行政院國家科學委員會補助專題研究計畫成果報告

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（計畫名稱）一種不經 NO 誘導即能刺激 soluble guanylate cyclase 的藥物 YC-1 對白兔陰莖海綿體平滑肌的影響

Effects of YC-1, an NO-independent stimulator of soluble guanylate cyclase, on rabbit corporal smooth muscle

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The Effects of YC-1, A Nitric Oxide independent Soluble Guanylate Cyclase Stimulator, On Rabbit Cavernous Smooth Muscle

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Abstract

Objective To evaluate the effects of a nitric oxide independent soluble guanylate cyclase stimulator, YC-1 on rabbit cavernous smooth muscle.

Materials and methods Sexually mature New Zealand white rabbits were used in this study. After the penis was removed, the corpus cavernosum was dissected free from the tunica albuginea. Two longitudinal corporal strips were used for isometric tension study. Each strip was stimulated with phenylephrine to yield a submaximal contractile response, then the relaxant effects of YC-1 with or without the presence of IBMX, and the relaxant effects of nitroprusside with or without the presence of YC-1 nitroprusside were determined. After treated with YC-1, sodium nitroprusside, IBMX, sodium nitroprusside + YC-1 and IBMX + YC-1, cGMP of the cavernous tissue was determined by EIA kits. Tissue protein was measured by the assay described by Lowry, DH et al.and the results were expressed as picomoles cGMP per miligram protein.

Results 1) YC-1 relaxed the phenylephrine precontracted corporal strips in a concentration dependent manner. 2) YC-1 significantly enhanced the relaxant effects of sodium nitroprusside on the phenylephrine precontracted corporal strips. 3) Compared with control, cGMP of the rat cavernous smooth muscle was significantly increased with the presence of YC-1.

Conclusions YC-1 induces and enhances cavernous smooth muscle relaxation. The mechanisms of the effects are through soluable guanylate cyclase stimulation and possibly phospodiesterase inhibition.

Keywords: YC-1, soluble guanylate cyclase, cyclic GMP, phosphodiesterase

中文摘要

研究目的：研究一種不經 NO 誘導即能刺激 solable guanylate cyclase 的新藥物---YC-1 對白兔陰莖海綿體平滑肌的影響。

研究對象與方法：把陰莖自成熟雄性白兔身上取下後，打開白膜並取出陰莖海綿體，將海綿體製成條狀後懸掛於 organ bath 進行等長張力試驗，先以 phenyiephrine 使其收縮後加入 YC-1、sodium nitroprusside、少量 YC-1 + sodium nitroprusside 及少量 IBMX + YC-1 後，觀察並記錄其放鬆情形。最後測量在各種狀態（無添加物質、加入 YC-1、加入 sodium nitropresside、加入 IBMX、加入 sodium nitropresside + YC-1、加入 IBMX + YC-1）下陰莖海綿體平滑肌組織中 cGMP 的量。

結果：一）YC-1 和 sodium nitroprusside 均以 concentration dependent 的方式放鬆被先行收縮的白兔陰莖海綿體條。在少量 YC-1 的存在下，sodium nitroprusside 的放鬆效果明顯地被增強。二）少量的 IBMX 存在下，YC-1 的放鬆效果並未被增強。三）YC-1 的存在使得白兔陰莖海綿體內的 cGMP 大為增加而少量 sodium nitroprusside 和 IBMX 的存在使得該效果更加顯著。

結論：YC-1 這種國內新合成的藥物具有明顯放鬆白兔陰莖海綿體的效果並可加強 sodium nitroprusside 的放鬆效果。該藥物也因此可能具有治療男性勃起功能障礙的潛力。

關鍵詞：YC-1，soluble guanylate cyclase，cyclic GMP

Introduction

It has now become apparent that nitric oxide (NO) released from the non-adrenergic non-cholinergic neurons and from the endothelium plays an important role in mediating penile erection.[1,2] By stimulating the soluable guanylate cyclase (sGC) to convert GTP to cyclic GMP (cGMP), NO triggers cavernous smooth muscle relaxation resulting in penile erection. cGMP was hydrolyzed by phosphodiesterase (PDE) and phosphodiesterase inhibition (PDEI) will lead to accumulation of cGMP.[1,2]

Theoretically, drugs that enhance the NO-sGC-cGMP-PDEI axis will have therapeutic potential for the treatment of ED.

YC-1 is a novel agent, which has been proved to be a potent NO-independent sCG stimulator of platelets and vascular smooth muscle. [3-5] The current study was designed to investigate the effects of YC-1 on cavernous smooth muscle.

Materials and methods

Sexually mature New Zealand white rabbits were used in this study. After the rabbit was anesthetized, the penis of the rabbit was removed at the level of the attachment of the corporal bodies to the ischium. The corpus cavernosum was sharply dissected free from the tunica albuginea and two corporal bodies were prepared. The corporal bodies were used either for isometric tension study in organ baths or for intracellular cGMP measurement.

Isometric tension study in organ baths: Two longitudinal strips with unstretched length of about 8 mm were made from the isolated corporal bodies. The corporal strips were placed in organ baths. One end of each strip was fixed and the other end was connected to a force displacement transducer. Each strip was stimulated with phenylephrine (3×10^{-5} M) to yield a submaximal contractile response, then the relaxant effects of YC-1 with or without the presence of IBMX (a PDE inhibitor), and the relaxant effects of nitroprusside with or without the presence of YC-1 nitroprusside were determined.

cGMP measurement: Measurement of cGMP of the cavernous tissue was performed as described by Itoh, T et al and Kauffman, Rf et al. It was summarized as follows: After being treated with YC-1, sodium nitroprusside, IBMX, sodium nitroprusside + YC-1 and IBMX + YC-1 for 2, 5, 10 and 15 minutes, the cavernous tissue was frozen with liquid nitrogen and stored at-70 ° C for future use. The cavernous tissue was put in the 10% trichloroacetic acid/4mM EDTA solution and homogenized with a Potter glass/glass homogenizer. The homogenate was filtered through gauze, then centrifuged at 10000 *g* for 5 minutes. Diethyl ether was used to extract cGMP from the supernatant and the amount of cGMP was determined by EIA kits. Tissue protein was measured by the assay described by Lowry, DH et al. Results was expressed as picomoles cGMP per miligram protein.

Data analysis and Statistics: Data for the relaxant effects of chemical agents on phenylephrine induced cavernous smooth muscle contraction were presented as percent maximal inhibition. All values presented in the results represented the mean + standard error. Student's t test was used for comparison of data. A probability level of

< 0.05 was required for statistical significance.

Results

Figure 1 shows YC-1 relaxed phenylephrine-induced contraction of rabbit coporal strips significantly and in a concentration dependent manner. 30 μM of YC-1 resulted in more than 75% of relaxation. ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one) (a specific sGC inhibitor) almost completely reversed the relaxant effects of YC-1.

Figure 2 shows sodium nitroprusside also relaxed phenylephrine-induced contraction of rabbit coporal strips significantly and in a concentration dependent manner. Pretreatment of a low concentration of YC-1 (3 μM) significantly enhanced the relaxant effects of sodium nitroprusside. The concentration response curve of sodium nitroprusside was shifted to the left.

Figure 3 shows the relaxant effects of YC-1 on phenylephrine-induced contraction of rabbit coporal strips was not significantly affected by pretreatment of IBMX (a nonselective PDE inhibitor)..

Table 1 shows cGMP of the rabbit corporal tissue was significantly increased by YC-1. Compared with the control, 30 μM of YC-1 increased intracellular cGMP approximately by 20 folds. Also shown in Table 1, sodium nitroprusside and IBMX both increased cGMP of the rabbit corporal tissue significantly. With pretreatment of YC-1, cGMP increased significantly more by sodium nitroprusside and IBMX.

Discussion

Due to its complexity, intracellular signal transducion in cavernous smooth muscle had not been the major interest of the investigators working on the physiology and pathophysiology of erectile dysfunction (ED). It was when sildenafil (a specific phosphodiesterase) was introduced as an effective oral medication for ED few years ago, that cavernous intracellular signal transduction started to draw world-wide attention. [6,7]

The NO-sGC-cGMP-PDEI axis has been recently well investigated and proved to be the main pathway stimulating penile erection. [1,2] Yet one key enzyme in the pathway, sGC, had not drawn too much attention from the investigators since experiments had been difficult without a specific sGC inhibitor available. Methylene blue and LY 83583 (6-anilino-5,8-quinolinedione) are two agents that inhibit sGC. Yet, they are both considered to be nonspecific since they had other actions like

superoxide anion generation and NO-synthases inhibition.[4,8] Recently, a new sGC inhibitor, ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one), was developed and it has been referred as a potent and specific sGC inhibitor.[9]

Beside the two natural sGC stimulators, NO and CO (carbon oxide), one agent, isoliquiritigenin, has been reported to be sGC enhancing but it is more likely this agent works via phosphodiesterase inhibition.[4] YC-1 is a domestically developed novel agent that has been proved to be a potent NO-independent sGC stimulator.[3-5] In amimal experiments, YC-1 was shown to inhibit platelet aggregation and to relax the precontracted aortic rings and cerebral arterioles via sGC stimulation of platelets and vascular smooth muscle.[3-5] The current study showed that YC-1 also affected rabbit cavernous smooth muscle. It significantly and remarkably relaxed the precontracted rabbit corporal strips. Taking consideration that the relaxing effects of YC-1 could be reversed by ODQ and cGMP of the rat cavernous tisse was significantly increased by the presence of YC-1, the relaxing effects of YC-1 on the corporal strips must be working by the mechanism of sGC stimulation.

Since YC-1 and NO stimulate sGC via different mechanisms, it is not surprising to see that the relaxant effects of nitroprusside on precontracted rabbit corporal strips was significantly enhanced by the presence of YC-1. In other words, their effects on sGC seemed to be synergic. One recent study by using rabbit aortic rings has shown that YC-1 is not only a sGC stimulator but also exhibits possible phosphodiesterase inhibition properties.[10] In this regard, it can not only explain why the relaxant effect of IBMX on the precontracted corporal strips was not significantly enhanced by the presence of YC-1 since both agents are phosphodiesterase, but also add another reason for YC-1's enhancing effects on the relaxant effects of nitroprusside.

In conclusion, YC-1 induces cavernous smooth muscle relaxation and enhances the relaxant effects of nitroprusside. The mechanisms of the effects are through soluble guanylate cyclase stimulation and possibly phospodiesterase inhibition.

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Legends

Fig.1. Relaxant effects of YC-1 on precontracted rabbit corporal strips. Relaxation is presented as % inhibition of the submaximal contractile response. Each bar is the mean + SEM of 4 to 6 individual rabbits. * < 0.05, ** < 0.01, *** <0.001 compared with control.

Fig.2. Relaxant effects of sodium nitroprusside with and without pretreatment of 3 uM of YC-1 on precontracted rabbit corporal strips. Relaxation is presented as % inhibition of the submaximal contractile response. Each bar is the mean + SEM of 4 to 6 individual rabbits. * < 0.05, ** < 0.01, *** <0.001 compared with control.

Fig.3. Effects of pretreatment of 0.3 uM of IBMX on precontracted rabbit corporal strips. Relaxation is presented as % inhibition of the submaximal contractile response. Each bar is the mean + SEM of 4 to 6 individual rabbits.

Table.1. Effects of YC-1, sodium nitroprusside and IBMX on cGMP accumulation in rabbit corpus cavernosum. * < 0.05, ** < 0.01, *** <0.001 compared with control.

計畫結果自評

一）研究內容與原計畫相符
二）已達成預期目標
三）已多次於國外學術討論會上發表
四）適合學術期刊發表

Table 1. Effects of YC-1, sodium nitroprusside (SNP) and 3-isobutyl-1-methylxanthine (IBMX) on cGMP accumulation in rabbit corpus cavernosum.

Drugs	cGMP formation (fmol/mg tissue/15 min)
Control	12±3
YC-1 3 μM	27±5*
YC-1 10 μM	137±31**
YC-1 30 μM	225±16***
SNP 10 μM	125±37*
+YC-1 3 μM	131±12***
+YC-1 10 μM	590±113***
+YC-1 30 μM	1544±608*
IBMX 300 μM	98±31*
+YC-1 3 μM	122±39*
+YC-1 10 μM	191±60*
+YC-1 30 μM	498±145**

Data are expressed as mean±s.e.m. of three determinations. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with control.

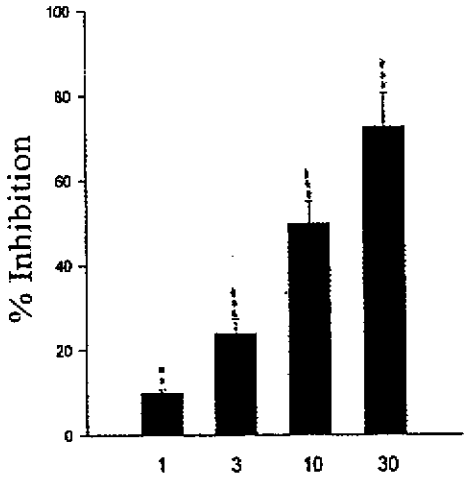

Fig. 1

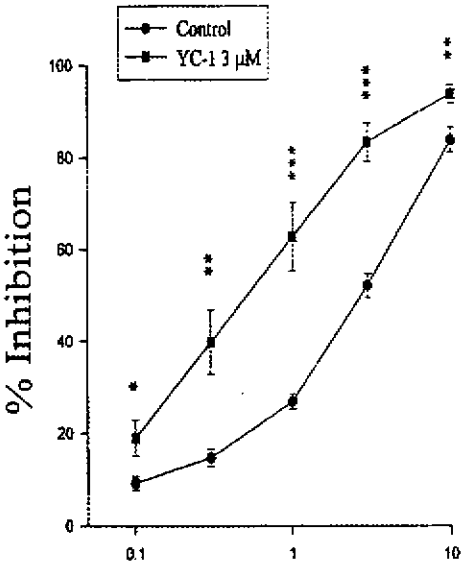

Fig. 2 Sodium nitroprusside (μM)

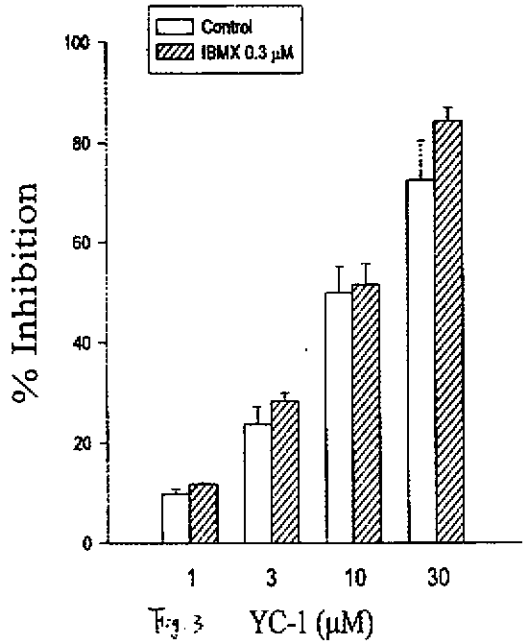

Fig. 3