Receptor-specific influence of endothelin-1 in the erectile response of the rat

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Dai, Y., D. M. Pollock, R. L. Lewis, C. J. Wingard, V. S. Stopper, and T. M. Mills. Receptor-specific influence of endothelin-1 in the erectile response of the rat. Am J Physiol Regulatory Integrative Comp Physiol 279: R25–R30, 2000.—Specific receptor antagonists were used to examine the role of endothelin-1 (ET-1) in the erectile response of the rat. In these studies, intact rats were cannulated to permit the continuous recording of mean arterial pressure (MAP) and intracavernosal pressure (CCP). Erection was induced by electrical stimulation of the autonomic ganglion, which regulates blood flow to the penis. The animals were subjected to intracavernosal injection with vehicle only (Cont) or with an antagonist to the endothelin-A receptor (ET_A) or to the endothelin-B receptor (ET_B). Blockade of the ET_A or the ET_B had no effect on the erectile response of CCP/MAP during maximal ganglionic stimulation. When ET-1 was injected into Cont rats, there was a marked vasoconstriction with a sharp rise in MAP and a decline in CCP as the cavernosal arterioles constricted and limited inflow. The injection of the ET_A antagonist prevented the vasoconstriction after ET-1 injection into Cont rats, whereas blockade of the ET_B had no effect on the erectile response of ET-1. Similar results were obtained during submaximal ganglionic stimulation. With minimal levels of ganglionic stimulation, ET-1 injection led to a moderated degree of vasodilation in the presence of the ET_A antagonist. The ET_B antagonist failed to alter the CCP response during minimal stimulation, but it did have a marked effect on the MAP response to ET-1 injection. The results of these studies confirm that cavernosal tissue of the rat penis is highly sensitive to ET-1. However, the failure of the ET-A antagonists to affect penile erection in response to ganglionic stimulation reflects a minimal role of ET-1 in the erectile response in the rat.

endothelin-A and endothelin-B receptors; penile erection; corpus cavernosum; vasoconstriction

Penile erection occurs when there is a change in the contractile state of the smooth muscle cells, which are located in the cavernosal arterioles that convey blood into the erectile tissue. Smooth muscle cells are also located in the walls of the cavernous sinuses, and their contractile state controls the volume of blood that fills these sinuses during erection. The venoocclusive mechanism that controls the blood outflow is dependent on the pressure within the sinuses and likewise dependent on the contractile state of the smooth muscle; when the smooth muscle is relaxed, the cavernous sinuses readily fill and expand against the tunica albuginea. This expansion of the sinuses against the tunica albuginea partially occludes the outflow veins, and the limiting of outflow results in erection.

A variety of neurotransmitters has been implicated in the regulation of the cavernosal and arteriolar smooth muscle tone and hence regulation of the erectile response. Nitric oxide (NO) is considered to be the principal neurotransmitter that causes smooth muscle relaxation (1), whereas other molecules including vasoactive intestinal polypeptide (II), calcitonin gene-related peptide (6), and prostaglandin E1 may be involved as well to a lesser extent (1). The predominant agent responsible for contraction of cavernosal smooth muscle has long been considered to be norepinephrine (NE), and most investigators believe that continuous sympathetic activity maintains the penis in the non-erect state (9, 26). However, it has also been suggested that endothelin (ET)-1 may be an important vasoconstrictive agent in the circulation of the penis (1). Endothelin receptors have been reported in cavernosal tissue (3, 24), and injection of endothelin can cause both vasoconstriction and vasodilation in the rat penis (2). In other vascular systems, ET-1 has been reported to cause both contraction and relaxation, depending on the experimental conditions and the vascular bed (12, 25). The underlying basis for this dual action of ET-1 appears to be the presence of two receptor types; the endothelin-A receptor (ET_A) binds ET-1 resulting in contraction of smooth muscle, and the endothelin-B receptor (ET_B) binds ET-1 resulting in vasodilation via an increased synthesis and release of NO (25). In some vascular beds, ET_B receptors located on vascular smooth muscle cells can produce vasoconstriction (21). The extent to which ET-1 and the A and B receptors are involved in erection has not been fully elucidated. In the present study, specific antagonists to the ET_A (20, 30) and ET_B (28) receptors were utilized to determine...
the extent to which the ET-1 system is operative during the erectile response of the rat.

MATERIALS AND METHODS

Animals. A total of 33 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 have previously described the procedure used to measure the corpus cavernousum pressure (CCP) and mean arterial pressure (MAP) (18, 19, 22, 23). In this procedure, rats were anesthetized with intramuscular ketamine (57 mg/kg body wt) plus xylazine (13 mg/kg) with anesthesia maintained with supplemental ketamine as needed. The left carotid artery was cannulated for continuous monitoring of MAP. Once this cannula was in place, the abdominal cavity was opened, the viscera retracted, and the right major pelvic ganglion (MPG) was exposed. The shaft of the penis was then 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 a 30-gauge needle attached to a 10-µl syringe via a short length of PE-10 tubing and used for administration (intracavernosal injection) of the endothelin and the receptor antagonists. Stainless steel bipolar electrodes were positioned on the MPG, and their position was adjusted during ganglionic stimulation until the maximal CCP was achieved. The ganglion was stimulated stepwise at 1–5 V with a duration of 5 ms and a frequency of 12 Hz with each 1-min stimulation followed by a 1-min rest period (22, 23). In nearly all instances, we have observed that stimulation of the MPG at 5 V yields a maximal and reproducible increase in CCP (data not shown). All pressure data were collected for analysis using Polyview Data Acquisition Software (AstroMed, Grass Instrument Division).

Drugs. The ETₐ antagonist (A-127722, Abbott Laboratories, Abbott Park, IL) was dissolved in vehicle 1 (0.1 mM bicarbonate buffer, pH 8.2) so that a 5-µl injection in vehicle 1 contained 1 mg/kg body wt. A similar volume of vehicle 1 was injected as control. The ETᵦ antagonist (A-1292621, Abbott Laboratories) proved to be sparingly soluble in vehicle 1 so a saturated solution (4.8 mg/ml) was prepared, and 100 µl were injected per rat. ET-1 was obtained from American Peptide (Sunnyvale, CA) and dissolved in saline containing 0.1% bovine serum albumin (vehicle 2). ET-1 was administered at a rate of 50 pmol/rat in 5 µl. Control injections were an equal volume of vehicle 2.

Experimental design. The experiments were completed according to the following protocol (Fig. 1). In each rat, the voltage-response relationship was first established by stimulating the MPG from 1 to 5 V (1-V increments, 1-min duration of stimulation, and 1-min rest between subsequent stimulations), whereas the CCP and MAP were continuously monitored. Next, each animal received an intracavernosal injection of 5 µl vehicle 1 and, after 2 min, the MPG was stimulated and CCP and MAP were recorded. After a 5-min rest period to allow CCP and MAP to return to prestimulation levels, the ETₐ antagonist (0.5 mg/kg in 5 µl vehicle 1) or ETᵦ antagonist (0.48 mg/kg in 100 µl vehicle 1) was injected. After an additional 2 min, the erection was stimulated and CCP and MAP were recorded. Both CCP and MAP were allowed to return to prestimulation levels before injection of vehicle 2 and followed 2 min later by ganglionic stimulation. Again after a rest period of 5 min, each animal was injected with ET-1 (50 pmol/kg in 5 µl vehicle 2) followed by ganglionic stimulation 2 min later. Control (Cont) rats received a second vehicle 1 injection in lieu of the receptor antagonist, but the vehicle 2 and ET-1 injections were the same in all groups. In separate groups of rats treated with the ETₐ antagonist, the experimental protocol was repeated, except that the level of ganglionic stimulation was submaximal (3 or 4 V) or minimal (1 or 2 V). The selection of the voltage for submaximal and minimal stimulation was on the basis of the initial voltage-response determination (Fig. 2).

Results of these experiments are expressed as means ± SE. Changes in CCP, MAP, and the CCP/MAP ratio were analyzed with the use of one-way ANOVA for repeated measures with post hoc analysis by Newman-Keuls test. The paired variable t-test was also used to analyze some of the results as appropriate (29). Statistical significance was set at P < 0.05.

RESULTS

Figure 2 shows the erectile response to graded levels of electrical stimulation in 33 animals expressed as the ratio of CCP relative to MAP. This method of expression of the erectile response (CCP/MAP) is used because
MAP is the force that drives CCP, and stimulation of MPG can lead to a small but transitory change in the MAP in some animals. Figure 2 also shows that 4- or 5-V stimulation of MPG led to a maximal erectile response and that stimulation of the ganglion in the range of 3-4 V resulted in an intermediate level of erectile response, which was significantly less than the maximal response. There was greater variability in the minimal voltage able to elicit a response, with some animals responding at 1 V, whereas others responded only at 2 V. Due to this variability, submaximal and minimal voltages were always determined individually.

The results in Fig. 3 show the effects of treatment with the ET\textsubscript{A} and ET\textsubscript{B} antagonists on the ratio of CCP to MAP without ganglionic stimulation. These measurements showed that injection of the ET\textsubscript{A} receptor antagonist caused an elevation in the CCP/MAP ratio (0.03 ± 0.01 before and 0.06 ± 0.01 after, n = 16, P < 0.01 by paired t-test); CCP was significantly elevated (3.3 ± 0.7 before and 6.9 ± 1.5 after, P < 0.01), whereas MAP was not changed by treatment with the ET\textsubscript{A} receptor antagonist (113 ± 3.3 before and 111 ± 3.5 after, not significant), suggesting cavernosal vasodilation. Figure 3 also shows that control treatment and injection of the ET\textsubscript{B} antagonist was without effect on the CCP/MAP ratio.

Figure 4 depicts results of experiments to determine if blockade of the ET\textsubscript{A} or the ET\textsubscript{B} receptors altered the erectile response to ganglionic stimulation. Compared with the Cont rats, treatment with either of the antagonists failed to cause a statistically significant change in the CCP/MAP ratio during maximal stimulation of the MPG. Individual analysis of the CCP and MAP measurements reveals that treatment with the ET\textsubscript{A} antagonist led to a significantly higher CCP during erection (765 ± 54 before and 850 ± 5.0 mmHg after antagonist, n = 6, P = 0.04) and an elevated MAP (119 ± 5.5 before and 124 ± 5.0 mmHg after, n = 6, P = 0.03), but the increase in the two parameters was proportional so that the CCP/MAP ratio remained unchanged (0.64 ± 0.04 before and 0.69 ± 0.02 mmHg after, n = 6, P = 0.09).

Having demonstrated that treatment with the ET\textsubscript{A} and the ET\textsubscript{B} antagonists had no effect on the erectile response during maximal ganglionic stimulation in the intact rats (Fig. 4), we next sought to determine if the antagonists altered the response to exogenously administered ET-1. Figure 5 shows representative tracings of CCP and MAP in a Cont, an ET\textsubscript{A} antagonist-, and an ET\textsubscript{B} antagonist-treated rat. This figure shows that the rise in CCP during ganglionic stimulation is markedly inhibited after the ET-1 injection in the Cont and ET\textsubscript{B} antagonist-treated rats (Fig. 5, A and C). However, when the ET\textsubscript{A} receptors are blocked, injection of ET-1 no longer reduces the magnitude of the CCP response to ganglionic stimulation (Fig. 5B). These results also demonstrate a transitory decrease in MAP in Cont and ET\textsubscript{A} antagonist-treated rats, although this decline in MAP failed to occur in the ET\textsubscript{B} antagonist-treated animals. After this short-lived decrease in MAP, ET-1 caused systemic vasoconstriction, resulting in an increase in MAP to the same degree in all groups. Average values for the maximal erectile response in the Cont, ET\textsubscript{A}-, and ET\textsubscript{B} antagonist-treated rats are presented in Fig. 6 (n = 6 for each group). In the absence of either antagonist, ET-1 had a potent vasoconstrictor effect, resulting in a reduced CCP/MAP ratio. Direct intracavernous injection of ET-1 (50 pmol/rat) resulted in a 21 ± 3 mmHg decline in CCP between vehicle 2 and ET-1 treatment, whereas the MAP rose 38 ± 6 mmHg during this same period. When the ET\textsubscript{A} antagonist was given, ET-1 injection elevated CCP by 30 ± 7 mmHg, whereas MAP was raised 33 ± 4 mmHg. After treatment with the ET\textsubscript{B} receptor antagonist, ET-1 injection reduced CCP by 17 ± 7 mmHg, whereas the MAP was elevated by 56 ± 14 mmHg compared with the vehicle 2 control.
measurements (data not shown but represented by tracings in Fig. 5).

Additional experiments were conducted using submaximal ganglionic stimulation to further characterize the influence of ET-1 on the erectile response (n = 5 for each treatment). The results in fig. 7 show that with submaximal stimulation, the ETA antagonist failed to alter the erectile response compared with vehicle 1 controls. However, as was the case at maximal level of stimulation, injection of ET-1 only (Cont) during submaximal stimulation reduced the CCP/MAP ratio, and prior treatment with the ETA antagonists prevented this reduction (Fig. 7). When ET-1 was injected into rats treated with the ETA antagonist, the minimal stimulation of the MPG led to a significant increase in the erectile response compared with the vehicle 1-treated rats.

**DISCUSSION**

The studies presented here contribute to our understanding of the involvement of ET-1 in the erectile response. We find that exogenous ET-1 given into the corpus cavernosum has a strong vasoconstrictive action on the cavernosal vasculature as well as the systemic circulation. This action results in an attenuated increase in CCP and a rise in MAP during submaximal, electrically induced erection. We also report here that the vasoconstrictor actions of exogenous ET-1 are blocked by prior treatment with an ETA antagonist. Thus these experiments demonstrate that the ET-1 responses in the cavernosal circulation are dependent on activation of ETA receptors. However, despite this demonstration that the erectile response is sensitive to the actions of exogenous ET-1, our results also suggest
that endogenous ET-1 plays only a minor role in normal erection. This is supported by our findings that blockade of ET_A and ET_B during neurologically induced erection had no effect on the magnitude of the CCP increase or on MAP.

If the actions of ET-1 were critical to erection, then the ET_A antagonist would have blocked the endogenous ET-1-induced contraction and led to an enhancement of the rise in CCP during erection. In addition, blockade of the ET_B receptor might be expected to reduce the magnitude of the CCP rise if ET-1 were exerting a vasodilatory action via the ET_B receptors. There are, however, other possible explanations for the failure of ET_A and ET_B treatment to alter erection. For example, it is known that ET-1 binds irreversibly to its receptors, and the receptor-ligand complex may be internalized or dissociate very slowly (8). Because the receptor turnover is very slow and ET_A and ET_B antagonists would block only the interaction of ET-1 with an unoccupied receptor, an extended period of exposure to the antagonist may be necessary to see an effect. It may be that several days of treatment with the antagonist is required to realize measurable blockade of ET-1 binding.

Another possible explanation for the lack of ET_A and ET_B antagonist effect on erection is that ET-1 may act to enhance the responsiveness of the tissue to another vasoconstrictor such as NE (13, 15). Sympathetic agonists are known to be strong vasoconstrictive agents in the cavernosal circulation, and α-adrenergic receptors have been identified in the tissue (10, 27). In our prior studies (22), we reported that intracavernosal injection of the α-adrenergic agonist phenylephrine led to a rapid, but short-lived, contraction of the penile vasculature and a transitory depression in the CCP when injected during erection. An interaction between ET-1 and α-adrenergic agonists has also been reported in another animal model of penile erection. With the use of rabbit cavernosal strips in vitro, ET-1 enhanced the constrictor action of NE (13). Recently, Christ and co-workers (7) used strips of human cavernosal tissue in vitro and reported that ET-1 caused constriction when used in conjunction with phenylephrine. These authors found that at low doses of phenylephrine, which caused minimal contraction, ET-1 strongly enhanced the contracting effect of phenylephrine. If this enhancing effect is active during the normal tumescence-detrusumence cycle for the penis in other mammals, then the principal action of ET-1 may be to heighten the vasoconstrictor action of NE and, in doing so, to maintain the cavernosal smooth muscle in a state of partial contraction and to prevent erection. If this mechanism exists, then for erection to occur, NO and other agents that cause vasodilation would have to inhibit NE action, thereby permitting the cavernosal smooth muscle to relax and blood to flow into the cavernosal sinuses.

A third possible explanation for the failure of the antagonists to block normal erection is that the action of ET-1 is simply overridden during erection (5) by one of the vasodilatory agents such as NO, which is released during erection. We suggest that NO may act in two capacities. NO is known to cause relaxation of arteriolar and cavernosal smooth muscle cells by activating guanylate cyclase, and the resulting cGMP lowers intracellular calcium (4), relaxing the contractile apparatus (16). In addition, NO may inhibit ET-1-induced vasoconstriction via a direct action on the ET-1-sensitive pathway, leading to vasodilation.

In other vascular beds, ET-1 has been reported to cause both vasoconstriction and vasorelaxation (12, 25), and two distinct receptors have been described (17). The ET_A receptor on smooth muscle cells binds ET-1 and raises myoplasmic Ca^{2+}, resulting in contraction (25, 31), whereas the ET_B receptors on endothelial cells bind ET-1, resulting in vasodilation via an increase in the synthesis of NO (25). In the present studies, injection of the ET_A antagonist produced a small but significant rise in CCP without any change in MAP. This resulted in a significant rise in the CCP/ MAP ratio, suggesting that when the ET_A receptors are blocked, endogenous ET-1 may exert a slight vasorelaxation action (Fig. 5). The possibility that ET-1 may act as a vasodilator is supported by our finding that the erectile response to minimal stimulation of the MPG during ET_A antagonist treatment was significantly increased, indicating vasorelaxation (Fig. 5).

Previously, Ari and co-workers (2) used a rat model of induced erection to investigate the actions of the endothelins. These investigators used ET-1 and ET-3 along with N^\text{\textsuperscript{\text{-}}}\text{-}\text{nitrilo-L-arginine methyl ester (L-NAME)} to block the synthesis of NO and indomethacin and to disrupt prostaglandin production. They reported that in the dose range of 0.5–5 µg/kg, both ET-1 and ET-3 caused vasodilation and enhanced the erectile response. At 10 µg/kg, ET-1 exerted a vasoconstrictor action, whereas ET-3 continued to cause relaxation. Furthermore, L-NAME treatment significantly reduced the vasorelaxation resulting from ET-1 treatment, whereas indomethacin had no effect on the endothelin-induced relaxation. On the basis of these studies, Ari et al. (2) concluded that ET-1 causes vasorelaxation via ET_B and that this effect was mediated by NO. Additionally, at some doses, ET-1 acted as a vasoconstrictor via stimulation of ET_A. In our studies, we used receptor-selective antagonists to confirm ET_A-mediated vasoconstriction produced by ET-1 and extended these investigations by examining the role of endogenous ET-1. In an in vitro study of ET-1 and cavernosal tissue, both ET_A and ET_B receptor activities were demonstrated, but these authors found that ET-1 only caused contraction of the strips of cavernosal tissue (14, 24).

**Perspectives**

Taken together, the results of these studies show that exogenous ET-1 can exert both a vasoconstrictor and a vasodilator action during the erectile response of the rat. Our studies along with previously published reports support the presence of ET_A and ET_B receptors in the rat penile tissue. However, despite our demonstration of the presence of the ET-1 receptors and the finding that the rat penile tissue will respond to exogenous ET-1, our results do not support a central
role of ET-1 in the normal erectile response. In our experiments, treatment with antagonists to ETA or ETB receptors was without effect on erection (CCP/MAP) induced by electrical stimulation of the MPG. However, on the basis of the results presented, we hypothesize that ET-1 plays a critical role in the maintenance of flaccidity in the penis. We further suggest that conditions in which ET-1 is elevated may be associated with erectile dysfunction.

**REFERENCES**


