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

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DOI: 10.1111/j.1743-6109.2011.02296.x

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 (EC_{50} [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), 1*H*-[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 Ca^{2+} release or extracellular Ca^{2+} 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 Ca^{2+}-free solution; then, $CaCl_2$ (0.5–2.5 mM) were cumulatively added into the organ bath, and the contraction–response curve of $CaCl_2$ 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 Ca^{2+} release and extracellular Ca^{2+} 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 Ca^{2+}, respectively. To test the effect of 9-HC-6-one on voltage-sensitive Ca^{2+} 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