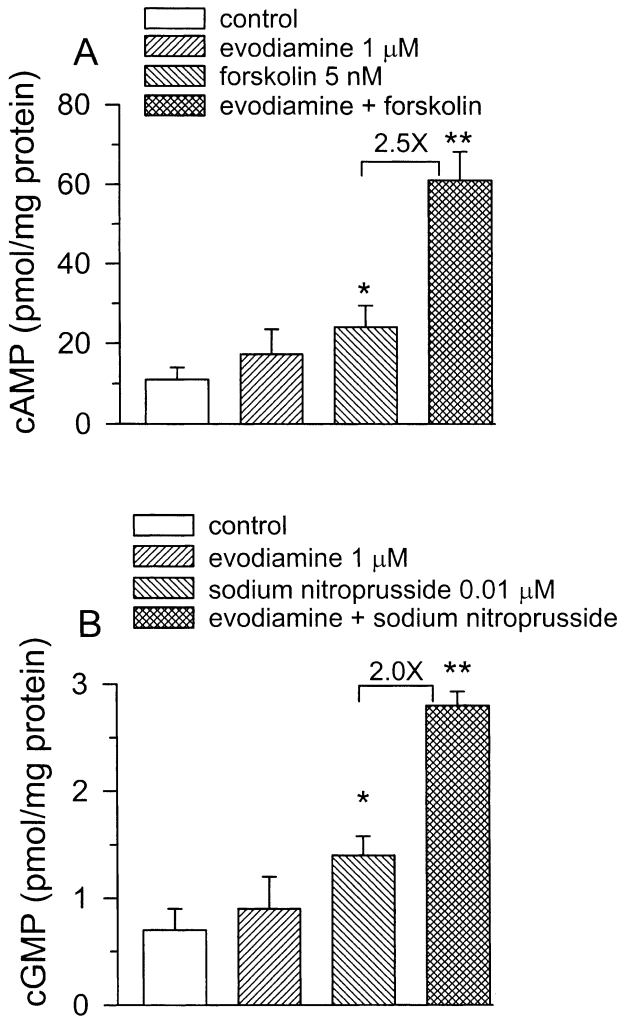

Fig. 4. Changes in cyclic nucleotide levels by vehicle control, evodiamine, forskolin, sodium nitroprusside, or combined application of evodiamine + forskolin and evodiamine + sodium nitroprusside treatment, respectively. (A) cAMP levels. (B) cGMP levels. Cyclic nucleotide (cAMP and cGMP) was measured with a commercially available kit (see Materials and methods). Data are shown as mean ± S.E.M. for four preparations. * $P < 0.05$, ** $P < 0.01$ versus control.

laxation (data not shown), however, it did not affect significantly the evodiamine-evoked response.

3.3. Effect of evodiamine on cyclic nucleotide levels in corpus cavernosum

The following experiments were performed in endothelium-deprived preparations. Evodiamine stimulated the accumulation of cAMP in phenylephrine-precontracted cavernosal strips to degree dependent on concentraion, however, statistical significance was observed started at the concentrations of 10 μM. As shown in Fig. 3A, cAMP level was increased fourfold after exposure to 10 μM evodiamine over the group without evodiamine. Tissue cGMP was also enhanced 2.5-fold in response to same concentration of evodiamine (Fig. 3B). Adenylyl cyclase inhibitor *cis-N*-(2-phenylcyclopentyl)-azacyclotridec-1-en-2-amine monohydrochloride (MDL-12,330A) (Kanda and Watanabe, 2001; Lippe and Ardizzone, 1991) or guanylyl cyclase inhibitor ODQ, when applied 30 min before evodiamine stimulation, did not attenuate the increased effect in cyclic nucleotide levels by evodiamine.

3.4. Effect of evodiamine on forskolin- and sodium nitroprusside-induced cyclic nucleotide levels and corporal relaxation

Treatment of rabbit corpus cavernosum with evodiamine (1 μM) alone resulted in no significant changes in cyclic nucleotide levels (Fig. 4). The concentrations used for

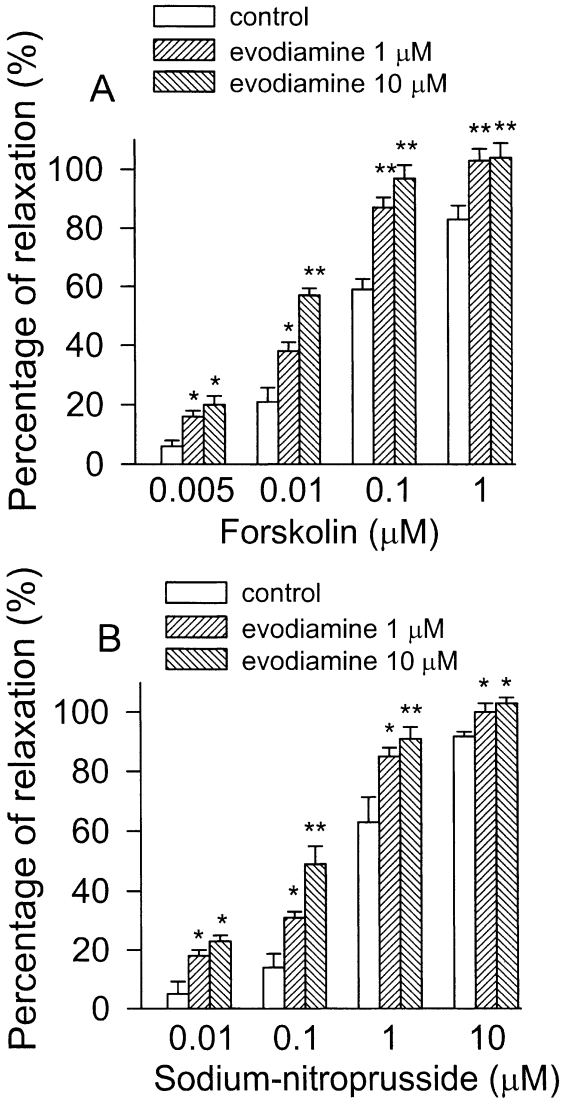

Fig. 5. Enhanced effects of evodiamine on the corporal relaxation evoked by (A) forskolin or (B) sodium nitroprusside in endothelium-denuded rabbit corpus cavernosum precontracted with phenylephrine. Data are shown as mean ± S.E.M. for five preparations. * $P < 0.05$, ** $P < 0.01$ versus control.

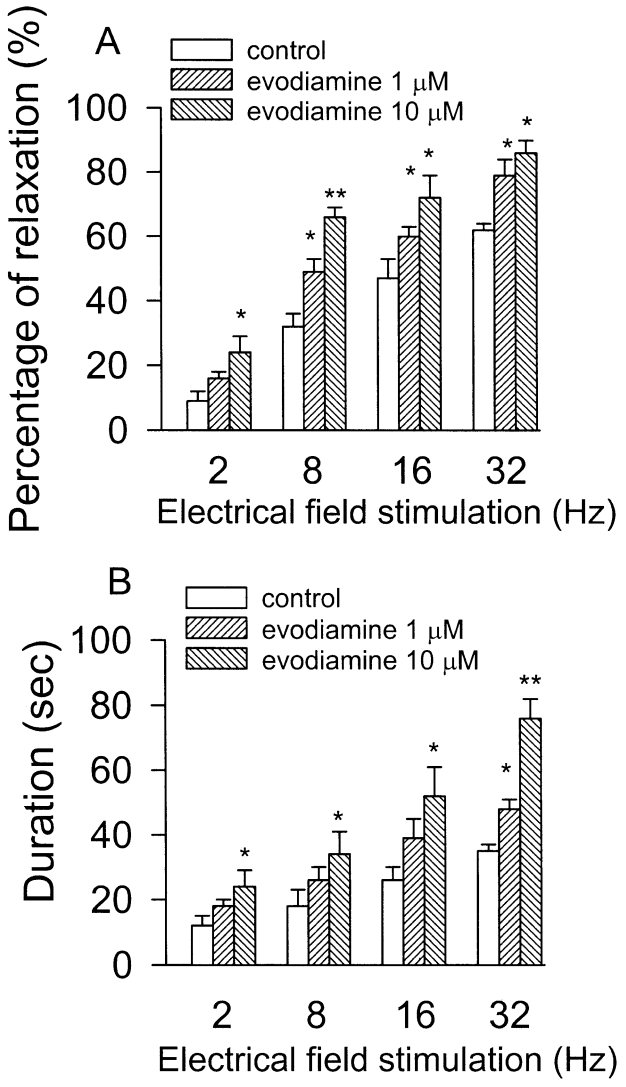

Fig. 6. Enhanced effects of evodiamine on the electrical field stimulation-induced changes in (A) relaxing magnitude and (B) duration in the endothelium-denuded rabbit corpus cavernosum precontracted with phenylephrine. Data are shown as mean ± S.E.M. for six preparations. * $P<0.05$, ** $P<0.01$ versus control.

forskolin (5 nM) and sodium nitroprusside (0.01 μM) also resulted in moderate increase in cAMP (from 11.2 ± 3.4 to 23.7 ± 5.4 pmol/mg protein) and cGMP (from 0.7 ± 0.2 to 1.4 ± 0.3 pmol/mg protein) levels, respectively. However, in the presence of 1 μM evodiamine, forskolin and sodium nitroprusside stimulated cAMP and cGMP levels 2.5-fold and 2.0-fold over itself alone, respectively.

Fig. 5 shows the effect of evodiamine (1 and 10 μM) on the corporal relaxation induced by forskolin and sodium nitroprusside, respectively. In the presence of active muscle tone induced by phenylephrine, the corpus cavernosum relaxed upon application of forskolin (5 nM to 1 μM) and sodium nitroprusside (0.01 to 10 μM) in a concentration manner. In the presence of evodiamine (1 and 10 μM) both agent-evoked relaxations were augmented significantly.

3.5. Evodiamine enhanced electronic field stimulation-induced relaxation

The effects of evodiamine on electrical field stimulation-induced NANC relaxation of the corpus cavernosum are summarized in Fig. 6. Electrical field stimulation caused frequency-dependent corporal relaxation. Pretreatment with evodiamine (1 and 10 μM) potentiated the electrical field stimulation-induced relaxation in a concentration-dependent manner (Fig. 6A). In addition to enhancing the magnitude of relaxation, evodiamine prolonged the duration of the relaxing response in a concentration-dependent manner, i.e. prolonged the time return to baseline. In the absence of evodiamine, the duration of the relaxation varied between 12 ± 3 and 28 ± 4 s at 2, 8, 16 and 32 Hz, respectively. In the presence of evodiamine, the duration of the response varied between 18 ± 2 and 47 ± 4 s and between 24 ± 5 and 76 ± 4 s by 1 and 10 μM evodiamine treatment, respectively.

3.6. Zaprinast attenuated the additive effect of the evodiamine on electrical field stimulation

Treated the cavernosal strips with 10 μM evodiamine caused a left shift of the stimulation–response curve to electrical field stimulation (Fig. 7, squares). However, preincubation of cavernosal strips with zaprinast (a cGMP-dependent phosphodiesterase inhibitor) (Saighi et al., 2000) abolished this additive effect of evodiamine. As shown in Fig. 7, zaprinast (triangles) significantly reversed the relax-

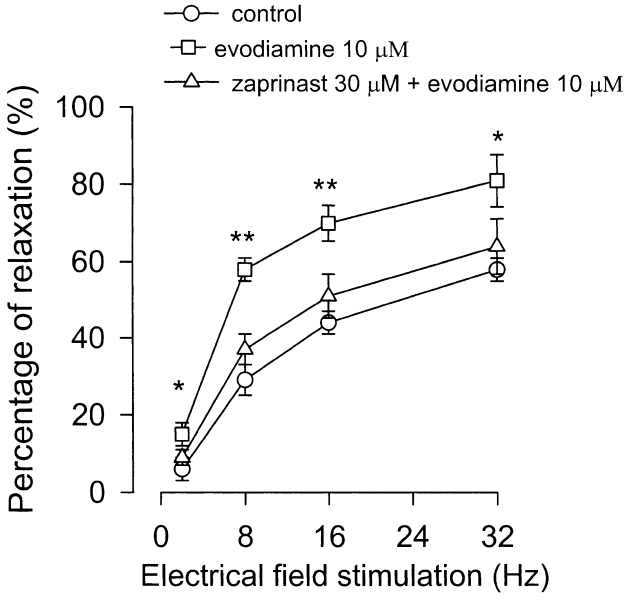

Fig. 7. Corporal relaxant effects of electrical field stimulation in absence (control), in presence of evodiamine, and in combined zaprinast and evodiamine in phenylephrine-precontracted endothelium-denuded corpus cavernosum. Data are shown as mean ± S.E.M. for six preparations. * $P<0.05$, ** $P<0.01$ versus control.

ation to electrical field stimulation that was not different from control tissues.

3.7. Effect of evodiamine of corpus cavernosum isolated from aged rabbits

A comparison study was performed to evaluate the corporal relaxation to acetylcholine (an endothelium-independent vasodilator), sodium nitroprusside (an endothelium-dependent vasodilator) and evodiamine in the specimen obtained from aged (3 years old) and adult young rabbits (4–5 months). In the intact corpus cavernosum obtained from aged animals (Fig. 8), the maximal corporal relaxation in response to acetylcholine (1 μM) was significantly less than in adult (11.4 ± 2.6% vs. 92.9 ± 7.6%). Sodium nitroprusside evoked responsiveness was also reduced in aged specimen. As shown in Fig. 8 (triangles), 1 μM sodium nitroprusside failed to relax and 10 μM sodium nitroprusside induced only moderate relaxation (20.1 ± 4.7%) as compared with adult (91.8 ± 1.6%, as shown in Fig. 5B). However, corporal relaxant response can be augmented when the concentration of sodium nitroprusside was further increased. As shown in Fig. 8, 100 μM sodium nitroprusside resulted in around 60% relaxation. In the present study, evodiamine was more potent than both agents to relax corpus cavernosum obtained from aged animals since 10 μM evodiamine still elicited almost complete relaxation (97.8 ± 4.5%). The EC_{50} value was 1.17 ± 0.38 μM.

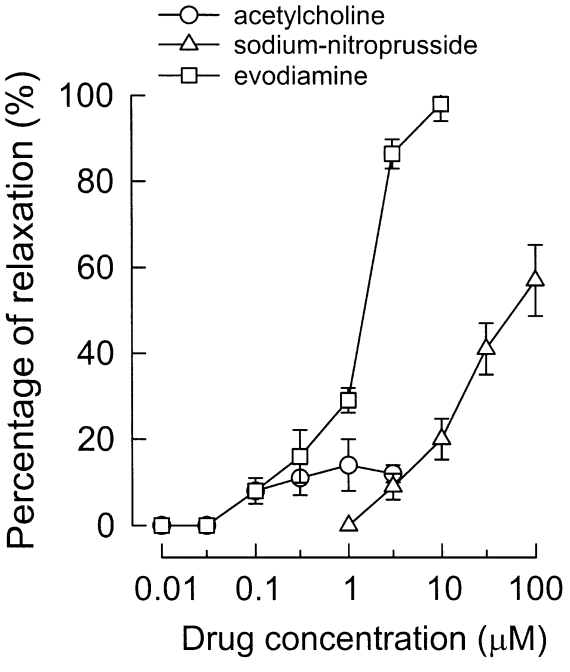

Fig. 8. Comparison the corporal relaxing effect of acetylcholine, sodium nitroprusside and evodiamine in the intact corpus cavernosum precontracted with phenylephrine isolated from aged rabbits (3 years old). Data are shown as mean ± S.E.M. for five preparations.

4. Discussion

This study represents the first attempt to describe the corpus cavernosum relaxing effect of evodiamine, a bioactive component isolated from aphrodisiac Chinese herb *E. rutaecarpa* (Wu-Chu-Yu). Cavernous smooth muscle cells have a key role in the control of penile erection and detumescence. In the present study, evodiamine showed a potent relaxing effect in isolated rabbit corpus cavernosum with an endothelium-NO-independent signal pathway.

It has become increasingly apparent that K^+ channel openers to hold promise as direct effectors of cavernosum smooth muscle relaxation (Lee et al., 1999; Malysz et al., 2001). Christ et al. (1998) examined the K^+ channels in regulating corporal smooth muscle tone, and demonstrated that K^+ channels play a significant role in maintaining penile erection. These authors further suggested that impairment in K^+ channel activity may contribute to erectile dysfunction. Thus, the possibility of K^+ channel activation by evodiamine was further investigated. The first evidence of the importance of K^+ channel-mediated hyperpolarization in the action of evodiamine was provided by the differential potency of evodiamine in relaxing phenylephrine- vs. 60 mM KCl-induced contraction. Vasodilators dependent on the K^+ channel mechanism lose their effects when exposed to high K^+ condition because an increase in extracellular K^+ concentration attenuates the K^+ gradient across the plasma membrane, thus rendering the K^+ channel-activating mechanism ineffective (Chiou et al., 1998; Fedida and Hesketh, 2001). Evodiamine is suggested to produce relaxation in this way since its effect was strikingly blunted in 60 mM KCl-precontracted cavernosal strips. Furthermore, evodiamine-evoked corporal relaxation was counteracted by charybdotoxin, a selective blocker that inhibits large-conductance Ca^{2+}-activated K^+ channels (K_{ca} channels) and voltage-gated potassium channels (Kv1.3) in smooth muscle and other tissues (Hanner et al., 1997, 1998; Jacob et al., 2000; Malysz et al., 2001; Mieyal et al., 1998). The lack of an effect of glibenclamide on evodiamine-induced corporal relaxation demonstrated the pharmacological selectivity of charybdotoxin. These data collectively identify charybdotoxin-sensitive K_{ca} channel as the possible mechanisms involved in the direct effect exerted by evodiamine on corpus cavernosal smooth muscle.

The primary intracellular effector pathway for cavernous smooth muscle relaxation appears to be mediated through the cyclic guanosine monophosphate (cGMP) system, and the cyclic adenosine monophosphate (cAMP) system apparently functions as a secondary pathway (Kim et al., 2000; Klotz et al., 2000; Recio et al., 1998). The good erectile responses caused by sodium nitroprusside and isoproterenol, the inhibition of their responsiveness by methylene blue and ethylmaleimide (an inhibitor of adenylyl cyclase), prove that the cGMP/cAMP system is involved as a second messenger of cavernous smooth muscle relaxation. The level of cGMP/cAMP is regulated by a balance between the rate of syn-

thesis by guanylyl cyclase or adenylyl cyclase and the rate of hydrolytic breakdown to guanosine 5′ -monophosphate (GMP) or adenosine 5′ -monophosphate (AMP) by cyclic nucleotide phosphodiesterase isozymes (Beavo, 1995), respectively. Therefore, agents that inhibit cGMP/cAMP hydrolysis may increase the cyclic nucleotide signal and could be expected to enhance relaxation of smooth muscle in the corpus cavernosum and thereby facilitate penile erection. The predominant phosphodiesterase in penile corpus cavernosum tissue are types 2, 3 and 5, with phosphodiesterase 5 being responsible for most of the cGMP specific hydrolytic activity (Uckert et al., 2001). Phosphodiesterase 2 is nonspecific and can break down both cAMP and cGMP, whereas phosphodiesterase 3 is preferential for cAMP. The present results showed that evodiamine has not only the ability to stimulate cyclic nucleotide (cGMP and cAMP) formation but also enhances both sodium nitroprusside- and forskolin-induced cGMP and cAMP production, respectively. Furthermore, evodiamine pretreatment augmenting significantly the forskolin-, sodium nitroprusside- and electrical field stimulation-induced corporal relaxation proved that inhibition of nonselective phosphodiesterase and subsequent deceleration of cyclic nucleotide hydrolysis may attribute to a part of the endothelium-independent signal pathway of evodiamine. In contrast, exposure of endothelial-denuded cavernosal strips to ODQ and MDL-12,330A did not inhibit both evodiamine-stimulated cyclic nucleotide formation. We hypothesized that evodiamine-evoked cyclic nucleotides increase would be attenuated after treatment with guanylyl cyclase inhibitor ODQ or adenylyl cyclase inhibitor MDL-12,330A if activation of guanylyl cyclase and/or adenylyl cyclase were involved in the signal pathway to evodiamine. However, there was a negative result obtained from Fig. 3A and B. Thus, we speculated that evodiamine-induced increase in cyclic nucleotides was not due to activation of guanylyl cyclase and adenylyl cyclase.

On the other hand, we had compared the additive effect of evodiamine on electrical field stimulation-evoked relaxation in the absence and presence of zaprinast, respectively. We found that zaprinast counteracted the enhancing effect of evodiamine on electrical field stimulation-induced relaxation toward control level. It had been reported that zaprinast, an inhibitor of CGMP-specific PDE (Saighi et al., 2000), enhanced NO-dependent relaxation of human and rabbit corpus cavernosum in vitro (Rajfer et al., 1992). Thus, the apparent reversal of the additive effect of evodiamine on electrical field stimulation evoked by relaxation by zaprinast may be due to interference with the common phosphodiesterase signal pathway. However, which type of phosphodiesterase is modulated by evodiamine is waiting to be defined in the future.

Sexual function commonly decreases with age (Kaiser, 1991). As the median age of the population increases, the number of men presenting with erectile dysfunction will probably increase. The impairment of endothelium integrity, nitric oxide synthase (NOS) activity (Bivalacqua et al., 2000) and/or the reduction in NOS-containing nerve fibers (Carrier et al., 1997) might account for these observations. A blunted effect of acetylcholine in aged rabbits observed in the present study indicates that the endothelium is functionless. Additionally, the guanylyl cyclase signal pathway seems to be less effective in aged specimen because sodium nitroprusside-induced responsiveness was also reduced. Thus, the combination of phosphodiesterase inhibitors with adenylyl/guanylyl cyclase stimulators or K^+ channel openers resulting to further enhancement of arterial blood supply into penis has become a new feature for pharmacological treatment of erectile dysfunction. (Martinez-Pineiro et al., 1996). Our potential finding in aged specimen indicates that evodiamine still relaxes cavernosal strips effectively and exhibits equal potency as compared with adult young rabbits. Although the mechanism of action of evodiamine has not been completely elucidated, it is believed that the activation of charybdotoxin-sensitive K^+ channel and prevention of cyclic nucleotide degradation may be contributed to the beneficial effect of evodiamine. The present observation correlates with the putative pharmacological activities of *E. rutaecarpa* and suggests that evodiamine is one of the active components.

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