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Possible role for endothelins in penile erection

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Abstract

The effects of endothelins (endothelin-1 and endothelin-3) on penile erection were studied in vivo in the pithed rat. Penile erectile responses were evaluated by measurements of intracorporal pressure via a needle inserted into one corpus cavernosum. Injection of endothelins (0.1–10 μg/kg i.v.) was followed by a rapid rise in corporal pressure. The responses to endothelin-1 and endothelin-3 were equivalent at the lower doses, but at 10 μg/kg the response to endothelin-3 was greater than that to endothelin-1. N^G-Nitro-L-arginine-methyl ester (20 mg/kg) but not indomethacin (10 mg/kg) antagonized the rise in corporal pressure caused by submaximal doses of endothelins. Electrical nerve stimulation-induced increase in corporal pressure was reduced after 5 μg/kg endothelin-1 but not after the same dose of endothelin-3. The results indicate that in corporal vasculature endothelin-3 has a predominantly vasodilator effect, while endothelin-1 has a vasodilator effect at lower doses and a vasoconstrictor effect at higher doses. The vasodilator action of endothelins is probably mediated by activation of endothelin ET_B receptors and local release of nitric oxide. Thus, endothelins may participate in initiation and/or maintenance of penile erection.

Keywords: Penile erection; Corpus cavernosum; Endothelin; Nitric oxide (NO); (Rat)

1. Introduction

Penile erection is mediated by coordinated function of the peripheral autonomic nerves. Activation of the pelvic parasympathetic nerve, together with reduction in sympathetic tone, results in relaxation of the smooth muscle of the helicine arterioles and the trabecular smooth muscle. Following this, blood flow into the lacunar spaces increases, venous resistance increases, and the flow out of the lacunar spaces decreases, leading to penile erection (Lue and Tanagho, 1987). Relaxation of the penile smooth muscles is caused by nitric oxide (NO), which may be released both from the parasympathetic nerves and the vascular endothelium (Burnett et al., 1992). Nitric oxide is thought to be the most important vasodilator neurotransmitter in this system, but other endogenous vasoconstrictor and vasodilator substances may participate in the physiological control of erection.

The endothelins are a family of peptides with potent vasoconstrictor and vasodilator properties. Three distinct genes that encode three isopeptides (endothelin-1, endothelin-2 and endothelin-3) of the family, were found in human and other mammalian genomes (Inoue et al., 1989). Two types of receptors, endothelin ET_A and endothelin ET_B, have been identified. Both receptor types are found in cardiovascular and non-cardiovascular tissues. In blood vessels, endothelin ET_A is expressed in the vascular smooth muscle and is responsible for vasoconstriction (Lin et al., 1991). Endothelin ET_B is found in the endothelial cells and may be responsible for vasodilation through the release of endothelium-derived mediators (Sakamoto et al., 1991) such as nitric oxide (NO)and prostacyclin (De Nucci et al., 1988).

It was shown by Saenz de Tejada et al. (1991) that human penile corpus cavernosum endothelium has the ability to synthesize and release endothelin. These authors also demonstrated that endothelins contract corporal smooth muscle, and that two distinct endothelin receptors, probably endothelin ET_A and endothelin ET_B, exist in this tissue. Holmquist et al. (1992) have shown the presence of endothelin-1 binding sites in human and rabbit corpus cavernosum and that endothelin induces contractions per se and enhances contractions induced by exogenously ap-

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plied noradrenaline in these tissues (Andersson and Holmquist, 1990).

In order to clarify the effects of endothelins on the corpus cavernosum we have studied erectile function using our in vivo rat preparation (Vardi et al., 1989). In this preparation the intracorporal pressure serves as an index of penile erectile response (corporal vasodilation). We were surprised to observe that intravenous administration of endothelins caused initially an increase in corporal pressure, indicative of corporal vasodilation. Subsequently, a vasoconstrictor response (inhibition of the increase in corporal pressure produced by indirect pelvic nerve stimulation) was also detected. The role of prostaglandins and NO in mediation of these responses has been investigated.

Some of these data were presented in a preliminary form as a poster at the winter meeting of the British Pharmacological Society (January, 1994).

2. Materials and methods

2.1. Pithed rat preparation

Corporal pressure response to spinal nerve root stimulation was determined essentially as described previously (Vardi et al., 1989). Rats were pithed under halothane (3% in oxygen) anaesthesia, by the use of a hollow trochar. Mean arterial blood pressure was measured from the carotid artery, and drugs were administered via a jugular vein cannula or infused into the corpus cavernosum as described below. (+)-Tubocurarine (1 mg/kg) was injected i.v. to prevent skeletal muscle contraction. An insulated stimulating electrode was passed down the spinal column to the level L5-S2, and a second electrode inserted under the skin of the back. The skin covering the penis was removed, a fine (26 gauge) needle was inserted into one corpus cavernosum, and connected to a pressure transducer (Statham p23Db) via a saline-filled polyethylene tube. Another needle was inserted into the contralateral organ for infusion of endothelins. Square wave pulses (1–20 Hz, 50 V, 1 ms duration for 1 min) were applied to the spinal electrode using a Grass 44B stimulator, and the position of the electrode adjusted for optimal corporal pressure response. The detailed protocol was approved by the Technion Animal Care and Use Committee.

2.2. Administration of drugs

Indomethacin was dissolved in 1 M Tris buffer, pH 8.4. All other drugs were dissolved in saline. Solutions of drugs were injected i.v. at volumes of 1 ml/kg, and then washed in with 0.30 ml saline, or infused into the corpus cavernosum (i.c.) at a rate of 30 μl/min for 2 min (total volume 60 μl) and then washed in with 30 μl saline. In a few experiments the infusion continued for 3–5 min at the above rate.

2.3. Determination of the response to endothelins

Increasing doses of endothelin-1 or endothelin-3 were administered (0.1–10 μg/kg i.v.) and corporal pressure and blood pressure responses were recorded. Blood pressure and corporal pressure were allowed to return to control levels before the administration of the next dose. The minimal time between two successive injections was 1 h, no more than 3 doses were administered to each rat, and rats which received 3 μg/kg did not receive 10 μg/kg.

The identity of the vasodilator mediator was investigated in separate experiments using a single submaximal dose of each drug (1 μg/kg for endothelin-1 and 3 μg/kg for endothelin-3). Each rat received an initial injection of endothelin, and a second injection 90 min later. N^G-Nitro-L-arginine-methyl ester (L-NAME, 20 mg/kg) was given 30 min and indomethacin (10 mg/kg) 75 min after the first endothelin administration. In control experiments, vehicle (saline or Tris buffer for L-NAME and indomethacin, respectively) was given at the same volume and time as the drugs.

The inhibitory effect of endothelin-1 or endothelin-3 on electrical stimulation (1–20 Hz) induced vasodilation was determined in a third set of experiments. An initial set of responses to electrical stimulation was determined, and stimulation was repeated 15 min after endothelin-1 or endothelin-3 administration, at a time when the initial rise in corporal pressure in response to endothelin was over. The doses of endothelin-1 and endothelin-3 used in these experiments were 1, 2 and 5 μg/kg, i.v., or 0.1 and 0.2 μg/rat, by slow intracorporal injection. In preliminary experiments, the variability with time of corporal pressure responses to electrical stimulation was examined. Responses were found to be stable for 3–4 h.

2.4. Calculation of responses

Arterial blood pressure and intracorporal pressure were recorded by a computer with an A/D converter at a sampling frequency of 20 Hz. Mean arterial blood pressure was calculated by digital averaging of blood pressure measurements. Since the value of corporal pressure achieved during cavernosal arterial vasodilation depends on the level of systemic arterial pressure, corporal pressure response is presented as the ratio of these two parameters (corporal pressure/mean arterial blood pressure; CP/BP). Corporal pressure response was the highest value measured during the first 10 min following administration of the drug. The corporal pressure responses to endothelins after L-NAME or indomethacin were compared to the responses following their respective vehicle.

2.5. Statistical analysis

Data are presented as means ± S.E.M. of the pressure measurements, or of the percent of baseline response.

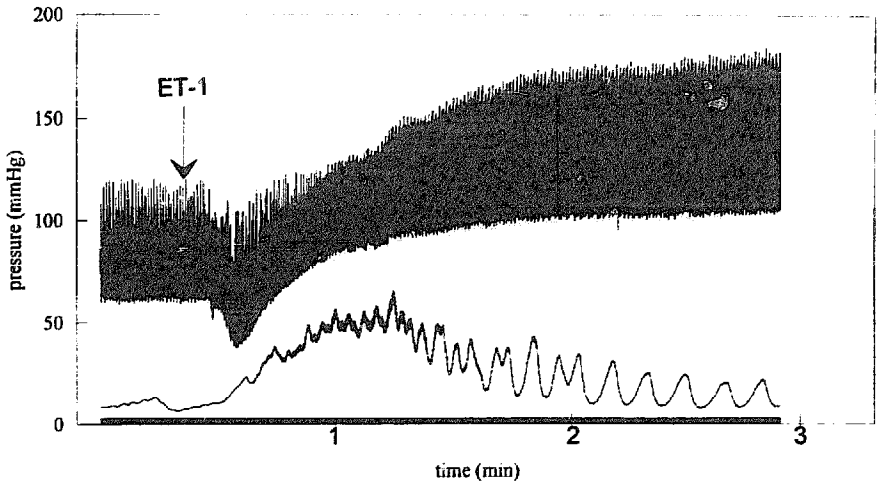

Fig. 1. Typical corporal (lower trace) and arterial blood pressure (upper trace) response of pithed rat to i.v. administration of endothelin-1 (3 μg/kg). Similar responses were obtained with endothelin-3.

Significance of difference between means of pressure responses was calculated by Student's *t* test.

2.6. Drugs

(+)-Tubocurarine was purchased from Taro, Israel; endothelins from Peninsula Laboratories, USA. N^G-Nitro-L-arginine methyl ester (L-NAME) and indomethacin were purchased from Sigma, Israel.

3. Results

3.1. Corporal pressure response to endothelins

Intravenous injection of endothelin-1 or endothelin-3 was followed by a transient systemic depressor response before the development of a prolonged pressor effect, and by a transient, dose-dependent increase in corporal pressure (Figs. 1 and 2). The rise in corporal pressure commenced during the depressor phase of systemic blood pressure, and so the CP/BP ratio was also increased. As blood pressure reached the maximal value, corporal pressure was returning to baseline values. In the decreasing pressure phase, oscillations in corporal pressure were seen (Fig. 1).

Endothelin-1 and endothelin-3 had equivalent potencies in elevation of CP/BP at low doses (0.1–3 μg/kg), but differed in the response obtained to the highest dose tested (10 μg/kg). Whereas in the case of endothelin-3, CP/BP ratio increased up to 10 μg/kg, with endothelin-1, CP/BP ratio increased at doses up to 3 μg/kg, but was lower at 10 μg/kg (Fig. 2).

3.2. Identity of vasodilator mediator

In these experiments a single submaximal dose of endothelin was administered twice, the first dose serving as a control. The depressor effect of endothelins on systemic blood pressure was not blocked by either indomethacin or L-NAME. L-NAME increased the pressor effect of endothelin-1 and endothelin-3, and indomethacin reduced the pressor component and increased the depressor component of the systemic blood pressure response to endothelin-3 (data not shown).

In control experiments, in which vehicle was injected between the two doses of endothelin, the response to the second dose was similar to the first. L-NAME significantly inhibited the increase in corporal pressure and CP/BP seen following submaximal doses of endothelin-1 or endothelin-3, although the corporal pressure response to endothelin-3 was inhibited to a greater extent than that to endothelin-1 (Fig. 3a). The corporal pressure response to endothelins was not affected by indomethacin (Fig. 3b).

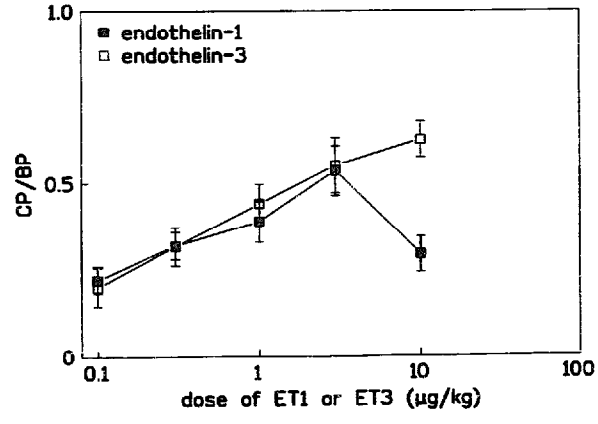

Fig. 2. Maximum increase in CP/BP ratio following endothelin-1 or endothelin-3 administration. Blood pressure and corporal pressure were allowed to return to control levels before the administration of the next dose. The minimal time between two successive doses was 1 h, no more than three doses were given to each rat, and rats which received 3 μg/kg did not receive 10 μg/kg. Each point is given as mean ± S.E.M. of 8–10 determinations.

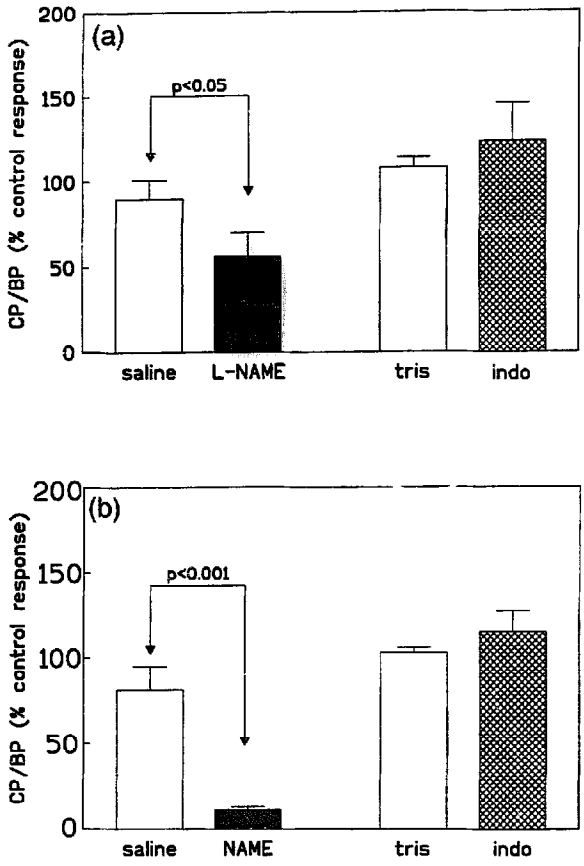

Fig. 3. Modification of CP/BP responses to endothelin-1 (*a*) or endothelin-3 (*b*) by L-NAME and indomethacin. The baseline CP/BP response was 0.513±0.028 to endothelin-1, and 0.589±0.051 to endothelin-3. L-NAME (20 mg/kg) or saline were given 30 min, and indomethacin (10 mg/kg) or tris buffer 75 min, after the first endothelin administration. Each bar shows response to second dose of endothelin as per cent of control response ± S.E.M. of 6–10 determinations.

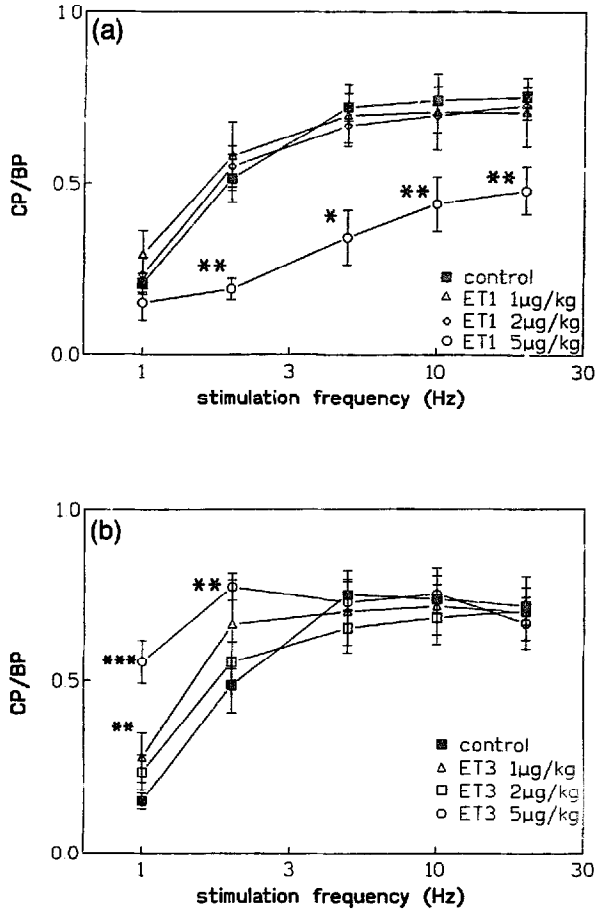

Fig. 4. Effect of i.v. administration of endothelin-1 (*a*) or endothelin-3 (*b*) on electrical stimulation-induced increase in CP. An initial set of responses to electrical stimulation (1–20 Hz, 50V, 1 ms duration for 1 min) was determined, and stimulation was repeated 15 min after endothelin-1 or endothelin-3 administration. Each point is mean ± S.E.M. of 4–6 determinations. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for difference from control values.

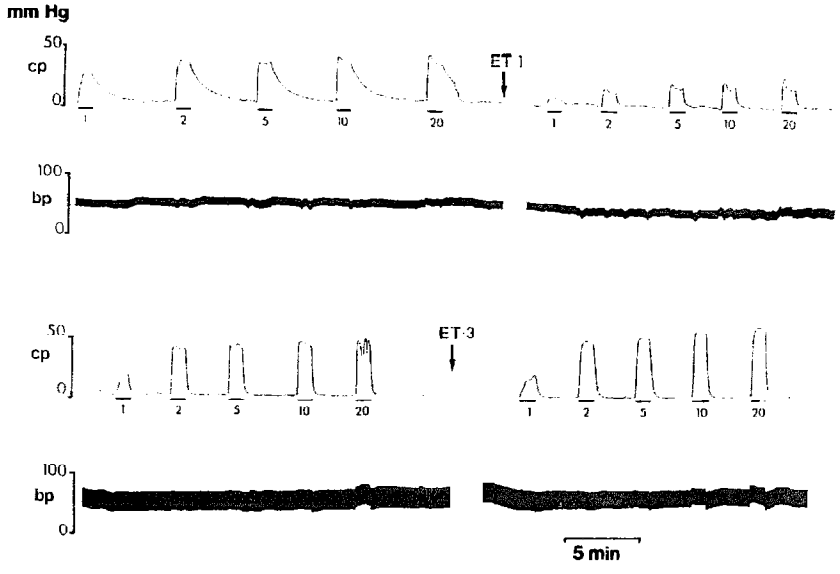

Fig. 5. Typical corporal pressure (CP) and blood pressure (BP) responses to electrical stimulation (1–20 Hz, 50 V, 1 ms duration for 1 min) of sacral part of spinal cord, before and 20 min after intracorporal infusion of endothelin-1 (upper record) or endothelin-3 (lower record). Peptides (1.67 µg/ml) were infused at 30 µl/min for 2 min. Similar results were obtained in four separate experiments with each peptide.

3.3. Responses to electrical nerve stimulation

The increase in CP/BP ratio caused by electrical stimulation of the pelvic nerves was not altered by i.v. administration of endothelin-1 (1 and 2 μg/kg), but was reduced at 5 μg/kg (Fig. 4a). On the other hand, the CP/BP response to pelvic nerve stimulation was not reduced following i.v. administration of any of the three doses of endothelin-3 (1, 2 and 5 μg/kg; Fig. 4b). At the low frequencies of stimulation (1 and 2 Hz) CP/BP response was even higher following i.v. administration of endothelin-3. This effect was dose dependent, and was more pronounced after 5 μg/kg than after 1 or 2 μg/kg of endothelin-3 (Fig. 4b).

Similarly, when endothelin-1 (1.67 μg/ml at 30 μl/min for 2 min, total dose 0.1 μg) was infused into the corpus cavernosum the CP/BP response to electrical nerve stimulation was inhibited. Intracorporal infusion of the same dose of endothelin-3 did not inhibit the CP/BP response to electrical nerve stimulation (Fig. 5).

4. Discussion

The biphasic hemodynamic response to endothelin in the rat, composed of an initial vasodilation followed by a potent and sustained vasoconstriction, has been described previously (De Nucci et al., 1988; Hoffman et al., 1989; Wright and Fozard, 1988; Yanagisawa et al., 1988). The nature of the response changes from predominantly depressor in anesthetised rats with high resting blood pressure to predominantly pressor in pithed rats. The vasodilation is thought to be mediated via release of nitric oxide and prostacyclin from endothelial cells in response to activation of endothelin ET_B receptors located on these cells (Sakurai et al., 1992; Haynes and Webb, 1993).

In the present study we found that i.v. administration of endothelin-1 or endothelin-3 causes a transient increase in corporal pressure. This response is indicative of a vasodilator effect of the endothelins in the helicine arteries of the corpora cavernosa (Vardi et al., 1989). Helicine artery vasodilation results in filling of the sinusoids (lacunae) with blood, and an increase in corporal pressure, which reflects sinusoidal pressure. In the presence of helicine artery vasodilation, a further increase in corporal pressure can result from contraction of the perineal striated muscles, principally the bulbospongiosus and ischiocavernosus muscles (McKenna et al., 1991), but contraction of these muscles alone does not produce an increase in corporal pressure (Giuliano et al., 1995). Since in our study, the rats were curarised, and since no suprasystolic pressure spikes were seen, the increase in corporal pressure following endothelin administration was almost certainly due to dilation of the helicine arteries. The similar potency of endothelin-1 and endothelin-3 to cause corporal vasodilation indicates the involvement of an endothelin ET_B receptor in mediation of this response, but further studies with selective antagonists will be required to prove this point.

The possibility that endothelins increased corporal pressure by constriction of emissary veins can largely be ruled out, because in the flaccid state, the helicine arteries are closed, and blood flow occurs through the capillary net rather than through the sinusoids (Banya et al., 1989). However, endothelins, as other vasoactive substances, could have an effect on venous resistance, by contraction of venous smooth muscle. Endothelins could also affect venous drainage from the corpora by contraction of the trabecular smooth muscle, which would be expected to decrease venous outflow resistance. Such a contractile effect on isolated corporal smooth muscle has in fact been observed (Holmquist et al., 1990, 1992).

We employed an inhibitor of NO synthase (L-NAME) and a cyclooxygenase inhibitor (indomethacin) to characterise the corporal vasodilator response. The corporal vasodilator effect of endothelin-3 was almost completely, while that of endothelin-1 was partially, blocked by L-NAME. The dose of L-NAME used could be submaximal which may explain the lack of complete blockade, although the dose used caused nearly maximal pressor effect in the rat (Rees et al., 1990). Alternatively, the fact that the increase in corporal pressure was similar with the doses of both peptides used, but the extent of blockade by L-NAME was different, could be indicative of a different mechanism of corporal vasodilation for the two peptides.

These results show that the corporal vasodilator response to endothelin is partially due to NO, but indicate the possible involvement of other vasorelaxant mediators. Indomethacin had no effect on corporal pressure responses to endothelin-1 and endothelin-3, so cyclooxygenase products are unlikely to be involved.

The ability of endothelin-1 to inhibit the corporal vasodilator response to nerve stimulation could be explained by either a corporal vasoconstrictor effect of endothelin-1, or by a presynaptic inhibitory effect, reducing release of the vasodilator neurotransmitter. The fact that this effect was seen after intracorporal, as well as intravenous, infusion of the endothelins indicates that it was a local effect in the corpora, and was not the result of vasoconstriction at an upstream site. Conclusive evidence for a presynaptic inhibitory effect of endothelins is lacking, but some observations concomitant with such an action have been made (Mutafova-Yambolieva and Westfall, 1995). The profound vasoconstrictor properties of the endothelins, together with the fact that inhibition of nerve stimulation-induced vasodilation was seen with endothelin-1 but not endothelin-3, point to the involvement of a corporal vasoconstrictor response mediated by endothelin ET_A receptors, but further experiments with selective antagonists will be required to confirm this.

The increased corporal pressure after endothelin-1 or endothelin-3 administration was transient, and corporal pressure rapidly returned to baseline values. When en-

dothelins were infused slowly i.v., the increase of corporal pressure and the systemic depressor response were not seen, although mean blood pressure increased (data not shown). Since the systemic depressor effect of endothelins is also transient, and since this transient behavior persists after endothelin-ET_A receptor blockade (Ihara et al., 1991), it seems that this is an intrinsic property of endothelin-ET_B activation after bolus injection.

In summary, the in vivo effect of endothelins shown in the present study, together with previously published data demonstrating specific endothelin binding, the existence of endothelin-like immunoreactivity (Saenz de Tejada et al., 1991; Holmquist et al., 1992), and endothelin mRNA expression in human and rabbit corpus cavernosum, implicates a physiological role for endothelin in penile vascular function. One possibility is that endothelins participate in the control of penile flaccidity, as can be derived from their vasoconstrictor and trabecular smooth muscle contractile effect. Another possibility arising from the present study is that endothelins, particularly endothelin-3, can participate in the initiation and/or maintenance of penile erection. The effect is dependent on the sites at which endothelins are released (arteries, veins, smooth muscle), on the isopeptide released (endothelin-1 or endothelin-3), and probably on the amount of endothelin released. The answers to these questions are important for further understanding the role of endothelins in the penile erectile process.

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References

Andersson, K.E. and F. Holmquist, 1990, Mechanisms for contraction and relaxation of human penile smooth muscles, Int. J. Impotence Res. 2, 209.

Banya Y., T. Ushiki, H. Takagane, H. Aoki, T. Kubo, T. Ohhori and C. Ide, 1989, Two circulatory route within the human corpus cavernosum penis: a scanning electron microscopic study of corrosion casts, J. Urol. 142, 879.

Burnett, A.L., C.J. Lowenstein, D.S. Bredt, T.S.K. Chang and S.H. Snyder, 1992, Nitric oxide: a physiologic mediator of penile erection, Science 257, 401.

De Nucci, G., R. Thomas, P. d'Orleans-Juste, E. Antunes, C. Walder, T.D. Warner and J.R. Vane, 1988, Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor, Proc. Natl. Acad. Sci. USA 85, 9797.

Giuliano, F., O. Rampin, J. Bernabe and J-P. Rousseau, 1995, Neural control of penile erection in the rat, J. Auton. Nerv. Syst. 55, 36.

Haynes, W.G. and D.J. Webb, 1993, The endothelin family of peptides. local hormones with diverse roles in health and disease?, Clin. Sci. 84, 485.

Hoffman, A., E. Grossman, K.P. Ohman, E. Marks and H.R. Keiser, 1989, Endothelin induced an initial increase in cardiac output associated with selective vasodilatation in rats, Life Sci. 45, 249.

Holmquist, F., K.E. Andersson and H. Hedlund, 1990, Actions of endothelin on isolated corpus cavernosum from rabbit and man, Acta. Physiol. Scand. 139, 113.

Holmquist, F., H.J. Kirkeby, B. Larsson, A. Forman, P. Alm and K.-E. Andersson, 1992, Functional effects, binding sites and immunolocalization of endothelin-1 in isolated penile tissues from man and rabbit, J. Pharmacol. Exp. Ther. 261, 795.

Ihara, M., K. Noguchi, T. Saeki, T. Fukuroda, S. Tsuchidaa, S. Kimura, T. Fukami, K. Ishikawa, M. Nishikibe and M. Yano, 1991, Biological profiles of highly potent novel endothelin antagonists selective for the ETA, Life Sci. 50, 247.

Inoue, A., M. Yanagisawa, S. Kimura, Y. Kasuya, T. Miyauchi, K. Goto and T. Masaki, 1989, The human endothelin family: Three structural and pharmacologically distinct isopeptides predicted by three separate genes, Proc. Natl. Acad. Sci. USA 86, 2863.

Lin, H.Y., E.H. Kaji, G.K. Winkel, H.E. Ives and H.F. Lodish, 1991, Cloning and functional expression of a vascular smooth muscle endothelin-1 receptor, Proc. Natl. Acad. Sci. USA 88, 3185.

Lue, T.F. and E.A. Tanagho, 1987, Physiology of erection and pharmacological management of impotence, J. Urol. 137, 829.

McKenna, K.E., S.K. Chung, and K.T. McVary, 1991, A model for the study of sexual function in anesthetized male and female rats, Am. J. Physiol. 261, R1276.

Mutafova-Yambolieva, V.N. and D.P. Westfall, 1995, Endothelin-3 can both facilitate and inhibit transmitter release in the guinea-pig vas deferens, Eur. J. Pharmacol. 285, 213.

Rees, D.D., R.M.J. Palmer, R. Schulz, H.F. Hodson and S. Moncada, 1990, Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo, Br. J. Pharmacol. 101, 746.

Saenz de Tejada, I., M.P. Carson, A. de las Morenas, I. Goldstein and A.M. Traish, 1991, Endothelin: localization, synthesis, activity, and receptor types in human penile corpus cavernosum, Am. J. Physiol. 261, H1078.

Sakamoto, A., M. Yanagisawa, T. Sakurai, Y. Takuwa, H. Yanagisawa and T. Masaki, 1991, Cloning and functional expression of human cDNA for the ETB endothelin receptor, Biochem. Biophys. Res. Commun. 178, 656.

Sakurai, T., M. Yanagisawa and T. Masaki, 1992, Molecular characterization of endothelin receptors, Trends Pharmacol. Sci. 13, 103.

Vardi, Y., M.B.H. Youdim, R.D. Levin and J.P.M. Finberg, 1989, A model for the study of penile erection in the rat, Int. J. Impotence Res. 1, 11.

Wright, C.E. and J.R. Fozard, 1988, Regional vasodilation is a prominent feature of the haemodynamic response to endothelin in anaesthetized, spontaneously hypertensive rats, Eur. J. Pharmacol. 155, 201.

Yanagisawa, M., H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitsui, Y. Yazaki, K. Goto and T. Masaki, 1988, A novel potent vasoconstrictor peptide produced by vascular endothelial cells, Nature 332, 411.