Inhibition of nerve stimulation-induced vasodilatation in corpora cavernosa of the pithed rat by blockade of nitric oxide synthase

¹John P.M. Finberg, Shulamit Levy & *Yoram Vardi

Department of Pharmacology, Bruce Rappaport Faculty of Medicine, Technion, POB 9649, Haifa, Israel and *Department of Urology, Rambam Medical Centre, Haifa, Israel

1 The effect of inhibition of nitric oxide synthase by N^G-nitro-L-arginine methyl ester (L-NAME) on nerve stimulation-induced vasodilatation in corpora cavernosa was studied in the pithed rat. Corporal vasodilatation was estimated by the increase in ratio (corpora cavernosal pressure/systemic blood pressure; CP/BP) following electrical stimulation of the sacral part of the spinal cord.

2 L-NAME (2, 5, 10 and 25 mg kg⁻¹) caused an increase in BP and a dose-dependent inhibition of the rise in the CP/BP ratio following stimulation.

3 The inhibitory effect of L-NAME (25 mg kg⁻¹) on the corporal response to spinal cord stimulation, as well as the pressor response, was partially prevented by prior administration of L- but not D-arginine (400 mg kg⁻¹, i.v.).

4 L-NAME (20 mg kg⁻¹, i.v.) did not inhibit the rise in corporal pressure resulting from direct intracavernosal administration of papaverine (400 µg over 2 min). However, this response was inhibited by 5-hydroxytryptamine (20 µg kg⁻¹, i.v.).

5 The results are indicative of a role of nitric oxide (NO) in the corporal vasodilator response to erectile stimulation.

Keywords: Penile erection; nitric oxide; papaverine; 5-hydroxytryptamine; corpora cavernosa; N^G-nitro-L-arginine; pithed rat

Introduction

Penile erection is initiated by neuronal impulses in parasympathetic pelvic nerves which cause arteriolar vasodilatation and relaxation of smooth muscle elements in the corpora cavernosa, the erectile tissue of the penis. Inflow of blood in the corporal lacunae, together with relaxation of the smooth muscle surrounding the lacunae, and venous outflow obstruction (Lue & Tanagho, 1987; Aoki *et al.*, 1989), leads to turgor of the erectile tissue. Although the corpora are extensively innervated with cholinergic nerves (Dail & Hamill, 1989), acetylcholine is not thought to play a major role as a vasodilator neurotransmitter, because atropine-like drugs (which effectively antagonize other vasodilator effects of acetylcholine), have little effect on the erectile response (Brindley, 1986; Stief *et al.*, 1989). Peptide neurotransmitters, especially vasoactive intestinal peptide (VIP; Willis *et al.*, 1981) have also been proposed to play a role in the arteriolar vasodilatation but there is marked tachyphylaxis to the vasodilator effect of VIP and the peptide causes a relatively poor vasodilatation even after direct administration to the corpora (Roy *et al.*, 1990).

Nitric oxide (NO) is a vasodilator and smooth-muscle relaxant substance which is produced in a number of vascular and non-vascular tissues by the enzyme nitric oxide synthase (see Moncada *et al.*, 1991, for review). It has been proposed that release of NO mediates the smooth muscle relaxant effect of non-adrenergic, non-cholinergic (NANC) nerve stimulation in tissues such as human corpora cavernosa (Ignarro *et al.*, 1990; Kim *et al.*, 1991; Pickard *et al.*, 1991), rat anococcygeus muscle, bovine penile artery and bovine retractor penis (Liu *et al.*, 1991). Although evidence exists for a role of NO in mediating the NANC relaxation of isolated muscle strips of corporal tissue, it is still not clear whether NO is responsible for the vasodilator phase of penile erection.

We have studied the effect of the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; Moore *et al.*, 1990) on erectile stimulation in our *in vivo* model of erectile function in the pithed rat (Vardi *et al.*, 1989). In this preparation, electrical stimulation of the sacral part of the spinal cord causes a rise in corporal pressure, which is the result of active vasodilatation in the corporal arterioles. The response is blocked by chlorisondamine but not by atropine. In this preparation, our preliminary data showed that L-NAME antagonized the corporal response to spinal stimulation (Finberg & Vardi, 1991). Holmquist *et al.* (1991) reported that intra-corporal L-NAME antagonized the penile erection and increase in corporal pressure caused by direct stimulation of the cavernous nerve in the anaesthetized rabbit. Recently, Burnett *et al.* (1992) reported inhibition of penile erectile response to direct stimulation of the cavernous nerve by L-NAME in the anaesthetized rat, as well as describing the location of NO synthase in penile tissue. Since L-NAME causes powerful peripheral vasoconstriction, we have examined the possibility that inhibition of erectile stimulation by L-NAME results from generalized vasoconstriction, as opposed to a specific antagonism of neuronally released NO.

Methods

Pithed rat preparation

Rats (Sprague-Dawley derived, 250–280 g body weight) were pithed under halothane anaesthesia, respired with oxygen-enriched room air at a rate of 1 ml kg⁻¹, 60 strokes min⁻¹, and prepared for preganglionic stimulation of the spinal outflow to the pelvic nerves as described previously (Vardi *et al.*, 1989). Rectal temperature was maintained at 37°C with a small electric pad underneath the animal, and (+)-tubocurarine (1 mg kg⁻¹) was administered i.v. to prevent contraction of skeletal muscle during stimulation. The

¹ Author for correspondence.

stimulating electrode (diameter 1.5 mm) was positioned in the sacral part of the spinal cord, and its optimal location determined by monitoring corporal response to test stimuli. The indifferent electrode was inserted underneath the dorsal skin. Corporal pressure was measured from a 26 gauge needle inserted into one corpus cavernosum, after removing the penile skin. Drugs were administered by intravenous injection via the jugular vein, and systemic arterial pressure was recorded from a carotid artery. Pressures were measured with Statham P23dB transducers, and monitored on a Graphtec direct-writing recorder. Since the hydrostatic pressure measured in the corpora (CP) during a vasodilator response is influenced by the level of the systemic arterial blood pressure (BP), corporal pressure was expressed as a function of mean blood pressure (CP/BP ratio).

Experimental protocols

A 45 min equilibration period was allowed after pithing, and then spinal stimulation was applied for 60 s periods at 2, 5, 10 and 20 Hz, with square-wave uniphasic pulses (1 ms) at maximal voltage. Four min rest periods were allowed between individual stimuli. Following establishment of at least two control responses to stimulation, L-NAME was administered at one of the indicated dose levels. Fifteen min later, application of spinal stimulation was recommenced, and frequency-response curves were repeated, with intervals of 15 min between each set of stimuli. When L- or D-arginine was administered, the amino acid was given slowly by intravenous injection in two divided doses, with 2 min between each dose, 10 min before the second set of control electrical stimuli. Either L-NAME or an equivalent volume of diluent was administered i.v., and determination of frequency-response curves recommenced 15 min later.

In another series of experiments, vasodilator responses to papaverine were obtained by infusing this drug into one corpus cavernosum ($20 \mu\text{l min}^{-1}$ of a 10 mg ml^{-1} solution for 2 min) and measuring pressure from the contralateral corpus. Control CP values were measured 2 min after the papaverine infusion, and again 60 min later. L-NAME or physiological saline was then administered as above, and a second intra-corporal papaverine bolus was infused 30 min later. Corporal pressure was again recorded 2 min after the papaverine

bolus. Spinal stimulation was carried out before the first papaverine bolus, and again after administration of L-NAME or saline. In an additional series of experiments, the effect of 5-HT was examined on papaverine-induced corporal vasodilatation. In these experiments, 5-HT was injected i.v. 5 min after the papaverine bolus; at this time, in control experiments, CP was not significantly different from the value measured 2 min after the bolus.

Values of CP/BP ratio following administration of L-NAME or arginine are presented as a percentage of control values recorded at the start of the experiment, or, in the papaverine experiments, as a ratio. Corporal pressures used for calculation of CP/BP ratio during electrical stimulation were the average values determined between 30 and 60 s after the onset of electrical stimulation.

Statistical method

Significance of difference between group means was estimated by Student's *t* test. A probability of less than 0.05 was considered significant.

Drugs

N^G -nitro-L-arginine methyl ester (L-NAME), L-arginine, D-arginine and 5-HT creatinine sulphate were obtained from Sigma (St Louis, MO, U.S.A.). L-NAME, L-arginine and D-arginine were dissolved in saline. 5-HT was dissolved in 0.01 M HCl, and diluted in saline immediately before use. Papaverine HCl was obtained from Teva Inc. (Petah Tikva, Israel). All other reagents were Analar grade.

Results

Intravenous injection of L-NAME caused an increase in BP, together with a suppression of the CP response to spinal stimulation (i.e. a decrease in CP/BP ratio, see Figures 1 and 2). The increase in BP reached maximum after about 5 to 10 min, while maximal suppression of CP/BP response to stimulation was evident about 30 min after L-NAME injection. Corporal responses were suppressed for the duration of the experiment (up to 3 h), and did not show any indication of a return over this time period. Responses to the lower frequency stimulation used (2, 5 and 10 Hz) were suppressed to a greater degree than those to the highest frequency (20 Hz). Previous work with this model has shown that there is no significant decline of responses to spinal stimulation over a 1 to 2 h period in control animals (Finberg & Vardi, 1990). Prior injection of L-arginine (400 mg kg^{-1}), which itself had no significant effect on corporal pressure response to spinal stimulation, partially prevented the suppression of the corporal response and the increase in BP caused by L-NAME (Figures 3 and 4). The same dose of D-arginine, however, had no effect on the L-NAME response (see Figure 3).

In order to determine whether inhibition of the corporal response to stimulation was a result of vasoconstriction in the arterial vessels supplying the corpora cavernosa, another vasodilator, papaverine, was infused into the corpus cavernosum of the rat during treatment with L-NAME. Intra-corporal infusion of a pulse of papaverine ($20 \mu\text{l min}^{-1}$ of a 10 mg ml^{-1} solution for 2 min) produced an increase in corporal pressure with no change in arterial pressure. The corporal pressure remained at a stable level for about 15 min after stopping papaverine infusion, before dropping gradually towards control levels over a period of about 20 to 40 min. Following administration of L-NAME (20 mg kg^{-1} , i.v.), intra-corporal infusion of papaverine now produced a greater rise in corporal pressure as a result of the increase in BP, but the ratio CP/BP was equivalent before and after administration of L-NAME (Table 1). In addition, there was no significant difference in the mean duration of papaverine-

Figure 1 Inhibition of spinal stimulation-induced increase in corporal pressure following i.v. injection of N^G -nitro-L-arginine methyl ester (L-NAME) in the pithed rat. Corporal pressure (CP) during stimulation at frequencies shown was divided by simultaneous mean arterial pressure (BP) and expressed as percentage of control response measured before drug injection. Doses of L-NAME administered were 2 (○), 5 (●), 10 (■) and 25 (▲) mg kg^{-1} , i.v. Responses are shown as mean $\pm$ s.e.mean for $n = 4$ to 5 animals in each treatment group.

Figure 2 Experimental records of arterial blood pressure (BP) and corporal pressure (CP) in two separate pithed rat experiments. At horizontal bars, electrical stimulation was applied to sacral spinal cord at frequency shown in Hz. Pressure scales are given as mmHg. (a) Right hand portion of record 15 and 30 min after administration of N^G -nitro-L-arginine methyl ester (L-NAME; 25 mg kg^{-1} , i.v.). (b) After left hand part of record, L-arginine (400 mg kg^{-1} , i.v.) was administered, followed, 10 min later, by L-NAME (25 mg kg^{-1} , i.v.). Right hand portion of record was made 15 min after L-NAME.

Figure 3 Prevention of N^G -nitro-L-arginine methyl ester (L-NAME)-induced suppression of corporal response to spinal stimulation in the pithed rat by L-arginine. Graph shows inhibition of stimulation-induced increase in CP/BP ratio by L-NAME (25 mg kg^{-1}) alone (■), after prior injection of L-arginine (400 mg kg^{-1} , i.v.; ●) or after prior injection of D-arginine (400 mg kg^{-1} , i.v.; ▲). Also shown is effect of L-arginine alone on corporal response to spinal stimulation (□). Responses are shown as mean $\pm$ s.e.mean for numbers of animals shown in parentheses. * $P < 0.05$, ** $P < 0.01$ for significance of difference between L-arginine + L-NAME and L-NAME alone. Numbers of animals per group shown in parentheses.

Figure 4 Mean arterial blood pressure (BP) response to N^G -nitro-L-arginine methyl ester (L-NAME) in pithed rat. Mean BP was measured at indicated times before and after injection of either L-NAME 25 mg kg^{-1} (■—■), L-NAME 25 mg kg^{-1} after prior injection of L-arginine (400 mg kg^{-1} at -30 min ; ●—●), or saline after prior injection of L-arginine (400 mg kg^{-1} at -30 min ; ▲—▲). * $P < 0.05$ for significance of difference between L-NAME alone and L-NAME + L-arginine. Responses are shown as mean $\pm$ s.e.mean for numbers of animals shown in parentheses.

induced increase in CP following L-NAME (18 ± 3.5 min in control to 24.8 ± 4.4 min following L-NAME; mean $\pm$ s.e.mean for 6 experiments). Prior administration of papaverine did not alter the corporal response to spinal stimulation, given after return of CP to control levels, and prior administration of papaverine did not affect the inhibitory action of L-NAME on the increase in CP/BP induced by nerve stimulation (Table 1). The papaverine pulse produced a sub-maximal degree of vasodilatation, since an increased duration of the intra-corporal infusion of papaverine produced a greater increase in the ratio CP/BP.

In order to compare the effect of another vasoconstrictor substance with that of L-NAME, 5-HT was injected intravenously in pithed rats which had just received an intra-corporal infusion of papaverine. In these conditions, 5-HT ($20 \mu\text{g kg}^{-1}$, i.v.) caused a marked suppression of the CP/BP ratio (Figure 5).

Table 1 Corporal pressure responses to spinal stimulation and intra-corporal papaverine following systemic administration of N^G-nitro-L-arginine methyl ester (L-NAME) in the pithed rat

	Stimulation frequency (Hz)				Papaverine (400 μg)
	2	5	10	20	
(1) Control series ($n = 5$)					
Control	0.57	0.73	0.72	0.73	0.64
s.e.mean	0.09	0.06	0.06	0.06	0.07
Saline	0.49	0.71	0.76	0.82	0.57
s.e.mean	0.13	0.04	0.04	0.04	0.01
(2) L-NAME series ($n = 6$)					
Control	0.40	0.53	0.56	0.59	0.55
s.e.mean	0.07	0.06	0.05	0.04	0.07
L-NAME	0.00*	0.10*	0.22*	0.30*	0.61
s.e.mean	0.00	0.09	0.09	0.08	0.08

CP/BP (cavernosal:arterial blood pressure) ratios are shown during spinal stimulation at 2, 5, 10 and 20 Hz, and 2 min after start of the infusion of papaverine intra-corporally ($200 \mu\text{g min}^{-1}$ for 2 min). An initial set of stimuli was followed by papaverine infusion. Either saline or L-NAME (20 mg kg^{-1}) was then administered i.v., and a second set of stimuli carried out 30 min later. The second papaverine infusion was administered following completion of the second set of stimuli.

* $P < 0.01$ for difference between first and second set of stimuli (t test).

Figure 5 Effect of 5-hydroxytryptamine (5-HT) on papaverine-induced elevation in CP/BP ratio. Values of CP/BP ratio shown in control period (C), 2 min after intracorporal papaverine ($200 \mu\text{g min}^{-1}$ for 2 min; P1), and 5 min after 5-HT ($20 \mu\text{g kg}^{-1}$, i.v.) or normal saline injected immediately after completion of papaverine infusion (P2). Control animals (open columns); 5-HT (hatched columns). * indicates significant difference ($P < 0.05$) between P1 and P2; $n = 6$ animals per treatment group.

Discussion

The results of these experiments are consistent with the hypothesis that stimulation of the pelvic nerve induces the release of NO, which causes corporal vasodilatation. The effect of L-NAME in inhibiting neuronally-mediated corporal vasodilatation is presumably the result of inhibition of NO production. The irreversible nature of the L-NAME-induced blockade of corporal vasodilatation is compatible with the characteristics of blockade of NO-synthase observed by other workers (Mulsch & Busse, 1990). The inhibition of CP/BP response to pelvic nerve stimulation by L-NAME could be prevented by pretreatment with a large dose of L-arginine. In preliminary experiments, we found that the 400 mg kg^{-1} dose of L-arginine was required in order to prevent effectively the inhibitory effect of L-NAME. The stereo-selective reversal of the effect of L-NAME by L-arginine is strongly indicative of a selective action of L-NAME inhibiting NO generation. Reversal of the inhibitory action of L-NAME on corporal vasodilator response to erectile stimulation by L-arginine but not by D-arginine was also demonstrated by Burnett *et al.* (1992) but not by Holmquist *et al.* (1991). In the latter study, L-NAME was administered intra-corporally, and the ability to demonstrate reversal by L-arginine could have resulted from insufficient concentration of L-arginine, or unfavourable timing of the L-arginine administration.

The inhibitory effect of L-NAME on cavernosal vasodilatation could be the result of inhibition of NO released as a NANC neurotransmitter from pelvic nerve endings, or a result of a direct vasoconstrictor action of L-NAME in the cavernosal arterioles. Administration of L-NAME caused extensive systemic vasoconstriction, as shown by the large pressor response. The pressor effect of L-NAME has been well described in conscious rats (Gardiner *et al.*, 1990); however, it is interesting that even in the pithed rat preparation, which is devoid of neural tone, we still find a marked pressor response. This effect is evidence of the degree of basal NO release. Such a profound vasoconstrictor effect of L-NAME in cavernosal vessels, therefore, would antagonize the effect of any vasodilator substance released from the parasympathetic nerves. In an attempt to distinguish between such a physiological antagonism at the post-synaptic level, and blockade of neuronally-released NO, we also examined the ability of L-NAME to antagonize the effect of a direct vasodilator, papaverine. It is interesting that L-NAME did not antagonize the papaverine-induced vasodilatation, while 5-HT did. The lack of antagonism of papaverine-induced vasodilatation by L-NAME provides evidence that the inhibition of stimulation-induced vasodilatation by L-NAME did not result from generalized vasoconstriction at a site central to the origin of the corporal blood vessels.

This finding adds credence to the hypothesis that production of NO is important for neuronally induced corporal vasodilatation. Further evidence that NO plays a neurotransmitter role in corporal vasodilatation is provided by the demonstration by Burnett *et al.* (1992) that nitric oxide synthase is localized to neuronal cells in the corpora cavernosa. It is, however, impossible to rule out completely the possibility that physiological antagonism between vasoconstrictor and vasodilator influences plays a role in the inhibitory effect of L-NAME seen in the present study. The NO generated by pelvic nerve stimulation could be released from the nerve endings themselves, and, in addition, could be released from the corporal endothelial cells in response to a neuromodulator released from the nerves. Localization of nitric oxide synthase to endothelial layer of cavernosal vessels was also demonstrated by Burnett *et al.* (1992). The identity of such a neuromediator is at present unknown, but it is unlikely to be acetylcholine, since atropine is largely ineffective in blocking penile erection in this (Vardi *et al.*, 1989) and other models of penile erection (Brindley, 1986; Stief *et al.*, 1989).

It is interesting to compare the effect of L-NAME with

that of 5-HT. The latter has previously been shown by us to inhibit the effect of spinal stimulation on CP/BP elevation in the pithed rat preparation (Finberg & Vardi, 1990). Again, this inhibitory effect of 5-HT could be the result of a direct post-synaptic vasoconstriction of the cavernosal arterioles, or pre-synaptic inhibition of released NANC neurotransmitter. The fact that 5-HT antagonized papaverine-induced vasodilatation, is evidence in favour of the former possibility.

The pithed rat model used in these experiments is devoid of CNS mediated peripheral nervous tone. It is interesting, therefore, that corporal arterioles are maintained in a state of vasoconstriction. This vasoconstriction may be the result of local production of vasoactive substances such as endothelin in the endothelial cells of the corporal vasculature. 5-HT apparently does not play a role in this tonic vasoconstriction, since 5-HT antagonists did not produce an increase in the CP/BP ratio (Finberg & Vardi, 1990).

The relatively greater effectiveness of L-NAME in antagonizing low frequency stimulation may indicate the presence of an additional vasodilator neurotransmitter, which is

released to a greater extent at the higher frequency. Alternatively, since we used a fixed duration of stimulation, the apparent resistance of 20 Hz stimulation to L-NAME may simply be the result of release of a greater amount of neurotransmitter, by a greater number of pulses. Candidate substances for alternative neurotransmitters have been mentioned above. Cholinergic and peptidergic (VIP) nerves have been localized in the corpora by immunohistochemical techniques (Dail & Hamill, 1989; Willis *et al.*, 1981), but the role of these neurotransmitters in control of trabecular, as opposed to vascular, responsiveness in the corpora is at present unknown.

Our findings are therefore indicative of a physiological role for NO as a NANC neurotransmitter in erectile function.

We wish to thank the Israel Ministry of Health, and Technion, Vice-President for Research Fund, for financial support. We also wish to thank Prof. T. Bennett for helpful criticism which prompted the work with papaverine.

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(Received July 6, 1992
Revised December 1, 1992
Accepted December 3, 1992)