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Effects of the nitric oxide synthase inhibitor N^G-nitro-L-arginine on the erectile response to cavernous nerve stimulation in the rabbit

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HOLMQUIST, F., STIEF, C. G., JONAS, U. & ANDERSSON, K.-E. 1991. Effects of the nitric oxide synthase inhibitor N^G-nitro-L-arginine on the erectile response to cavernous nerve stimulation in the rabbit. *Acta Physiol Scand* **143**, 299–304. Received 6 May 1991, accepted 8 July 1991. ISSN 0001–6772, Department of Clinical Pharmacology, Lund University Hospital, Lund, Sweden and Department of Urology, School of Medicine, Hannover, Germany.

Using a rabbit model, the involvement of the L-arginine/nitric oxide pathway in penile erection was investigated. The mean basal intracavernous pressure was 21 cm H_2O. Cavernous nerve stimulation (4–8 V, 20–30 Hz) increased the pressure to approximately 130 cm H_2O. This response was highly reproducible and usually associated with full penile erection. The pressure increase could be quantified in terms of: (1) the slope of the initial, ascending part of the pressure increase; (2) ΔP, which was defined as the maximal pressure obtained by the stimulation minus the basal pressure before the stimulation; (3) T_{90}, which was defined as the time to reach 90 per cent of ΔP. Intrapenile administration of the L-arginine/nitric oxide synthesis inhibitor N^G-nitro-L-arginine had no effect on systemic arterial blood pressure. However, N^G-nitro-L-arginine (0.22 and 2.19 mg), administered via the same route, abolished the erectile response induced by cavernous nerve stimulation; T_{90} increased and slope and ΔP decreased significantly. N^G-nitro-D-arginine (2.19), on the other hand, had no inhibitory effect. L-arginine (21.07 mg), given either directly or after N^G-nitro-L-arginine had no consistent effect on the functional response to cavernous nerve stimulation.

The results suggest that pharmacologically induced effects on intracavernous pressure in the rabbit can be described quantitatively, and that this model may be useful to study the mechanisms controlling penile erection *in vivo*. The pronounced inhibitory action of N^G-nitro-L-arginine demonstrates the important role of the arginine/nitric oxide pathway in mediating relaxation of penile smooth muscles necessary for erection.

Key words: nitric oxide, penile erection, rabbit.

For erection to be induced, the penile arteries and sinusoids have to dilate, thereby decreasing the resistance to penile blood flow (Andersson & Holmquist, 1990). However, the mechanism of penile smooth muscle relaxation has not been fully elucidated. Nitric oxide (NO), which is believed to account for the biological actions of endothelium-derived relaxing factor (for review; Ignarro 1990, Marin & Sánchez-Ferrer 1990), was recently suggested to be of importance in the regulation of penile smooth muscle tone, both in the flaccid state (Holmquist *et al.* 1991b) and during erection (Ignarro *et al.* 1990, Holmquist *et al.* 1991a, b). This was based on experiments utilizing isolated preparations of human and rabbit corpus cavernosum. For instance, in both human (Holmquist *et al.* 1991a, b) and rabbit (Ignarro *et al.* 1990, Holmquist *et al.* 1991b)

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preparations, N^G-nitro-L-arginine (L-NOARG), an inhibitor of the synthesis of NO from L-arginine (Moore *et al.* 1989, Mülsch & Busse, 1990), almost abolished the relaxations elicited by electrical field stimulation. Furthermore, L-NOARG produced a tension-increase when given to preparations contracted by noradrenaline (Holmquist *et al.* 1991b). However, to the best of our knowledge, the possible involvement of the L-arginine/NO pathway in the control of penile blood flow has never been investigated *in vivo*.

It has previously been shown that the rabbit is a useful model for the study of erectile mechanisms in the intact animal (Sjöstrand & Klinge 1979, Stief *et al.* 1990). Using the experimental set-up previously described (Stief *et al.* 1990), we wanted to investigate the effect of NO synthase inhibition on the erectile response induced by electrical stimulation of the cavernous nerve in the rabbit.

MATERIALS AND METHODS

Animals. Eleven rabbits (New Zealand White) weighing 4–5 kg were used for the investigation. After sedation with i.m. ketamine (10 mg), the animals were anaesthetized with i.v. pentobarbital (15 mg kg^{-1}) through a 21-gauge needle introduced into an ear vein. Anaesthesia was maintained with 3 mg kg^{-1} i.v. bolus injections of pentobarbital as needed. During the course of the experiment, the rabbits also received warm saline (2–3 ml kg^{-1} h^{-1}) and 10% glucose in saline (0.5 ml kg^{-1} h^{-1}) i.v. The animals breathed spontaneously.

The rabbits were placed in a supine position on a thermoregulated operating table (model 11A, Hugo Sachs Elektronik, Germany). Additional heat was provided with a heating lamp. The abdomen was opened by a midline incision, and the bladder was emptied. The rectum was tied, and the intestines were put in a pad soaked with saline and placed in the upper abdomen. By gentle dissection, the cavernous nerves were exposed in the dorso-lateral aspect of the prostate on both sides.

The penile skin was removed by blunt dissection and a 21-gauge needle was inserted into the left corpus cavernosum for pressure recording. The needle was connected to a fluid line via a threeway stopcock, which allowed for intracavernosal application of drugs. To prevent clotting, 50 I.U. heparin was given through this route every 2–3 h. This dose of heparin is well below the doses needed to induce changes in penile haemodynamics (Kirkeby *et al.* 1990). In some experiments, arterial blood pressure was recorded from one of the femoral arteries. Pressure was measured using Statham transducers (model P23XL) connected via DC Bridge Amplifiers Type 660 to a Watanabe Mark VII recorder (Hugo Sachs Elekronik, Germany).

Experimental procedure. The cavernous nerve on one side was stimulated electrically using a movable contact electrode. Square wave pulses were delivered by a Stimulator IZ (Hugo Sachs Elektronik, Germany). Upon stimulation, the intracavernous pressure increased rapidly, and the penis usually became tumescent or erect. The stimulation was continued for 60 s or until a maximal, stable intracavernous pressure had been obtained. The increase in intracavernous pressure during cavernous nerve stimulation was described in terms of: (1) the slope of the initial, ascending part of the pressure increase; (2) ΔP, which was defined as the maximal pressure obtained by the stimulation minus the basal pressure before the stimulation; and (3) T_{90}, which was defined as the time to reach 90% of ΔP (Fig. 1). After stimulation and the pressure had returned to baseline, 1 ml saline was given intracavernosally in order to flush drugs away and to avoid clotting. The time interval between stimulations was approximately 15 min.

In every animal, different stimulation parameters were investigated in a randomized manner to obtain the optimal functional response. This response was reproducible for several hours and used as control. To study the effects of a drug on the functional response to cavernous nerve stimulation, the aorta and v. cava were clamped (30 s) while the drug (dissolved in 1 ml saline) was applied intracavernously through the 21-gauge needle. An incubation-time of at least 10 min was then allowed until the next stimulation was conducted. This incubation time was chosen on the basis of previous studies showing that the maximal effect on arterial blood pressure after intravenous administration of different NO synthase inhibitors is obtained within 5–10 min (Rees *et al.* 1989, 1990, Persson *et al.* 1990). The lowest concentration of a drug was always given first.

Drugs. The following drugs were used: Ketamine (Parke-Davis, USA), pentobarbital (WDT, Germany), heparin (Roche, Switzerland), D-NOARG (Bachem, Switzerland), L-NOARG and L-arginine hydrochloride (Sigma, USA). When appropriate, the drugs were dissolved in saline and stored at −70 °C.

Calculations. When appropriate, results are given as mean values ± standard error of the mean (SEM) or 99% confidence intervals (as specified). Statistical determination of the effect of a drug on penile erection was performed by using the confidence intervals of the quotients of the slope, ΔP and T_{90} before and after application of the drug. Since the actions of all the drugs were compared with the same control response,

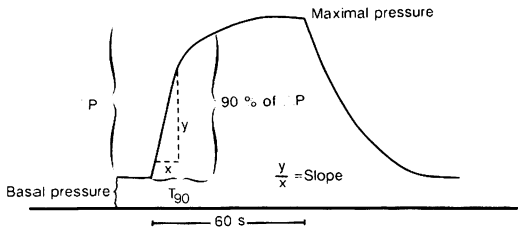

Fig. 1. Schematic drawing showing the increase in intracavernous pressure induced by unilateral cavernous nerve stimulation in the rabbit. The increase in pressure was described in terms of: (1) the slope of the initial, ascending part of the pressure increase (y/x); (2) ΔP, which was defined as the maximal pressure obtained by the stimulation minus the basal pressure before the stimulation; and (3) T_{90}, which was defined as the time to reach 90 per cent of ΔP.

99% confidence intervals were chosen. Student's two-tailed *t*-test was used to evaluate the drug-effects on basal intracavernous pressure. A probability level < 0.05 was regarded as significant.

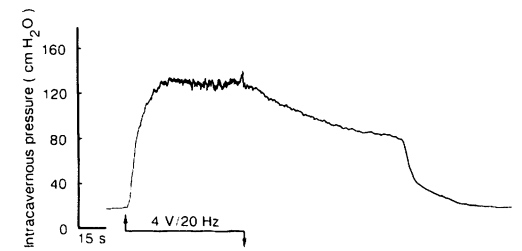

Fig. 2. Tracing showing the increase in intracavernous pressure induced by unilateral cavernous nerve stimulation (4 V, 20 Hz) in the rabbit. In this case, the pressure did not fall immediately after the stimulation was stopped, but declined gradually until it suddenly returned to baseline.

RESULTS

The baseline intracavernous pressure recorded on 64 different occasions was 21 ± 1 cm H_2O. Stimulation of the cavernous nerve induced a rapid intracavernous pressure increase, usually associated with tumescence or full penile erection. In half of the animals used, the intracavernous pressure did not fall directly after cessation of the stimulation, but declined gradually until it suddenly dropped to baseline (Fig. 2). The slope, ΔP and T_{90} were dependent on the voltage and frequency of stimulation (Table 1), whereas the pulse width (0.5–2.0 ms) seemed to be of less importance (1 ms was chosen for the investigation). Optimal functional responses were obtained with 4–8 V and 20–30 Hz.

L-NOARG (2.19 mg), D-NOARG (2.19 mg) and L-arginine (21.07 mg) had no effect on systemic arterial blood pressure when applied intracavernosally ($n = 3$). L-NOARG (2.19 mg), but not D-NOARG (2.19 mg), decreased the intracavernous basal pressure from 21 ± 3 to 16 ± 2 cm H_2O ($n = 7$). However, this effect was not significant. Pretreatment with D-NOARG (2.19 mg) before cavernous nerve stimulation had no effect on ΔP or T_{90}, but significantly increased the slope compared to control responses (Fig. 3 & Table 2). L-NOARG (2.19 mg), on the other hand, decreased ΔP and increased T_{90} and the slope significantly, and abolished the erectile response (Fig. 3 & Table 2). The effect of L-NOARG was long-lasting and persisted for at least 60 min. The functional response to cavernous nerve stimulation was also impaired by L-NOARG at a lower dose (0.22 mg), although the effect was less pronounced (Fig. 3 & Table 2). L-arginine (21.07 mg), given either directly or after 2.19 mg L-NOARG, had no

Table 1. Effect of different stimulation frequencies on the increase in intracavernous pressure induced by unilateral cavernous nerve stimulation at 4 V. Results are given as mean values ± SEM

Frequency (Hz)	Maximal pressure (cm H_2O)	Pressure increase, ΔP (cm H_2O)	T_{90} (S)	Slope	*n*
2.5	78 ± 16	60 ± 14	106.5 ± 20.9	0.42 ± 0.05	4
5	86 ± 19	66 ± 20	55.7 ± 9.6	1.18 ± 0.53	5
10	118 ± 10	98 ± 10	35.6 ± 7.1	3.64 ± 1.36	4
20	123 ± 6	103 ± 5	23.7 ± 3.0	4.51 ± 0.54	15
30	130 ± 11	107 ± 7	15.8 ± 0.2	5.31 ± 1.51	4

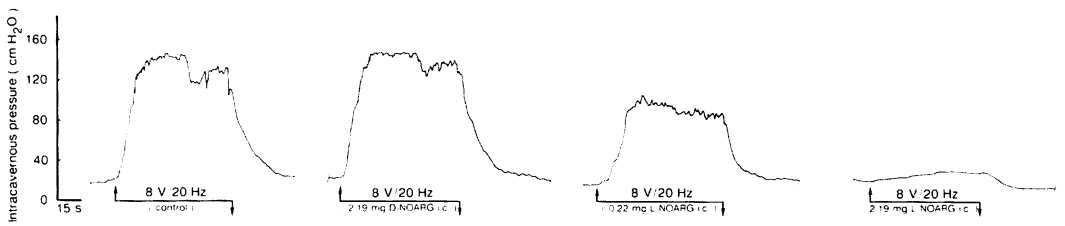

Fig. 3. Tracing showing the effects of D-NOARG and L-NOARG on the increase in intracavernous pressure induced by unilateral cavernous nerve stimulation (8 V, 20 Hz) in the rabbit. The drug investigated was injected intracavernosally at least 10 min before stimulation.

Table 2. Effects of intracavernosally injected D-NOARG and L-NOARG on the increase in intracavernous pressure induced by unilateral cavernous nerve stimulation using optimal stimulation parameters (4–8 V, 20–30 Hz). Results are given as mean values ± SEM, or 99% confidence intervals (within parentheses).

	Pressure increase, ΔP (cm H_2O)	T_{90} (s)	Slope	$\Delta P_{(NOARG)}/\Delta P_{(control)}$	$T_{90(NOARG)}/T_{90(control)}$	$Slope_{(NOARG)}/Slope_{(control)}$	*n*
Control	103 ± 7	21.3 ± 2.1	4.23 ± 0.75	—	—	—	7
2.19 mg D-NOARG i.c.	109 ± 11	22.4 ± 2.5	4.48 ± 0.78	1.02 (0.91–1.14)	1.02 (0.71–1.33)	1.16 (1.05–1.27)	5
0.22 mg L-NOARG i.c.	73 ± 10	39.3 ± 6.5	2.14 ± 0.51	0.72 (0.49–0.96)	1.78 (1.10–2.46)	0.54 (0.39–0.69)	6
2.19 mg L-NOARG i.c.	32 ± 12	55.3 ± 5.9	0.49 ± 0.20	0.31 (0.04–0.58)	2.88 (1.74–4.01)	0.13 (−0.01–0.28)	7

consistent effect on the functional response to cavernous nerve stimulation ($n = 6$).

DISCUSSION

The present study confirms and extends previous findings *in vitro*, suggesting that NO, released either directly from nerves or from the endothelium via the action of some yet unidentified transmitter, is involved in the control of penile smooth muscle tone (Ignarro *et al.* 1990, Holmquist *et al.* 1991a, b). Indeed, the pronounced inhibitory effect of L-NOARG on the functional response induced by cavernous nerve stimulation clearly demonstrates the crucial role of the L-arginine/NO pathway in the mechanisms leading to penile smooth muscle relaxation necessary for erection. At the doses used, intrapenile administration of L-NOARG did not cause any pressor effects. This is in agreement with previous investigations where intravenous injections of low doses of L-NOARG methyl ester had no or only minor effects on blood pressure and heart rate (Gardiner *et al.* 1990, Rees *et al.* 1990). It is therefore reasonable to assume that the local penile effect of L-NOARG observed in the present study was not influenced by any systemic haemodynamic changes. D-NOARG had no effect on ΔP and T_{90} thus confirming the enantiomer-specific nature of the action of L-NOARG (Ignarro *et al.* 1990, Holmquist *et al.* 1991a & b). However, D-NOARG significantly increased the slope of the intracavernous pressure increase evoked by cavernous nerve stimulation. The reason for this is unknown.

Also the basal intracavernous pressure was decreased by L-NOARG, although this effect was not significant. Previous results *in vivo* indicate that there is a continuous release of NO, or a NO-containing compound, modulating vascular tone and thereby the systemic blood pressure (Vallance *et al.* 1989, Rees *et al.* 1989, 1990, Gardiner *et al.* 1990, Persson *et al.* 1990). Based on experiments in isolated preparations, a similar mechanism, opposing the effect of noradrenaline and other possible contractant factors during the flaccid state (Andersson & Holmquist 1990), was proposed to be of importance also in the penis (Holmquist *et al.* 1991b). However, considering that electrical stimulation of the sympathetic trunk (L_6–S_1) induced an intracavernous pressure increase in the rabbit (Stief *et al.* 1990), it can be questioned whether or not the pressure

decrease observed with L-NOARG reflects an impaired synthesis of basally released NO. The possible involvement of the L-arginine/NO pathway in regulating penile blood flow in the flaccid state remains to be established.

Since L-arginine given intravenously had no direct effect on blood pressure (Rees *et al.* 1989, 1990, Gardiner *et al.*, 1990, Persson *et al.* 1990), and since the concentration of endogenous L-arginine in endothelial cells was as high as 0.8 mM (Gold *et al.* 1989), it was concluded that the enzymatic conversion of L-arginine to NO is saturated and not rate limiting under normal conditions (Gold *et al.* 1989, Rees *et al.* 1989, 1990). However, high concentrations of L-arginine could reverse the haemodynamic changes induced by inhibition of the NO synthesis (Rees *et al.* 1989, 1990, Gardiner *et al.* 1990, Persson *et al.* 1990). In accordance with previous findings, intracavernous administration of L-arginine had no effect *per se* on the penile response induced by cavernous nerve stimulation. In addition, however, L-arginine also failed to reverse the inhibitory effect of L-NOARG. It may be speculated that under the present experimental conditions, the L-arginine dose used, which was 10 times higher than that of L-NOARG, was not sufficient to induce any measurable effects. One must also keep in mind that since the penile blood flow reduction, using the present experimental design, is not complete during drug administration, and since the cavernous bodies constitute an unknown volume, it is difficult to determine the actual intrapenile concentration of a drug injected intracavernosally. Furthermore, L-NOARG, but not L-arginine, is known to act in an irreversible manner (Mülsch & Busse 1990). Thus, quantitative comparisons regarding the different drug doses used cannot be done.

Despite the difficulties in estimating intrapenile drug concentrations, the present results further emphasizes the rabbit as an appropriate model for the study of penile erection. Upon electrical stimulation of the cavernous nerve, the intracavernous pressure increased rapidly until it reached a plateau, which was maintained during the whole period of stimulation. In some cases, the pressure did not fall to baseline immediately after the cessation of stimulation, but declined gradually until it suddenly dropped. During erection, the venous blood flow from the penis is greatly reduced due to compression of the subalbugineal venular plexus and postcavernous venules against the relatively indistensible tunica albuginea. As the intracavernous pressure declines in the detumescent phase, the penile veins become open, with a subsequent increase in venous blood flow. In speculation, it is possible that the different patterns of pressure decrease after electrical stimulation, as observed in this study, reflect interindividual variations in the intracavernous pressure at which the penile veins are opened.

The response to cavernous nerve stimulation in the rabbit is reproducible for several hours using the optimal stimulation parameters. The increase in intracavernous pressure can be described in terms of ΔP, T_{90} and slope, all of which reflect the erectile response fairly well. By doing so, the effects of different drugs interfering with the erectile mechanism can easily be described and quantified. Future studies will show if this model also can be used to characterize agents of potential use in the treatment of erectile dysfunction.

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