Endothelin-1-induced vasoconstriction is inhibited during erection in rats

T. M. MILLS,1,2 D. M. POLLOCK,1,3 R. W. LEWIS,2 H. S. BRANAM,1 AND C. J. WINGARD1,3

Departments of 1Physiology and of 2Surgery (Urology Section), 3Vascular Biology Center, Medical College of Georgia, Augusta, Georgia 30912-3000

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Mills, T. M., D. M. Pollock, R. W. Lewis, H. S. Branam, and C. J. Wingard. Endothelin-1-induced vasoconstriction is inhibited during erection in rats. Am J Physiol Regulatory Integrative Comp Physiol 281: R476–R483, 2001.—Recent evidence indicates that endothelin-1 (ET-1) might be a principal vasoconstrictor in the penis. We report that ET-1 injection into the cavernous sinuses before erection sharply reduced the magnitude of subsequent erections. Corpus cavernosum pressure-to-mean arterial pressure ratios (CCP/MAP), with maximal ganglionic stimulation, were 0.62 ± 0.05 before ET-1 injection and 0.31 ± 0.05 after, indicating that ET-1 acted as a vasoconstrictor. When ET-1 was injected during a maximal neurally induced erection, the ability of ET-1 to attenuate subsequent erections was diminished (CCP/MAP 0.75 ± 0.02 before ET-1, 0.61 ± 0.03 after). At submaximal stimulation voltages, injection of ET-1 during erection also attenuated its vasoconstrictive effect. Similarly, when ET-1 was injected during erection induced by intracavernosal injection of the nitric oxide (NO) donor NOR-1, subsequent erections were not significantly suppressed (CCP/MAP 0.53 ± 0.04 before ET-1, 0.45 ± 0.04 after). These findings that ET-1-induced vasoconstriction is attenuated during erection are consistent with the hypothesis that NO mediates erection both by initiating pathways that cause smooth muscle relaxation and by inhibiting the vasoconstrictive actions of ET-1.

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ERECTION RESULTS FROM RELAXATION of the smooth muscle in the arterioles and cavernous sinuses of the penis (19). This relaxation permits increased flow of blood into the sinuses and, as the sinuses fill, they expand against the tunica albuginea to activate the veno-occlusive mechanisms that limits outflow of blood (17). The principal agent causing relaxation of smooth muscle leading to the erectile response is nitric oxide (NO) (14, 33), although vasoactive intestinal polypeptide (7, 34, 43) and prostaglandin E may participate as well (5). However, most of the time the penis is flaccid due to contraction of the smooth muscle of the cavernosal arterioles and sinuses that limits blood flow through the penis. The factors that maintain the smooth muscle in the contracted state have not been studied to the same extent as the agents that lead to erection through smooth muscle relaxation. Studies from several laboratories (5, 10, 15, 44) have shown that norepinephrine is a potent vasoconstrictor in the cavernous circulation and could be important in the maintenance of the flaccid penis. Endothelin-1 (ET-1) has also been proposed as the principal agent that maintains cavernosal smooth muscle in the contracted state preventing erection (1, 4, 9, 18, 28, 48). Publications from several laboratories have demonstrated the presence of both ETα- and ETβ-receptor subtypes in erectile tissue (13, 41). We recently reported that ET-1 causes vasoconstriction in the penile circulation of the rat and that an antagonist to the ETα receptor prevents the vasoconstriction (20).

Although there have been published discussions about the individual actions of vasodilators or vasoconstrictors during the erectile response, less is known about how these agents interact to initiate and sustain erection. It is not known, for example, to what extent NO can cause vasodilation and erection in the presence of a potent vasoconstrictive agent such as ET-1. The present studies were undertaken to examine changes in the vasoconstrictor effect of ET-1 during penile erection induced by stimulation of the autonomic enervation of the cavernous vasculature or by exposure to the NO donor drug NOR-1.

MATERIALS AND METHODS

Animals

Intact male Holtzman rats (Harlan Laboratories), 90–120 days of age, were used in these studies. All procedures were performed in accordance with the Guiding Principles in the Care and Use of Animals established by the American Physiological Society and approved by the Medical College of Georgia Committee on the Use of Animals in Research and Education.

Measurement of the Erectile Response

We previously described the methods for measurement of corpus cavernosum pressure (CCP) and mean arterial pressure (MAP) (36, 37, 39, 40). In this procedure, rats were anesthetized with intramuscular ketamine (87 mg/kg body wt) plus xylazine (13 mg/kg) and maintained on supplemen-
tal ketamine as needed. The left carotid artery was cannulated for continuous monitoring of MAP. The shaft of the penis was freed of skin and fascia, and the right corpus cavernosum was cannulated by insertion of a 30-gauge needle connected via PE tubing to a pressure transducer to permit continuous monitoring of CCP. The left corpus cavernosum was cannulated with 30-gauge needles attached to 10-μl syringes via short lengths of PE-10 tubing and used for administration (intracavernosal injection) of vasoactive drugs. The abdominal cavity was opened, and the right major pelvic ganglion (MPG; contains autonomic nerve fibers that innervate the cavernosal vascular tissue) was exposed; stainless steel bipolar electrodes were positioned on the MPG, and their positions were adjusted during electrical stimulation (5 V) until the maximal CCP was achieved. During the experiment, stimulatory voltages applied to the MPG ranged from 1 to 5 V delivered in 5-ms pulses at a frequency of 12 Hz. The duration of stimulation was 1 or 2 min with minimal rest periods of 2–3 min between subsequent stimulations. All pressure data were collected for analysis using Polyview data-acquisition software (AstroMed, Grass Instruments Division).

Calculation of CCP and MAP

The Polyview data-acquisition software continuously recorded CCP and MAP at a rate of 5 samples/s. The CCP and MAP responses were integrated over the specific measurement time period. The resulting integrated values were then converted to pressure units (mmHg) based on a conversion factor obtained by integrating the response to a standard 100-mmHg pressure for 1 min (mercury manometer).

Experimental Design

The design of the experimental protocols is described as follows.

Protocol 1: effect of ET-1 when given before erection induced by ganglionic stimulation. In this experiment, vehicle (1 μl of 0.9% NaCl containing 0.1% bovine serum albumin) was injected into the cavernous sinuses followed 3 min later by ganglionic stimulation (2 min at 5 V) to induce erection (before ET-1). After a rest period of 5–7 min, ET-1 was injected into the cavernous sinuses (50 pmol in 1 μl) followed 3 min later by 2-min ganglionic stimulation to induce erection (+3 min) and then 3 min later by final ganglionic stimulation (+8 min).

Protocol 2: effect of ET-1 when given during erection resulting from ganglionic stimulation. During erection induced by a 2-min, 5-V maximal or 2- to 3-V submaximal stimulation, 1 μl vehicle was injected into the cavernous sinuses 30 s after the start of the ganglionic stimulation (before ET-1). After a 5- to 7-min rest period, the ganglionic stimulation was repeated with ET-1 (50 pmol in 1 μl vehicle) injected into the cavernous sinuses at 30 s after the start of ganglionic stimulation. After 3 min of recovery, the ganglionic stimulation was repeated to induce erection (+3 min) and this was followed 3 min later by a final ganglionic stimulation (+7 min).

Protocol 3: effect of ET-1 when injected during erection resulting from intracavernosal administration of an NO donor drug. One microliter ethanol vehicle was injected into the cavernous sinuses followed 2 min later by ganglionic stimulation (2 min at 5 V) resulting in erection (before ET-1). After a brief rest period, NOR-1 (20 μg/kg body wt in 1 μl ethanol) was injected. One minute later, ET-1 (50 pmol in 1 μl vehicle) was injected so that the ET-1 was administered during the NOR-1-induced increase in CCP. Two minutes after the ET-1 injection, the MPG was stimulated (2 min at 5 V) to cause erection (+3 min) and this was followed 3 min later by a final ganglionic stimulation (+7 min).

In all these experimental protocols, vehicle-only injections were found to have no effect when made before or during ganglionic stimulation-induced erection or erection resulting from NO donor drug injection. A separate group of animals was used for each of the protocols.

Drugs

ET-1 was obtained from American Peptide (Sunnyvale, CA). The NO donor drug (±)-(E)-methyl-2-(E)-(hydroxyimino)-5-nitro-6-methoxy-3-hexenamide (NOR-1) was purchased from BIOMOL Research Laboratories (Plymouth Meeting, PA) and dissolved in ethanol to prevent liberation of NO before injection. NOR-1 is stable when dissolved in an organic solvent but releases NO in an aqueous environment (32).

Statistical Analysis

Data were analyzed using ANOVA for repeated measures with post hoc comparisons made by Student-Newman-Keuls test (46). Student’s t analysis was used where appropriate. Statistical significance was set at $P < 0.05$.

RESULTS

Figure 1 illustrates the effects on the erectile response (CCP/MAP) of stimulation of the major pelvic ganglion between 1 and 5 V. Figure 1 shows that with increasing voltage (1–4 V) there is a continual increase in the erectile response. However, the response reached a plateau in most animals, and no further statistical increase was seen between 4 and 5 V. Thus, to ensure maximal response, 5-V stimulation was used in all subsequent studies except where noted. Submaximal stimulation of the MPG was accomplished using 2- to 3-V stimuli.

We previously reported that intracavernosal injection of ET-1 results in a reduction in the magnitude of
subsequent erectile response (20). The results in Fig. 2 confirm this observation and show a characteristic pattern in MAP after ET-1 injection (Fig. 2, A and C). This pattern is characterized as a transitory vasorelaxation indicated by the fall in MAP followed by a rapid rise as the vasoconstrictive action of ET-1 predominated. Figure 2A demonstrates that the electrically induced increase in CCP that follows ET-1 administration is markedly reduced, suggesting that ET-1 caused constriction of the arteriolar and sinusoidal smooth muscle to limit inflow. The results from these experiments are summarized in Fig. 2B and demonstrate that the erectile response (CCP/MAP) after ET-1 injection is significantly lower than the response in the same animals measured before ET-1 injection.

To determine if erection itself altered the responsiveness to ET-1, 50 pmol ET-1 was injected during erection caused by 5-V ganglionic stimulation. As shown in Fig. 2C, ET-1 administration resulted in the typical MAP response with transitory vasodilation followed by vasoconstriction. However, it is clear from this tracing that when ET-1 was administered during erection, the subsequent CCP response was not suppressed to the same extent as was seen when ET-1 was injected before erection (Fig. 2A). Figure 2D summarizes the vasoconstrictor effects of ET-1 when administered during maximal MPG stimulation. The erectile response was diminished by less than 20% when injected during erection (Fig. 2D) compared with a 50% reduction when ET-1 was administered before erection (Fig. 2B).

Figure 3 illustrates the impact of injection of NOR-1 in 1 μl ethanol into the cavernous sinuses on the erectile response. When the NOR-1 comes in contact with an aqueous environment such as blood, NO is

![Fig. 2. Effects of endothelin (ET)-1 injection on CCP, MAP, and erectile response (CCP/MAP) when administered before or during ganglionic-stimulated erection. A: representative pressure responses when 50 pmol ET-1 was administered before a ganglion-stimulation-induced erection. B: summarized data on the erectile response when 50 pmol ET-1 was administered before a ganglion-stimulation-induced erection; n = 6. C: representative pressure responses when 50 pmol ET-1 was administered during a ganglion-stimulation-induced erection. D: summarized data on the erectile response during this protocol when 50 pmol ET-1 was administered during a ganglion-stimulation-induced erection. Open bars, erectile response before ET-1 injection; solid bars, response after ET-1 injection. Reported are means ± SE; n = 6. *Statistical significance, P < 0.05, comparing before ET-1 to +3 min ET-1. Stim, periods of 5-V ganglionic stimulation.](http://ajpregu.physiology.org/2001/281/0000000000.pdf)
released. The generated NO can then act on the cavernosal smooth muscle, causing relaxation and resulting in increased erectile response. The CCP/MAP values were significantly elevated within 1 min of NOR-1 injection, were maximal after 2 min, and declined significantly from the maximum by 3 min.

In preliminary studies, injection of NOR-1 into the cavernous sinuses did not augment the erectile response induced by maximal stimulation of the MPG (data not shown), with average CCP/MAP values for 14 rats of 0.54 ± 0.03 before NOR-1 and 0.58 ± 0.03 after NOR-1 injection. Figure 4A illustrates that when injected during NOR-1-induced erection, ET-1 failed to exhibit its strong vasoconstrictor effects on subsequent erections while inducing the typical effect on MAP (vasodilation followed by vasoconstriction). In these experiments, NOR-1 was injected into the cavernous sinuses and, 1 min later, when the CCP had started to increase, ET-1 was injected into the sinuses. Figure 4B summarizes the results and reveals that when injected during NO-induced erection, the vasoconstrictor effect of ET-1 was significantly suppressed. This reduction of ~20% was similar in magnitude to that reduction in the erectile response when ET-1 was injected during maximal MPG stimulation (Fig. 2D).

Evaluations of a series of neurally induced erectile responses subsequent to the injection of ET-1 are shown in Fig. 5. In each experimental protocol, a second MPG stimulation erectile response (3 min after the cessation of the first) induced a similar erectile response compared with the earlier stimulation. Figure 5C illustrates the impact of submaximal (2–3 V) MPG stimulation on the capacity of ET-1 to suppress the erectile response when injected during the erection. The results could be interpreted to show that with submaximal stimulation, less NO is liberated into the cavernosal circulation and is less effective at preventing ET-1 vasoconstrictive action.

Figure 6 summarizes the CCP/MAP response from the different experiments in which ET-1 was administered before erection, during erection induced by maximal ganglionic stimulation, and during erection resulting from the administration of the NO donor drug (NOR-1). In Fig. 6, the erectile response soon after ET-1 administration is expressed as the percentage of the response measured before ET-1 administration. Injection of ET-1 decreased the response by ~50% when given before erection. However, when administered during erection resulting from ganglionic stimulation or resulting from administration of the NO donor drug, the vasoconstrictor effect of ET-1 was equally diminished.

**DISCUSSION**

The results from the present study confirm our earlier observation that ET-1 exerts potent vasoconstrictive action in the penile circulation and sharply reduces the erectile response. Our current results extend this observation and demonstrate the vasoconstrictive effects of ET-1 are significantly reduced when administered during NO-induced erection. The CCP/MAP values for the different experimental protocols are shown in Fig. 6. The erectile response soon after ET-1 administration is expressed as the percentage of the response measured before ET-1 administration.

**Fig. 3.** The effects of intracavernosal injection of the nitric oxide (NO) donor NOR-1 on the erectile response (CCP/MAP). Bars represent the means ± SE of consecutive measurements made in 4 rats. Statistical analysis (ANOVA for repeated measures) revealed that all means are significantly different from one another with the exception of the 1- and 3-min time points, which are not different.

**Fig. 4.** The effects of ET-1 on CCP and MAP and erectile response (CCP/MAP) when administered during an NO donor (NOR-1)-induced erection. A: representative pressure responses when 50 pmol ET-1 was administered during a 20 μg/kg NOR-1-induced erection. B: summarized data on the erectile response during this protocol. Reported are means ± SE; n = 14. *Statistical significance comparing the erectile responses before and after ET-1 administration, P < 0.05.
istered during erection resulting from ganglionic stimulation or administration of an NO donor drug. These results point to two possible routes by which NO can influence the erectile response: the first by a direct activation of smooth muscle relaxation pathway(s) and the second by also inhibiting the vasoconstrictive action of ET-1.

ET-1 acts via specific receptors (ETA) to cause constriction and thereby minimize inflow and prevent activation of the veno-occlusive mechanism in the penis. In some vascular beds, ET-1 can also exert a paracrine effect on the endothelial cells via the ETB receptors to increase NO production contributing to smooth muscle relaxation (45). In endothelial cells, shear stress and stretch (21) combined with the paracrine effects of ET-1 activate NO production. NO has also been reported to inhibit ET-1-induced vasoconstriction in a variety of vascular beds (12, 24, 42). NO may antagonize ET-1 effects by inhibiting the release of ET-1 from endothelial cells (31), by inhibiting ET-1 binding to its receptor or postreceptor pathway calcium mobilization (23), and/or by reducing ET-1 gene expression (16). These physiological antagonisms between the action of ET-1 and NO may exist in the penis to regulate penile blood flow and thus control the erectile response. For erection to occur, the balance between vasoconstriction and vasorelaxation must shift in favor of NO-induced vasorelaxation with increased inflow and veno-occlusion. What is not clear in the process, however, is the fate of vasoconstrictive agents such as ET-1 during vasorelaxation leading to erection. Two possibilities can be identified: 1) the NO-induced vasorelaxation may simply override the ET-1-induced contraction or 2) the NO may interfere directly with the constractive action of ET-1. The present studies were designed to test the hypothesis that NO acts both as a principal vasorelaxation agent in erection and to modulate ET-1-induced vasoconstriction.

Prior studies from this laboratory used intracavernosal injection of ET-1 along with treatment with specific antagonists to the ETA or ETB receptors to study

Fig. 5. Summary of effects of ET-1 injection on the erectile response when administered before maximal ganglionic stimulation, during maximal (5 V) and submaximal (2–3 V) ganglionic stimulation, and during erection induced by NOR-1 exposure. Open bars, mean ± SE erectile response before administration of ET-1 (50 pmol/kg); solid bars, mean ± SE erectile response 3 min after ET-1 administration; shaded bars, mean ± SE erectile response 7 or 8 min after ET-1 administration. *Statistical difference from before ET-1 CCP/MAP values within that panel. †Statistical significance of the during maximal stimulation +3 min ET-1 value from before maximal stimulation, during submaximal stimulation, and during NOR-1 stimulation values. ‡Statistical significance of the during maximal stimulation +7 min ET-1 value from before stimulation, during submaximal stimulation, and during NOR-1 stimulation values. §Statistical difference from the before ET-1 value of B. Sample numbers for A, B, and C were n = 6 and for D, n = 14.

Fig. 6. Summary of effects of ET-1 on subsequent erection when given before erection, during erection (Stim) resulting from ganglionic stimulation, and during erection (NO) resulting from NOR-1 injection. The results are expressed relative to the erectile response before ET-1 injection. Reported are means ± SE for 6–8 samples. Based on ANOVAs for repeated measures, only the administration of ET-1 before erection resulted in a statistically significant decrease in the magnitude of the erectile response (*P < 0.05).
the influence of ET-1 in rat erection (20). These studies showed that blockade of the ET<sub>A</sub> receptor prevented ET-1-induced vasoconstriction, whereas blockade of the ET<sub>B</sub> receptors had no effect on the response to ET-1 injection. Furthermore, neither the antagonist to ET<sub>A</sub> or ET<sub>B</sub> receptors had an acute effect (1–3 min) on erection in response to ganglionic stimulation (20). On the basis of these observations, we suggested that either endogenous ET-1 was not critically involved in the rat erectile response or the vasoconstrictor actions of ET-1 could be attenuated during erection induced by NO.

The results reported here confirm the previous observation that when injected into the cavernous sinuses, ET-1 acted as a vasoconstrictor and suppressed the magnitude of subsequent erections (20). Although the dose of ET-1 administered in these studies is in the pharmacological range, it was chosen for its ability to suppress the maximal ganglionic-induced erectile response by ~50%. The systemic circulating levels of ET-1 have been reported in rats to be below the dose administered here (2). Although it is generally recognized that circulating levels of ET-1 do not necessarily reflect de novo production in the tissue where may be very high local concentrations of ET-1.

The suppression of the erectile response (CCP/MAP) occurred despite a large increase in MAP. Because the systemic blood pressure is the force that drives blood into the cavernous sinuses during erection, a rise in MAP would be expected to also increase intracavernosal pressure if cavernosal smooth muscle activity remained unchanged (36). It can be seen in the representative tracings of Figs. 2 and 4 that when MAP was elevated, there was a slight elevation of CCP values during the periods between ganglionic stimulation. Despite the increased MAP after ET-1 administration before erection, the suppression in CCP/MAP suggests an increase in constrictive activity of the smooth muscle of the cavernosal circulation. However, when the same dosage of ET-1 was injected into the penis during erection resulting from both maximal and submaximal stimulations of the MPG, the vasoconstrictive effect of ET-1 was diminished despite a similar increase in MAP. This suggests that the vasoconstrictor effect of ET-1 was actively suppressed during erection.

Reports from other laboratories have pointed to a role for ET-1 in the regulation of penile blood flow. These include reports showing specific binding of ET-1, enhancement of intracellular Ca<sup>2+</sup> levels (41), and contraction of strips of human and rabbit penile tissue in vitro (26, 27, 29, 41). Conversely, when infused into the cavernosal sinuses of rats at low concentrations, ET-1 had a vasorelaxation effect and a vasoconstrictive action at very high concentration (9).

The circulatory pattern in the penis changes during erection, resulting in an increased transient time as veno-occlusion slows blood escape. Thus the erection resulting from maximal MPG stimulation would be expected to cause retention of ET-1 in the cavernosal sinuses and provide even greater opportunity for receptor occupancy and vasoconstriction. Although equivalent dosages of ET-1 were administered directly into the cavernosal tissue both before and during erection, we observed a statistically greater suppression of the vasoconstrictive effect under conditions when transient time should be longest. Hemodynamic differences in flow though the penile circulation may still complicate our interpretation. However, the results presented in Fig. 5C may help to explain our findings. Previous studies reported different blood flow patterns in the penis associated with submaximal and maximal MPG stimulation (35). At submaximal stimulations, cavernosal blood flow is high where veno-occlusion is incompletely activated, whereas at maximal stimulations, the flow is transiently elevated at the onset of erection but rapidly falls to a very low level during the continued stimulation. The current studies reveal little difference in the erectile responses when ET-1 was administered under stimulation conditions with reported different flow patterns. This lends support to the hypothesis that NO released during MPG stimulation might in fact be capable of modulating the ET-1 vasoconstrictive actions and is not a reflection simply of different flow patterns impacting the distribution of ET-1 in and through the penis. Our data suggest hemodynamic changes are not sufficient to give the observed result and that some other mechanism was responsible for the impaired erection when ET-1 is administered during erection.

The α-adrenergic agonist methoxamine, when administered in the same fashion as ET-1 in this study, produced a similar result. We found when injected before erection methoxamine had a strong vasoconstrictive effect but when given during erection it was much less effective at constricting the penile circulation (47).

NO has also been reported to inhibit ET-1-induced vasoconstriction in other vascular beds including the renal (12), mesenteric circulation (24), and other tissues (42). NO may antagonize ET-1 effects by inhibiting the release of ET-1 from endothelial cells (31), by inhibiting ET-1 binding to its receptor or postreceptor pathway calcium mobilization (23), and/or by reducing ET-1 gene expression (16). When NOS activity was reduced with specific inhibitors, ET-1 release was increased (25). Although receptor binding assays or gene expression were not examined in these studies, our results are consistent with an action of NO to interfere with the ET-1-induced vasoconstriction in the cavernosal circulation.

Further support for the role of NO in the modulation of ET-1-induced vasoconstriction was found when ET-1 was injected during erection induced by an NO donor drug (NOR-1). When injected during NOR-1-induced erection, the vasoconstrictive action of ET-1 was also blunted. Thus, when taken together, our results can be interpreted to suggest that NO may have two routes of action facilitating erection: 1) directly stimulating cavernosal smooth muscle relaxation pathway(s) and 2) inhibiting the vasoconstrictive action of ET-1.

In summary, we used an intact animal model to examine the antagonistic effect of exogenous and en-
dogenous NO production on the vasoconstrictive actions of ET-1. Although our approach does not distinguish the endogenous mechanisms normally used in intracavernosal pressure maintenance, it does suggest a dual action of NO where NO can directly relax cavernosal smooth muscle and can interfere with the contractile action of ET-1; both actions could then contribute to the erectile response.

**Perspectives**

The studies presented here support the hypothesis that a balance between the actions of ET-1 and NO regulates penile blood flow and penile erection. It is generally believed that an imbalance between the actions of NO and ET-1 contributes to vascular dysfunction (11, 12) and could be responsible for some forms of erectile dysfunction (30). For example, in both diabetic and nondiabetic men, there was a significantly higher level of ET-1 in cavernosal blood and peripheral blood in patients with erectile dysfunction than in those without erectile dysfunction (22). These authors suggest that local release of ET-1 during the early stages of atherosclerosis could also contribute to pathogenic changes in the cavernosal tissue and result in erectile dysfunction.

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