응용약물학회지, The Journal of Applied Pharmacology 2, 136-140(1994)

Another Evidence for Nitric Oxide as Mediator of Relaxation of Isolated Rabbit and Human Corpus Cavernosum

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(Received June 16, 1994; accepted July 18, 1994)

Abstract—To prove the hypothesis that NO- and NO_2-carrying molecules potentiate photorelaxation by generating NO, investigation was carried out using isolated rabbit and human corpus cavernosum. Corporal smooth muscle, in the presence or absence of endothelium, relaxed only slightly upon ultraviolet light (366 nm) irradiation. But, NO-and/or NO_2-containing compounds such as streptozotocin and N^G-nitro-L-arginine methyl ester significantly ($p<0.01$) enhanced photorelaxation in this tissue. In addition, N^G-nitro-D-arginine methyl ester, known to lack inhibitory action on NO synthase, showed concentration-dependent potentiation of the photorelaxation. Oxygen radical generating system via copper+ascorbic acid and guanylate cyclase inhibitor, methylene blue, significantly ($p<0.05$) inbibited the streptozotocin-potentiated photorelaxation. Nitrite was accumulated by photolysis of streptozotocin, N^G-nitro-L-arginine methyl ester and N^G-nitro-D-arginine methyl ester, in a concentration and exposure time dependent manner. These observations indicate that NO is a potent relaxant of rabbit and human corpus cavernosum and further support the hypothesis that NO is released by photolysis from NO- and NO_2-carrying molecules.

Keywords □ Nitric oxide, photorelaxation, corporal smooth muscle.

Impotence is a major clinical problem in adult men. It has been shown that corporal smooth muscle relaxes markedly in response to stimulation by nonadrenergic noncholinergic (NANC) neurons. Growing lines of evidence indicate that nitric oxide (NO) is involved in mediating the inhibitory influence (smooth muscle relaxation) of NANC neurotransmitter in corpus cavernosum. In recent years it has been demonstrated that L-ARG/NO pathway is an important and ubiquitous effector system that plays a significant role in the regulation of a diverse set of mammalian physiological processes (Moncada *et al.*, 1991; Ignarro, 1990; Stuehr and Griffith, 1992). Recently, Chang *et al* (1993a) reported that photo induced adequate nitric oxide (PIANO) generating system such as photoactivated NO- or NO_2-carrying molecules can be applied as a tool to investigate the role of NO in relaxing vascular or non vascular smooth muscle. NO is also known as a potent relaxant of human and rabbit corpus cavernosum (Bush *et al.*, 1992) and the principal mediator of penile erection caused by NANC stimulation (Ignarro *et al.*, 1990; Bush *et al.*, 1992). The purpose of the present study, therefore, was to extend the hypothesis that photolysis of NO- and NO_2-containing compounds may be responsible for the relaxation of corpus cavernosum of the rabbit and human, possibly through photolysis-induced release of NO.

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Materials and Methods

Materials

Streptozotocin (STZ), N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-D-arginine methyl ester (D-NAME), l-ascorbic acid, phenylephrine (PE) and methylene blue (MB) were obtained from Sigma Chemical Co. (St. Louis, MO).

Muscle Preparation

Six New Zealand White rabbits weighing 2.5~3 kg were sacrificed and the penis was removed en bloc. A ventral incision was made on the right and left corpora, the tunica was dissected and the corpus cavernosum tissue was exposed. Human cavernosal tissue was obtained from three patients who carries penile resection due to cancer. Strips of corpus cavernosum tissue measuring approximately 2 mm×2 mm×10 mm were submerged in 10 m*l* organ chambers containing Krebs-

Ringer bicarbonate solution.

Measurement of Photorelaxation

The strips were suspended with a glass capillary rod to force transducer on one end, and fixed with silk ties to a metallic support on the opposite end. The physiologic solution was gassed with 95% O_2-5% CO_2 and had the following composition (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.18; KH_2PO_4, 1.18; $NaHCO_3$, 24.9; glucose, 10 and EDTA, 0.03. The strips were equilibrated at 1 g resting tension for 90 minutes prior to the drug additon. Isometric tension was induced using a submaximal concentration of PE and was recorded on a Grass physiograph (model 79 E, Grass Instruments, Quincy, MA) using a force displacement transducer. After reaching a plateau of contration, corporal smooth muscles were exposed to UV light (5~60 sec) using a long wavelength UV lamp (366 nm, Minelalight UV GL 58, San Gabriel, CA) in the presence or absence of test compounds.

Nitrite Assay

In a series of test tubes containing 1 mM of STZ, L-NAME and D-NAME was exposed to UV light from 10 min to 60 min, respectively. At indicated time, nitrite production was quantified by the method of Stuehr *et al.* (1992), using the Griess reagent (1% sulfanilamide/0.1% naphthylethylenediamine dihydrochloride/ 2.5% H_3PO_4). Nitrite concentrations were calculated from a standard curve using sodium nitrite as the standard.

Statistic

Results are expressed as the mean± S. E. M. Statistical evaluation was made using Student's t-test. Probabilities of less than 5% ($p<0.05$) were considered significant. Drug concentrations are expressed as final negative log molar concentrations.

Results

PIANO-Mediated Relaxation of Rabbit and Human Corpus Carvernosum

Corporal tissues precontracted with 10 μM PE were almost resistant to relaxation by UV irradiation in both rabbit and human tissues. The developed tone was slightly depressed as STZ (100 μM) was administered, but it soon stabilized. After STZ treatment, corporal smooth muscles relaxed upon UV irradiation (Fig. 1). The magnitude of the relaxation was dependent on the exposure time to UV light (Fig. 1 and 2). Exposure to 300 μM L-NAME caused a contraction of the cavernosum precontracted with PE. The magnitude of the potentiation of L-NAME on UV light-induced relaxations was dirctly related to the duration of UV light exposure (Fig. 1) and L-NAME concentration. Even though 3 times higher concentration was use, the potentiation of photorelaxation by L-NAME was less than that by STZ (Fig. 1, 2).

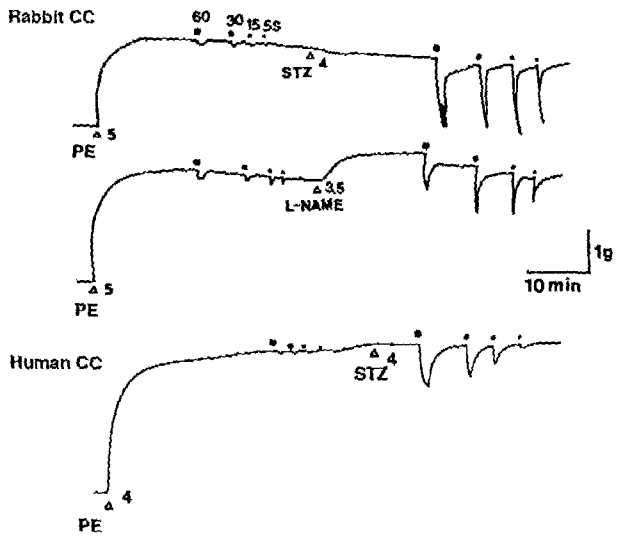

Fig. 1. PIANO-mediated relaxation of rabbit and human corpus cavernosum.

D-NAME Sensitization of Photorelaxation in Rabbit Corpus Carvernosum

Exposure of the carvernosum to 300 μM of D-NAME, stereiosomer of L-NAME, did not affect the developed tone. The magnitude of the potentiation of D-NAME on UV light-induced relaxations was directly related to the duration of UV light exposure (Fig. 1) and D-NAME concentraion (Fig. 3). The potentiation of the photorelaxation by D-NAME was also less than that by STZ (Figs. 1 and 2).

Inhibitory Action of Oxygen Free Radical System and MB on STZ-Enhanced Photorelaxation

Fig. 2B shows that oxygen free radical generating system by copper+ascorbic acid inhibits the potentiation of the UV light-induced relaxation due to STZ. Copper by itself did not affect the tone but addition of ascorbic acid significantly ($p<0.05$) inhibited STZ-enhanced photorelaxation. Methylene blue, concentration dependently, inhibited STZ-enhanced photorelaxation (Fig. 3 lower panel).

Accumulation of Nitrite by PIANO System

As shown in table 1, nitrite, spontaneous oxidation product of NO, has accumulated by photo activation of STZ, L- and D-NAME. Much greater nitrite was accumulated by UV exposure of STZ than the stereoisomers of NAME even in the same concentration (1 mM). Furthermore, bulb light (110 V, 60 W) exposure

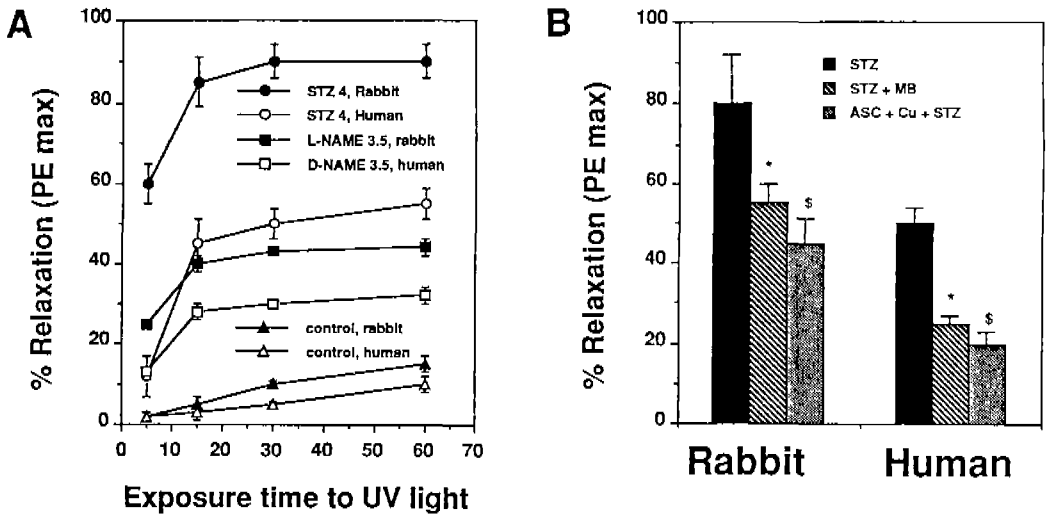

Fig. 2. Concentration- and UV exposure time-dependent potentiation (A) of STZ, L-NAME and D-NAME, and inhibition of STZ-enhanced potentiation by MB, copper+ascorbic acid (B) in rabbit and human coporeal smooth muscle. Each point represents mean± SEM of 3 experiments. *$p<0.01$ greater than control. $^{\$}p<0.05$ (STZ vs copper+ascorbic acid, and STZ vs MB).

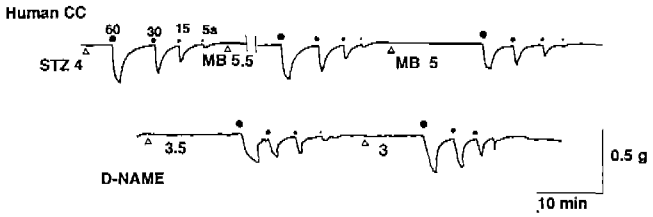

Fig. 3. Tracing of the inhibitory action of MB (3, 10 μM) on STZ-potentiated photorelaxation and concentration-dependent potentiation of photorelaxation of D-NAME on human corpus cavernosum.

Table I. Exposure time of UV irradiation dependent accumulation of nitrite to NO or NO_2-carrying molecules

Compounds	nitrite (μM)			
	10 min	20 min	30 min	60 min
STZ (1 mM)	16± 2	21± 3	26± 3	47± 5
L-NAME (1 mM)	7± 3	9± 3	11± 2	19± 4
D-NAME (1 mM)	5± 2	9± 3	11± 3	18± 5

of STZ, not L- or D-NAME, time dependently, produced nitrite (data not shown).

Discussion

The aim of the present study was to confirm of the earlier report that release of NO by photolysis is responsible for the enhancement of the photorelaxation of NO_2-carrying molecules, and to extend the hypothesis to corporal smooth muscle in which NO-mediated relaxation plays a very important role in penile erection (Bush *et al.*, 1992). The present results clearly show that isolated rabbit and human corpus cavernosum was photorelaxed in the presence of NO- and/or NO_2-carrying molecules. This was consistent with our previous report (Chang *et al.*, 1993a) and others (Chen and Gillis, 1993) in that UV irradiation, in the presence of NO or NO_2-carrying compounds, of nonvascular as well as vascular smooth muscle (Chang *et al.*, 1993a; Matsunaga and Furchgott, 1989) enhanced the relaxation by releasing EDRF-like substance. These observations indicate that NO may be responsible for the relaxation of corpus cavernosum smooth muscle.

In recent years, the relaxation of cavernosum elicited by authentic NO is pharmacologically very similar to that elicited by EFS (electrical field stimulation) (Bush *et al.*, 1992). EFS-elicited relaxation of precontracted strips of rabbit corpus cavernosum, in the presence of functional adrenergic and cholinergic blockade, has been reported to occur due to the endogenous formation and release of NO (Bush *et al.*, 1992). The relaxation elicited by EFS was inhibited by methylene blue, a guanylate cyclase inhibitor (Gruetter *et al.*, 1979), and structural analogues of arginine (Persson and Andersson, 1992; Thornbury *et al.*, 1992) stereoselectively inhibited the endogenous formation of NO from L-ARG, indicating the involvement of L-ARG/NO pathway in EFS-induced relaxation. However, L-NAME, a NO synthase inhibitor, potentiated rather than inhibited the photorelaxation. Moreover, copper +ascorbic acid system, oxygen radical generator (Winkler, 1987; Chang *et al.*, 1993c), inhibited STZ-potentiated photorelaxation of corporal strip of rabbit. From these findings, it seems reasonable to assume that the

photorelaxation and EFS-induced relaxation may be mediated by the same mediator, NO but the mechanism of the action in signal transduction system may be different. To support this idea. D-NAME which lacks the inhibitory action on NO synthase but carries NO_2 molecule, also showed enhancement of photorelaxation. This findings strongly support that NO may be released through photolysis from NO_2-carrying molecules and the photorelaxation mechanism seems not to involve the L-ARG/NO pathway (Venturini *et al.*, 1993; Chang *et al.*, 1993b). On the other hand, MB (3~10 μM) inhibited STZ-potentiated photorelaxation probably by blocking the soluble guanylate cyclase. Although there is a report that methylene blue enhanced photorelaxation upon UV-irraditaion (Chen and Gillis, 1993), the present finding could not confirm this. The reason for this discrepancy can not be adequately explained but different experimental conditions may account for the different results. There is considerable evidence to suggest that MB can generate superoxide anion under both *in vitro* and *in vivo* conditions. In the presence of electron donors, MB is known to undergo photoreduction with an associated generation of superoxide anion (Goodspeed *et al.*, 1965; Marshall *et al.*, 1988). Although, measurement of superoxide anion generation by MB was not performed in the present study, there is a possibility that MB may inhibit STZ-enhanced photorelaxation via generation of superoxide anion (Marczin *et al.*, 1992). The fact that pyrogallol, a superoxide anion generator, inhibited photorelaxation in rat aorta (Chang *et al.*, 1993a) further supports the fact that superoxide anion has an inhibitory action on photorelaxation.

In the present study, precontracted corporal smooth muscle was almost resistant to relaxation upon ultraviolet light (366 nm) irradiation in the presence or absence of endothelium. However, both endothelium-intact and -denuded corporal smooth muscle photorelaxation potentiated by the presence of STZ and L- or D-NAME, confirms that photorelaxation is independent of endothelium (Furchgott *et al.*, 1984). This findings may add another difference in the mechanism of action between photorelaxation and L-ARG/NO-mediated relaxation. Therefore, NO- and NO_2-carrying molecule-induced photopotentiation of relaxation appears to be a suitable model to study the effect of NO-mediated relaxation of smooth muscle.

In conclusion, evidence is presented that potentiation of relaxation of corpus cavernosum in the presence of NO- and NO_2-carrying molecule is due to the release of NO through photolysis of the molecules. The mechanism of photorelaxation action as well as its physiological significance is still unknown. However, the finding that NO and NO_2-carrying molecules potentiate relaxation of corpus cavernosum provides another evidence that NO is responsible for the penile erection.

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