

9-Hydroxycanthin-6-One Induces Penile Erection and Delays Ejaculation

Wen-Fei Chiou, PhD*^{†‡} and Tian-Shung Wu, PhD**^{§¶}

*National Research Institute of Chinese Medicine, Taipei, Taiwan; [†]Department of Biotechnology, Hungkuang University, Taichung, Taiwan; [‡]Institute of Traditional Medicine, National Yang-Ming University, Taipei, Taiwan; **Department of Chemistry, National Cheng Kung University, Tainan 701, Taiwan, Republic of China; [§]Department of Pharmacy, China Medical University, Taichung 404, Taiwan, Republic of China; [¶]Chinese Medicine Research and Development Center, China Medical University and Hospital, Taichung 404, Taiwan, Republic of China [Correction added after online publication 13-May-2011: Dr. Wu's affiliation has been updated.]

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ABSTRACT

Introduction. *Eurycoma longifolia* Jack (Simaroubaceae) has the reputation as a male aphrodisiac because it is claimed to increase virility and sexual prowess. Nevertheless, whether or not *E. longifolia* regulates directly the muscle tone of corpus cavernosa and/or seminal vesicle (SV) remains unclear. Even until now, the compositions that could account for its aphrodisiac property are still unknown

Aim. We examined the effect of 9-hydroxycanthin-6-one (9-HC-6-one), a β -carboline alkaloid isolated from *E. longifolia*, on penile erection and ejaculation, and further elucidated the mechanism of action.

Main Outcome Measures. 9-HC-6-one induces penile erection and delays ejaculation.

Methods. Drug's effect was studied on rat corpus cavernosum (CC) and SV in vitro, and on the changes in intracavernosal pressure (ICP) after IC injection and intraluminal pressure (ILP) of the SV after hypogastric nerve stimulation (HNS), respectively.

Results. 9-HC-6-one relaxed significantly phenylephrine (PE)-precontracted CC. Such response was not attenuated by endothelium disruption, N^G -nitro-L-arginine methyl ester, or 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one treatment, suggesting that a nitric oxide/cyclic guanosine monophosphate-dependent pathway was precluded. 9-HC-6-one attenuated PE-induced contraction by blocking cell surface and internal calcium channels with a higher potency for internal calcium release. This compound also antagonized calcium-evoked contraction in Ca^{2+} -free, high K^+ -depolarizing condition, suggesting that interfering with the entry of calcium through voltage-dependent channels also contributed to 9-HC-6-one-induced corporal relaxation. After IC application of 9-HC-6-one, a significant rise in ICP was observed as compared with the application of normal saline. 9-HC-6-one relaxed significantly norepinephrine (NE)- and KCl-precontracted SV, and antagonized NE-induced oscillatory contraction as potent as clomipramine. Finally, the HNS-evoked increase in ILP was dose-dependently repressed after challenge by 9-HC-6-one.

Conclusion. 9-HC-6-one might be the active component that contributed to the aphrodisiac effect of *E. longifolia* by antagonizing the smooth muscle tone of CC as well as SV probably through interfering with Ca^{2+} mobilization. **Chiou W-F and Wu T-S. 9-Hydroxycanthin-6-one induces penile erection and delays ejaculation. J Sex Med 2012;9:1027–1036.**

Key Words. 9-Hydroxycanthin-6-One; Penile Erection; Corpus Cavernosum; Ejaculation; Seminal Vesicle

Introduction

Research in male sexual dysfunction has predominantly focused on rapid ejaculation (RE) and erectile dysfunction (ED) [1]. The goal for RE therapy is to increase patient control over the timing of his ejaculation. Current research on the

treatment of RE has focused on centrally acting or topical desensitizing agents; however, no treatment has yet been approved [2]. An alternative approach could be to develop drugs that act directly upon the target organ itself, and our increasing knowledge of the molecular biology of the accessory sex organs makes this a realistic

possibility. Because the walls of the vas deferens, seminal vesicles, ejaculatory ducts, and prostate are lined with smooth muscle cells, it was suggested that the smooth muscle relaxant agent should be useful to treat RE [2]. However, for erection to take place, the penile arteries and erectile tissue (corpus cavernosum) have to dilate, thereby increasing the blood flow into the penis [3]. The degree of contraction of corpus cavernosal smooth muscle determines the functional states of penile flaccidity (or detumescence) and erection (tumescence). Thus, drugs that relax the corpus cavernosum may be beneficial to induce penile erection.

Eurycoma longifolia Jack (Simaroubaceae), also identified by the local name tongkat ali (in Malaysia), is a well-known plant for treating disease and enhancing health. It has also gained reputation as a male aphrodisiac because it is claimed to increase virility and sexual prowess [4,5]. In animal study, *E. longifolia* Jack was shown to enhance libido in sexually experienced, un-copulatory aged, and castrated male rats [6–8]. Nevertheless, it remains unclear whether or not *E. longifolia* regulates directly the muscle tone of corpus cavernosa and/or seminal vesicle. Even until now, which or what kinds of compositions account for its aphrodisiac property are unknown. Our preliminary study demonstrated that the methanol extract of *E. longifolia* evoked an obvious relaxation of corpus cavernosum (EC₅₀ [effective concentration]: 2.8 ± 0.5 mg/mL). After bioactivity-guided fractionation and isolation, seven β-carboline alkaloids were obtained. Among them, 9-hydroxycanthin-6-one (9-HC-6-one) displayed the most potent corporal relaxant activity. We therefore attempted to determine its mechanisms of action and evaluate the therapeutic application in ED and/or RE.

Methods

Animals

Male Sprague-Dawley rats (250–300 g) were used and housed in a light-controlled room with a 12-hour day/night cycle, and given free access to food and water. Experiments were approved by the Animal Care Committee of the National Research Institute of Chinese Medicine (number 97-P-06, 10/22/2008).

Materials

Acetylcholine hydrochloride (ACh), clomipramine, KCl, N^G-nitro-L-arginine methyl ester (L-NAME), norepinephrine (NE), 1H-[1,2,4]

oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), phenylephrine hydrochloride (PE), and sodium nitroprusside (SNP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The isolation and identification of 9-HC-6-one from *E. longifolia* Jack was published in our previous report [9,10]. 9-HC-6-one was dissolved in dimethyl sulfoxide (DMSO) at 0.1 M as a stock solution and further diluted with Krebs' solution. A final DMSO concentration was less than 0.05% and did not produce significant effects on the responsiveness of the tissues.

Preparation of Corpus Cavernosal Strips for Tension Recording

Tissue preparation and endothelium disruption were performed as described previously [11,12]. After equilibration, cavernosal strip was contracted with PE (3 μM) or KCl (40 mM). When the contractile response had stabilized, various tested agents were added cumulatively for the preparation during the tonic contraction. When evaluating the antagonist effect of 9-HC-6-one on PE- or KCl-induced concentration–constriction curve, 9-HC-6-one was added 15 minutes before the second concentration–response curve was obtained. The effect of 9-HC-6-one on receptor-sensitive intracellular Ca²⁺ release or extracellular Ca²⁺ influx was studied as described previously [13]. Briefly, the cavernosal strip was allowed to equilibrate for 20 minutes in calcium-free Krebs' solution (containing 1 mM EGTA [Ethylene glycol-bis(beta-aminoethyl ether)-N,N,N',N'-tetra acetic acid]). Then, each dose of PE (1, 10, or 100 μM) was added singly into the organ bath, and the contraction–response curve was subsequently constructed. The same test was made in the presence of 9-HC-6-one applied 15 minutes before the contraction test by PE. However, tissues were allowed to equilibrate for 20 minutes in PE (100 μM)-primed Ca²⁺-free solution; then, CaCl₂ (0.5–2.5 mM) were cumulatively added into the organ bath, and the contraction–response curve of CaCl₂ was subsequently constructed. The same test was made in the presence of 9-HC-6-one applied 15 minutes before the addition of calcium. The effects of 9-HC-6-one on intracellular Ca²⁺ release and extracellular Ca²⁺ influx were calculated as percentage of the maximal control response to 100 μM of PE and of the maximal control response to 2.5 mM Ca²⁺, respectively. To test the effect of 9-HC-6-one on voltage-sensitive Ca²⁺ influx, the experiment was performed in the presence of 1 μM of tetrodotoxin (TTX) to completely block depolarization-induced neuronal transmission from the nerve ending [12]. After

incubation with TTX for 30 minutes, cavernosal strips were equilibrated in Ca^{2+} -free potassium depolarizing (with 1 mM EGTA and 40 mM KCl) Krebs' solution for 20 minutes. Thereafter, Ca^{2+} was added cumulatively to obtain a concentration–response curve [13].

Animal Preparation for Intracavernous Pressure Recording

Rats were anesthetized with intraperitoneal pentobarbital sodium (30 mg/kg) and kept breath spontaneously. Mean arterial pressure (MAP), heart rate (HR), and intracavernous pressure (ICP) were monitored according to our previous report [12,14]. Increasing doses of 9-HC-6-one were injected intracavernously in a consistent volume of 0.1 mL. Normal saline (NS, 0.1 mL) was used as negative control. Erectile response was represented as ICP measured.

Preparation of Seminal Vesicle Strips for Tension Recording

Seminal vesicle strip was placed in tissue bath containing Krebs' solution. Silk thread was used to attach the seminal vesicle strips to a fixed hook and a force transducer, respectively. Isometric contractions were recorded using a force transducer [11,12]. After equilibration under an initial load of 0.5 g for 60 minutes, strip contractions were evoked by NE (100 μM) or KCl (60 mM).

Hypogastric Nerve Stimulation and Intraluminal Pressure Measurement

Rats were anesthetized and secured in the supine position. To continuously monitor systemic blood pressure, a 24-gauge angiocatheter was introduced into the carotid artery. Intravenous drug administrations were accomplished via the external jugular vein. Hypogastric nerve was mounted on a bipolar silver electrode (Grass SD9, Grass Instrument, Quincy, MA, USA). Nerves stimulation and intraluminal pressure (ILP) measurement of the seminal vesicle were prepared according to the methods described by Kim et al. [15].

Data Analysis

The relaxation induced by tested drugs were expressed as a percentage of relaxation against PE-, KCl-, or NE-evoked contractions running from 0% to 100% and used in the construction of the concentration–response curves. The EC_{50} (the concentration required to cause half-maximal relaxation) was calculated by PCS 4.0 software (Pharmacological Calculation System, Springer-

Verlag, New York, NY, USA). All data are expressed as mean \pm standard error of the mean of the indicated number of experiments. Data statistical analysis was done by one-way analysis of variance followed by the post hoc Dunnett's *t*-test. Values of $P < 0.05$ were considered significant.

Results

The Endothelium and Nitric Oxide/Cyclic Guanosine Monophosphate Pathway Are Not Involved in 9-HC-6-One-Evoked Corporal Relaxation

In endothelium-intact, PE-precontracted cavernosal strips, 9-HC-6-one evoked a concentration-dependent relaxation with an EC_{50} value of $6.7 \pm 0.9 \mu\text{M}$. The addition of L-NAME and ODQ fully blocked the ACh- and SNP-induced corporal relaxation (Figure 1A, B, inset), respec-

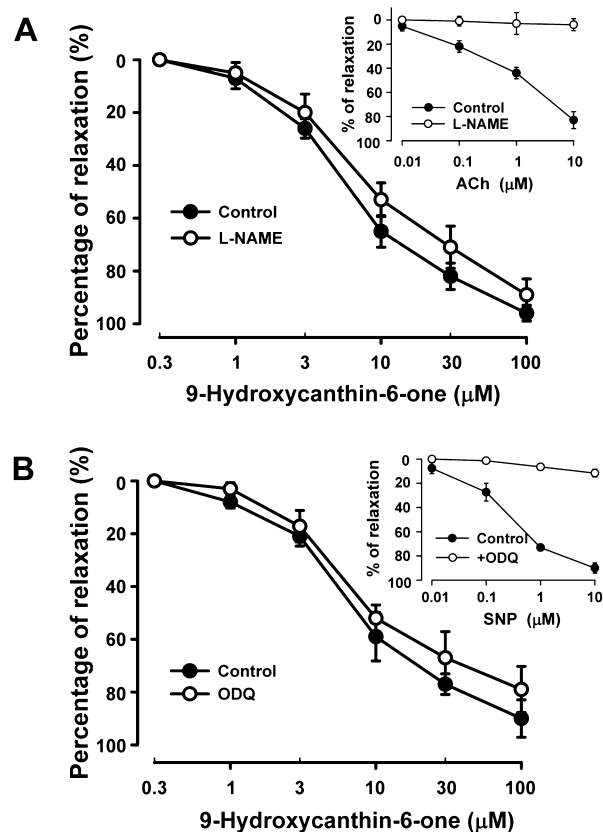


Figure 1 Effects of (A) N^G -nitro-L-arginine methyl ester (L-NAME) and (B) $1H$ -[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ) pretreatment on 9-hydroxycanthin-6-one-induced relaxation of the isolated rat corpus cavernosum precontracted with phenylephrine. Data are shown as mean \pm standard error of the mean (N = 6–8). The inset showed the effects of L-NAME and ODQ on acetylcholine (ACh)- and sodium nitroprusside (SNP)-induced corporal relaxation, respectively.

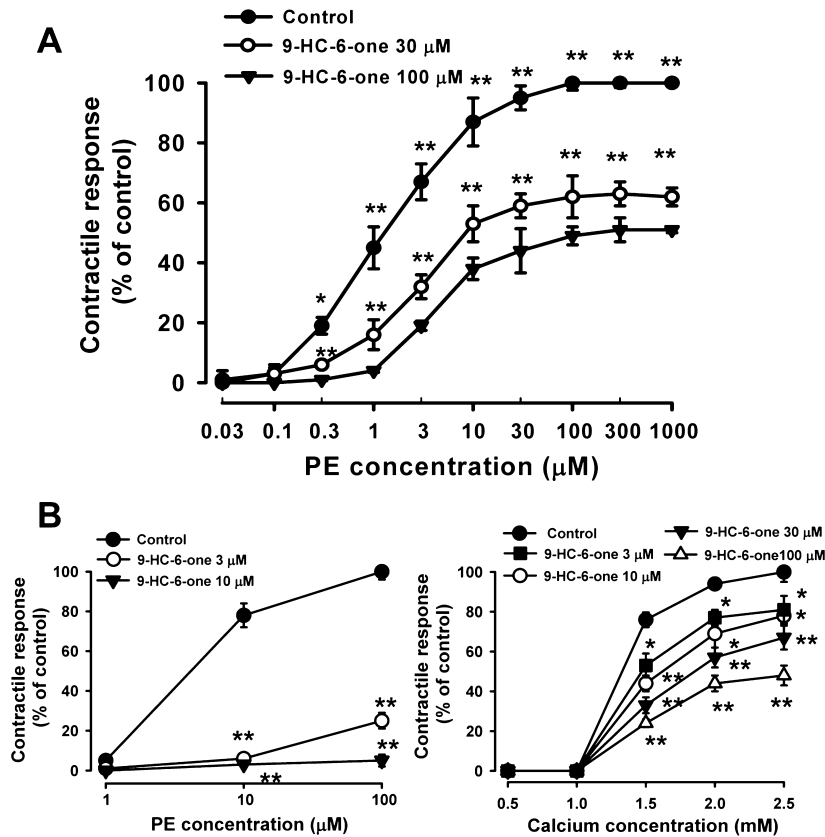


Figure 2 Effects of 9-hydroxycanthin-6-one (9-HC-6-one) pretreatment on the (A) phenylephrine (PE)-evoked concentration–response curves and (B) PE-sensitive intracellular Ca^{2+} release and extracellular Ca^{2+} influx in rat corpus cavernosum. Data are shown as mean \pm standard error of the mean ($N = 7-8$). * $P < 0.05$ and ** $P < 0.01$, response significantly different to control.

tively, but failed to modify 9-HC-6-one-induced responsiveness (Figure 1A, B). These suggested that nitric oxide/cyclic guanosine monophosphate (NO/cGMP)-dependent signal pathway was precluded in 9-HC-6-one-induced relaxation. However, the concentration–relaxant curve to 9-HC-6-one obtained in endothelium-denuded preparations was almost superimposed to those obtained in endothelium-intact strips (EC_{50} : 7.5 ± 1.1 vs. $6.7 \pm 0.9 \mu\text{M}$).

Intracellular Calcium Mobilization Is Repressed by 9-HC-6-One

9-HC-6-one inhibited serotonin and endothelin contraction (not shown) with the same potency as PE contraction (Figure 2A), suggesting that a mechanism other than alpha-adrenoceptor antagonism appears to be involved. Therefore, the inhibitory potency of 9-HC-6-one on receptor-sensitive calcium release and calcium influx was further studied. Both the calcium release (the contraction produced by PE in $0\text{-Ca}^{2+}/\text{EGTA}$ condition) and influx (resulting from the reintroduction of CaCl_2 to the bath) were all attenuated by 9-HC-6-one pretreatment. As shown in Figure 2B, 10 μM of

9-HC-6-one completely abolished the corporal constrictive activity of PE in Ca^{2+} -free condition; however, it only partially inhibited calcium influx. It appeared that 9-HC-6-one displayed predominant inhibitory effect on calcium release than influx through the receptor-sensitive Ca^{2+} channel. Also, the vasoconstriction produced by Ca^{2+} -induced contraction in calcium-depleted, potassium-depolarizing condition was concentration dependently, but moderately, attenuated by 9-HC-6-one pretreatment (Figure 3). In the presence of 100 μM of 9-HC-6-one, maximal constriction in response to 2.5 mM Ca^{2+} was reduced to 43% of the control.

ICP Is Effectively Raised by 9-HC-6-One Administration

The basal ICP recorded was 13.7 ± 4.2 mm Hg. Administration of 9-HC-6-one induced a dose-dependent erectile response as documented by a sustained increase in ICP and duration of tumescence. The peak ICP was increased, respectively, from basal to 22.8 ± 2.4 , 41.0 ± 3.2 , 53.2 ± 4.1 , 57.9 ± 3.2 , and 60.1 ± 4.3 mm Hg (Figure 4), with a duration of 10 ± 4 , 36 ± 3 , 54 ± 6 , 71 ± 9 ,

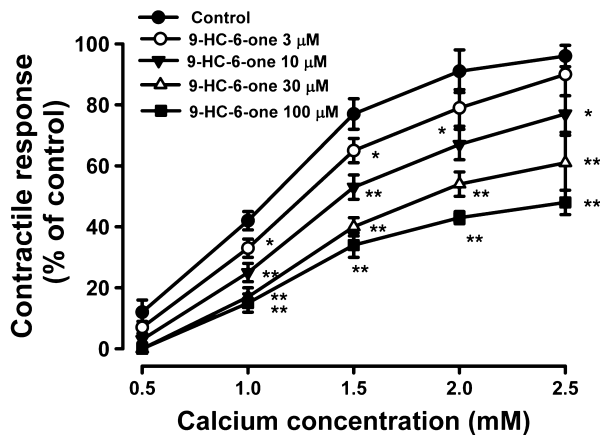


Figure 3 Effect of 9-hydroxycanthin-6-one (9-HC-6-one) on Ca^{2+} -induced contraction in Ca^{2+} -depleted, potassium-depolarizing medium. Data are shown as mean \pm standard error of the mean ($N = 6-8$). * $P < 0.05$ and ** $P < 0.01$, response significantly different to control.

and 90 ± 8 minutes, after being challenged by increasing doses of 9-HC-6-one. During the injection periods, the MAP and HR were unchanged (data not shown). Intracavernous injection of NS induced a transient rise in ICP, but the pressure rises often returned to the resting level within 3 minutes, and the spike-like pressure tracing curves were different from those of 9-HC-6-one. We believe that the transient rise in ICP was due to the volume effect of injection of NS.

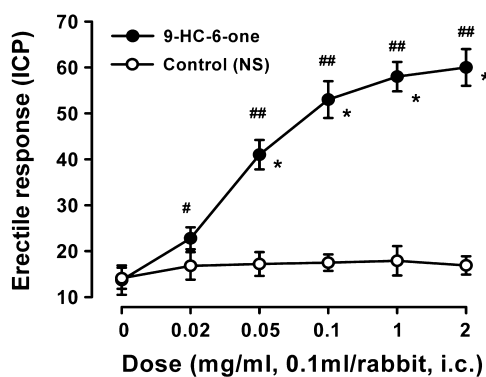


Figure 4 Intracavernous injections of 9-hydroxycanthin-6-one (9-HC-6-one) induced a dose-dependent elevation in intracavernosal pressure (ICP) in rats. The injected volume was 0.1 mL. Normal saline (NS) was used as a control. Results are expressed as mean \pm standard error of the mean for five animals. * $P < 0.01$, response significantly different to control; # $P < 0.05$ and ## $P < 0.05$, response significantly different to group without 9-HC-6-one injection (indicated as 0). i.c. = intracavernous.

9-HC-6-One Antagonized NE-Induced Contraction in Seminal Vesicle Strips and Evoked Relaxation of Precontracted Seminal Vesicle Is Significantly Antagonized by 9-HC-6-One

In seminal vesicle, NE contractions were characterized by a sudden rise in tension with superimposed rhythmic contractions that remained until the tissue was washed with the Krebs' solution (Figure 5A). We first assessed the effect of clomipramine on the contractile response of seminal vesicle strips to NE because selective serotonin-reuptake inhibitors (SSRIs) have emerged as an effective new treatment modality for premature ejaculation [16]. As shown in the upper trace of Figure 5A, pretreatment with clomipramine (100 μM) for 15 minutes completely abolished the contractile response to NE in four preparations. 9-HC-6-one also significantly suppressed the contractile response to NE in six preparations (bottom trace of Figure 5A). On the other hand, cumulative addition of 9-HC-6-one to a single submaximal dose of NE reduced the rhythmic contractions in a concentration-dependent manner (upper trace of Figure 5B, filled circles). This is a similar phenomenon observed in clomipramine-evoked responsiveness (upper trace of Figure 5B, triangles). According to the concentration-response curve, results indicated that 9-HC-6-one and clomipramine were equally potent to antagonize NE-mediated constriction. Cumulative addition of 9-HC-6-one and clomipramine to the KCl-precontracted seminal vesicle strips also induced significant and equal relaxations (bottom trace of Figure 5B).

Hypogastric Nerve Stimulation-Evoked Increase in ILP of the Seminal Vesicle Is Repressed by 9-HC-6-One

Hypogastric nerve stimulation (HNS) caused significant increases in ILP of seminal vesicle. Prior to drug administration, the peaks of ILP of the seminal vesicle subsequent to HNS were 15 ± 6 , 45 ± 4 , 74 ± 5 , and 102 ± 8 mm Hg under 10, 20, 40, and 60 Hz of stimulation, respectively. Repeated nerve stimulation, each preceded by a 20-minute rest interval, produced reproducible ILP recordings in each organ. The administration of clomipramine elicited a dose-dependent inhibition in the HNS-induced elevations of ILP (Figures 6A and 7). At 3 mg/kg, clomipramine virtually abolished any pressure response by HNS. 9-HC-6-one also demonstrated a significant repressing effect of the ILP. As shown in Figures 6B and 7, 0.5 mg/kg of 9-HC-6-one

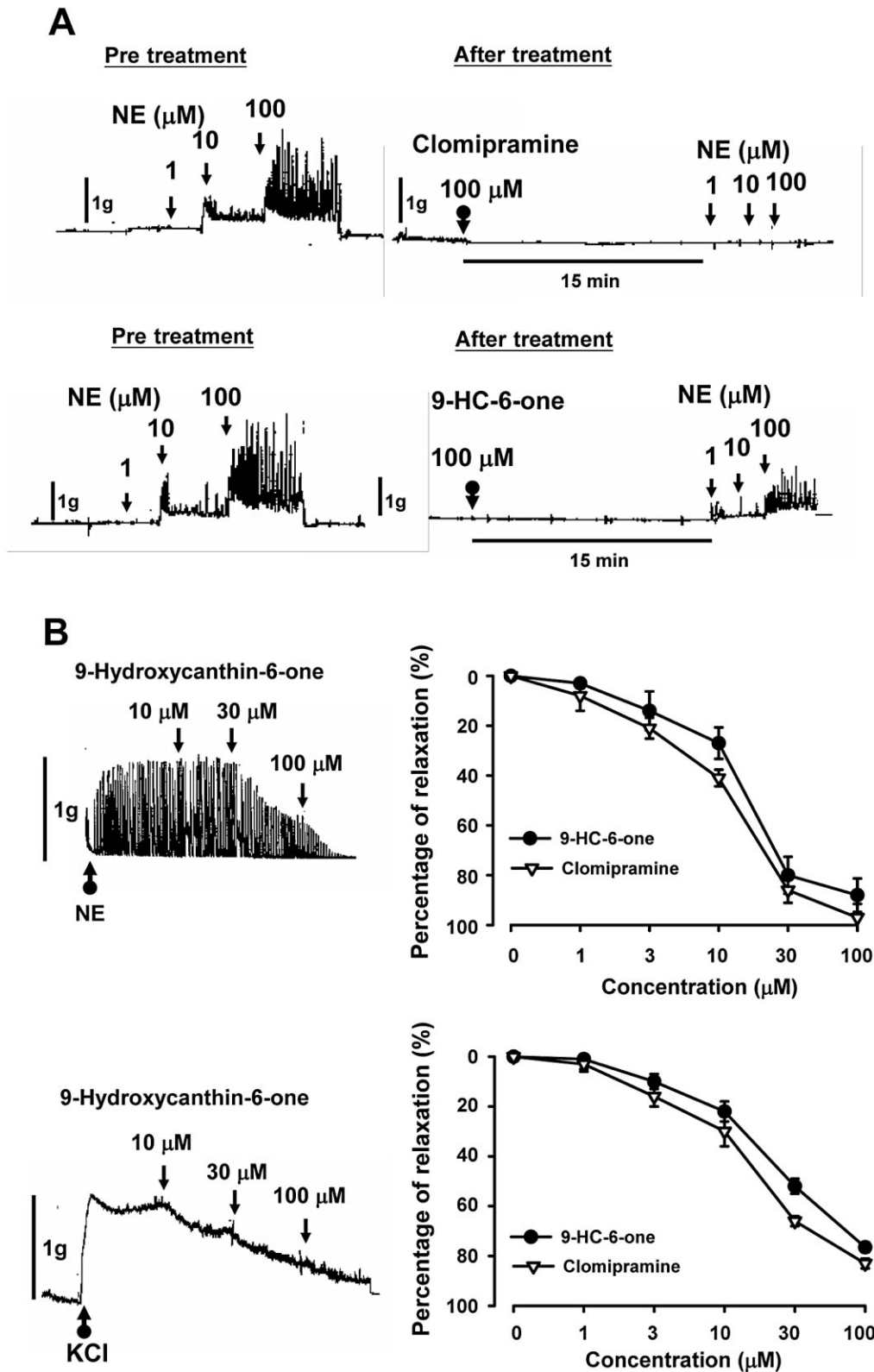


Figure 5 Traces showed (A) the effects of clomipramine and 9-hydroxycanthin-6-one (9-HC-6-one) pretreatment on the contractile activity of seminal vesicle strips in response to norepinephrine (NE, 1–100 μM), and (B) the relaxant effects of 9-HC-6-one and clomipramine on NE (100 μM)- and KCl (60 mM)-precontracted seminal vesicle strips. Data are shown as mean \pm S.E.M. (N = 6–8), respectively. Bars under the traces indicate the time.

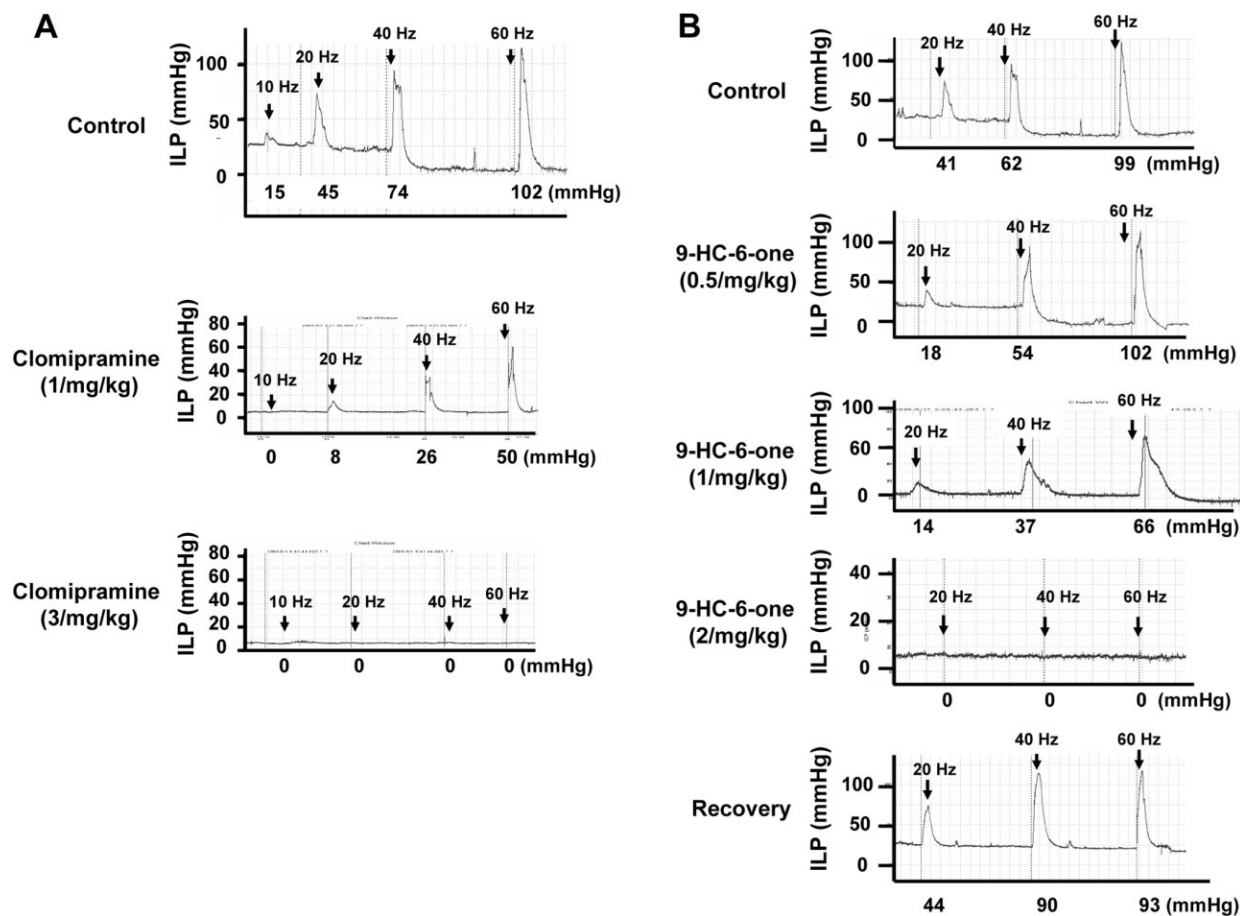


Figure 6 Trace of intraluminal pressure (ILP) of the seminal vesicle caused by electrical stimulation of the hypogastric nerve before (control) and after intravenous injection of (A) clomipramine (1 and 3 mg/kg) or (B) 9-hydroxycanthin-6-one (9-HC-6-one) (0.5, 1, and 2 mg/kg) in rat, respectively. Trace in the bottom showed that after 2 hours of recovery period, repeated nerve stimulation produced reproducible ILP recording. Stimulus parameters were described in Methods.

markedly antagonized low-frequency (10 Hz and 20 Hz), stimulation-evoked pressure effect, although much higher frequency (40 Hz and 60 Hz), stimulation-evoked increases in ILP were less affected. The inhibition of ILP by 1 mg/kg of 9-HC-6-one was equally potent to the same dose of clomipramine: approximately 40–50% of the pressure response remained. Surprisingly, 9-HC-6-one at a dose of 2 mg/kg completely abolished HNS-induced elevations of the ILP. Cutting the hypogastric nerve did not change the effect of 9-HC-6-one on seminal vesicle pressure (data not shown). Furthermore, the administration of clomipramine and 9-HC-6-one at the doses used did not significantly alter the MAP and HR in animals (data not shown). After 2 hours of recovery period, repeated nerve stimulation produced reproducible ILP (Figure 7).

Discussion

This study demonstrated that 9-HC-6-one can relax corpus cavernosum through NO/cGMP-independent signal pathway. Sexual function commonly decreases with age [17]. The impairment of endothelium integrity, NOS activity [18], and/or the reduction in NOS-containing nerve fibers [19] might account for these observations. An invariant effect of 9-HC-6-one in endothelium-denuded corpus cavernosum indicated that this agent might be useful in the elderly population. Results also showed that 9-HC-6-one produced noncompetitive antagonism of PE-induced contraction characterized by reduced maximal effect. Indeed, 9-HC-6-one attenuated receptor-mediated calcium mobilization by blocking PE-activated cell surface and internal calcium channels with a higher

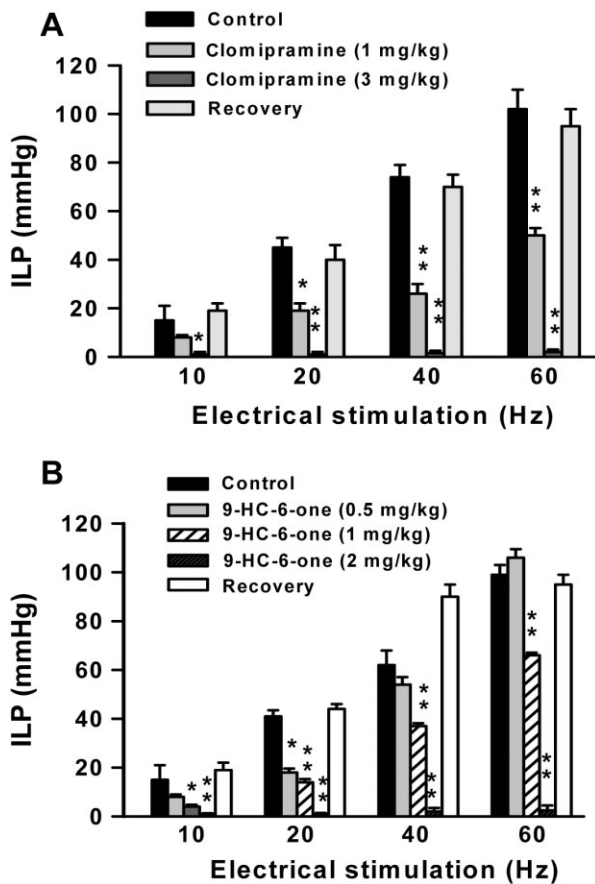


Figure 7 Dose–response effects of (A) clomipramine and (B) 9-hydroxycanthin-6-one (9-HC-6-one) on intraluminal pressure (ILP) of the seminal vesicle caused by hypogastric nerve stimulation. Stimulus parameters were described in Methods. Data are shown as mean \pm standard error of the mean for six animals. * $P < 0.05$ and ** $P < 0.01$, response significantly different to control.

potency for the internal calcium release channels. 9-HC-6-one also inhibited calcium-evoked contraction in Ca^{2+} -free, high K^+ -depolarizing solution, suggesting that interfering with the entry of calcium through voltage-dependent channels contributed to 9-HC-6-one-induced corporal relaxation. It was recently demonstrated that reduced relaxation in the corpus cavernosum of spontaneously hypertensive rat might be attributed to impaired neuronal NO-dependent relaxation in the corpus cavernosum [20]. Ushiyama et al. investigated whether treatment with hypotensive agents affected the impaired relaxation of the penile corpus cavernosum in hypertensive rats [21]. An interesting finding showed that treatment with Ca^{2+} channel blockers (amlodipine or imidapril) alone ameliorated significantly the relaxation response of the corpus cavernosum in a dose- and

blood pressure-dependent manner [21]. In addition to nitrogenic neuronal damage, vascular endothelial dysfunction has been implicated in secondary complications because of essential hypertension [22], congestive heart failure [23], diabetes mellitus [24], and hypercholesterolemia [25]. It has been noted that ED is highly associated with cardiovascular diseases, especially arterial hypertension [26]. The present results indicate that 9-HC-6-one possesses direct muscle relaxant in part via inhibiting Ca^{2+} influx through both receptor-operated and voltage-dependent Ca^{2+} channels. The ability of 9-HC-6-one to modulate Ca^{2+} mobilization may be considered as an alternative mechanism independent of the classical NO/cGMP pathway that could serve as a drug with pro-erectile effect even in the absence of pelvic nerve stimulation. This was proved by an obvious and lasting increase in ICP after intracavernous injection of 9-HC-6-one. Therefore, 9-HC-6-one might possess the potential to improve ED even in hypertensive patients.

Many behavioral studies have documented the effects of SSRIs on rat ejaculatory function. It was suggested that the smooth muscle relaxant agent should be employed to treat RE as an adjunct to SSRIs [27,28]. The delay in ejaculation time induced by the smooth muscle relaxant agent was attributable to its inhibitory effect on the contractility of seminal vesicles as well as to its inhibition of seminal emission through certain defined pathways [29]. Because seminal vesicle contractions in humans and rats may occur through different mechanisms, we used NE as the dominant agonist and added KCl to the organ bath to induce contractions in this study. Contractions induced by KCl are a direct effect of seminal vesicle smooth muscle because extracellular high concentrations of KCl cause cell depolarization and consequently calcium influx into smooth muscle cells because of activation of voltage-dependent membrane calcium channels [30]. In this study, we confirmed the peripheral effects of clomipramine (a SSRI) on the contractile response of rat seminal vesicles *in vitro*. Results showed that clomipramine resulted in a concentration-dependent inhibition of NE- and KCl-induced seminal vesicle contraction. We also observed an inhibitory effect of 9-HC-6-one on seminal vesicle contractions. By contrast, relaxant agents that increase cGMP had no marked effect on isolated rat seminal vesicle. Our preliminary data showed that $10 \mu M$ of SNP produced $94 \pm 3\%$ relaxation in PE-precontracted corpus cavernosum but evoked only $6 \pm 2\%$ relaxation in NE-precontracted seminal vesicle. The finding of

an inhibitory effect by 9-HC-6-one on agonist-induced contractility in the seminal vesicles is likely because of the blocking of intracellular calcium mobilization as suggested in corpus cavernosal tissues.

We also evaluated the effects of 9-HC-6-one on the seminal vesicle pressure induced by electrical stimulation of the hypogastric nerve in vivo. The administration of 9-HC-6-one blunted markedly the HNS-induced ILP increase but did not significantly alter systemic blood pressures. The basic hypothesis of our in vivo study was that the drugs for treating premature ejaculation produced their effects by inhibiting ILP elevations of the seminal tract. This was evidenced by the significant inhibition of pressure responses of seminal vesicle by clomipramine, which has been demonstrated to have a therapeutic effect on premature ejaculation. In conclusion, we identified a major active component of 9-HC-6-one from *E. longifolia* Jack and revealed that interfering with Ca^{2+} mobilization might account for its corporal relaxant effect and contribute to induce substantial penile erection. Moreover, 9-HC-6-one was able to antagonize the smooth muscle tone of certain ejaculated tissues (such as seminal vesicle) in vitro and reduce the ejaculation threshold by inhibiting the seminal vesicle pressure effect after HNS in vivo. The observation correlated with putative pharmacological activities of *E. longifolia* Jack, and 9-HC-6-one might bring into perspective the treatment strategy for those patients with ED and RE.

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Corresponding Authors: Wen-Fei Chiou, PhD, National Research Institute of Chinese Medicine, No. 155-1, Sec. 2, Li-Nung Street, Shipai, Taipei 112, Taiwan. Tel: 886-2-2820199 (4481); Fax: 886-2-28250743; E-mail: wfchiou@nricm.edu.tw

Tian-Shung Wu, PhD, National Cheng Kung University, No. 1, Ta-Shiueh Rd., Tainan, 701, Taiwan. Tel: +886-6-2757575-65333; Fax: 886-6-2740552; E-mail: tswu@mail.ncku.edu.tw [Correction added after online publication 13-May-2011: Acknowledgment and corresponding author information has been updated.]

Conflict of Interest: None.

Statement of Authorship

Category 1

- (a) **Conception and Design**
Wen-Fei Chiou; Tian-Shung Wu
- (b) **Acquisition of Data**
Wen-Fei Chiou
- (c) **Analysis and Interpretation of Data**
Wen-Fei Chiou

Category 2

- (a) **Drafting the Article**
Wen-Fei Chiou
- (b) **Revising It for Intellectual Content**
Wen-Fei Chiou

Category 3

- (a) **Final Approval of the Completed Article**
Wen-Fei Chiou; Tian-Shung Wu

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