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L-arginine-induced relaxation of the rat isolated penile bulb

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Abstract

The effects of L-arginine, the precursor in the synthesis of nitric oxide (NO), were investigated in the penile bulb isolated from saline (control) or lipopolysaccharide (20 mg/kg, i.p.)-treated rats. Four consecutive concentration–response curves for L-arginine were made at hourly intervals with the penile bulb. L-arginine ($10^{7}-10^{-3}$ M) elicited a concentration- and time-dependent relaxation response in the control group. The NO synthase (NOS) inhibitors, N^{G}-methyl-L-arginine (L-NMMA) and aminoguanidine, guanylate cyclase inhibitor, 1-*H*-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ) and protein synthesis inhibitor, cycloheximide, inhibited L-arginine-induced relaxation. In the lipopolysaccharide-group, L-arginine produced a pronounced non-time-dependent relaxation at the first concentration–response curve, which was not different from the fourth response of the control group. This response was also inhibited by aminoguanidine. These results show that L-arginine induced NO-mediated relaxation and suggest the presence of a biochemical pathway converting L-arginine to NO, which is probably an inducible type in the penile bulb.

Keywords: Penile bulb; Nitric oxide (NO); L-Arginine; Protein synthase inhibition; (Rat)

1. Introduction

L-arginine is the precursor amino acid in the synthesis of nitric oxide (NO) by either constitutive or inducible isoforms of NO synthase (NOS) (Palmer and Moncada, 1989; Bredt et al., 1991). The direct effects of L-arginine have been evaluated in various isolated vascular tissues. L-arginine itself causes endothelium-independent relaxation in intrapulmonary arteries (Peng et al., 1996). A slight relaxation response to L-arginine, which increased on in vitro incubation, has been demonstrated in isolated rat aorta (Schini and Vanhoutte, 1991; Moritoki et al., 1991, 1992). However, results of other studies suggested that L-arginine did not produce any relaxation in isolated vascular preparations (Thomas and Rammell, 1988; Thomas et al., 1989).

In the urogenital tract, locally released NO from nerves and/or endothelium has been proposed as the major transmitter in penile erection (Andersson, 1993; Klinge and Sjostrand, 1994). Immunohistochemical studies revealed the presence of NOS in penile erectile tissues such as corpus cavernosum (Burnett et al., 1992, 1993; Alm et al., 1993). The similar distribution of NOS-containing nerves has also been reported in the penile bulb, which is the proximal part of corpus spongiosum. NO released from these nerves results in inhibitory junction potentials by activating guanylate cyclase and stimulating sodium pump activity (Hedlund et al., 1995). Two layers of smooth muscle, the parenchyma layer, consisting of spongy cells, and the outer layer were identified in the penile bulb (Hashitani, 2000). All these structures of the penile bulb have been suggested to play a role in erection. The onset of the haemodynamic changes was found to be more rapid than in the corpus cavernosum and, therefore, an important function for the penile bulb in initiating the erection of glans penis has been proposed (Hedlund et al., 1995). Due to the established role of the penile bulb as a tissue and of NO as a mediator in erection, we now investigated the direct effects of L-arginine, the precursor of NO, and its possible mechanism of action in isolated rat penile bulb.

2. Materials and methods

2.1. Tissue preparation

Rats (male, 200–300 g) were treated with an intraperitoneal (i.p.) injection of either saline or lipopolysaccharide (20

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mg/kg) 4 h before they were killed by a sharp blow to the head. The penile bulb was isolated and mounted under a resting tension of 1 g in a 20-ml organ bath filled with Krebs–Henseleit solution at 37 °C and gassed with 95% O_2–5% CO_2. The composition of the Krebs–Henseleit solution was (in mM): NaCl, 118; KCl, 4.7; $MgSO_4$, 1.2; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.1. Isometric changes in tension were recorded with an isometric force transducer and a "MAY 95-transducer data acquisition system" (Commat, Ankara, Turkey) on an IBM-compatible personal computer.

2.2. Experimental protocol

The contractile response to phenylephrine and relaxation responses to acetylcholine, L-arginine and D-arginine were evaluated in precontracted tissues (5×10^{-5} M phenylephrine; 60–80 of the maximum contraction). The first concentration–response curve to each agonist was made at the end of the equilibration period of 1 h. Then, consecutive concentration–response curves to the agonists were made three more times at hourly intervals during which the tissues were rinsed every 10 min.

In some experiments, the penile bulbs were incubated with either N^G-methyl-L-arginine (L-NMMA) (10^{-4} M) or aminoguanidine (10^{-4} M) or 1-*H*-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ) (10^{-5} M) for 30 min before the fourth concentration–response curves to L-arginine were made. In another group of experiments, concentration–response curves to L-arginine were obtained in the presence of cycloheximide (10^{-5} M) or polymyxin B (30 μg/ml), added to the physiological solution in the beginning of the experiment.

2.3. Statistical analysis

The contractile response to phenylephrine is expressed as *g* contraction. The relaxation responses are expressed as percentages of the phenylephrine (5×10^{-5} M)-induced contraction. The maximum response elicited by the agonist (E_{max}) and the concentration required to achieve half-maximum contraction and relaxation (EC_{50}) were obtained from individual concentration–response curves. EC_{50} values are given as pD_2 values, which are defined as the negative logarithm of EC_{50} (p$D_2 = -\log EC_{50}$).

All data are expressed as means ± standard error of the mean (S.E.M.). Statistical analyses were performed by using an analysis of variance (ANOVA) with repeated measurements and the Bonferroni test. Paired samples were compared using Student's *t*-test. A *P*-value of less than 0.05 was considered significant.

2.4. Drugs used

L-phenylephrine hydrochloride, L-arginine hydrochloride, D-arginine hydrochloride, acetylcholine chloride, N^G-methyl-L-arginine (L-NMMA), 1-*H*-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ), aminoguanidine hemisulphate, polymyxin B, lipopolysaccharide (*E. coli* serotype 0.55:B5) were purchased from Sigma (USA). Cycloheximide was purchased from Fluka.

3. Results

The phenylephrine (10^{-8}–10^{-4} M)-induced contractile response was not altered significantly in four consecutive concentration–response curves obtained at hourly intervals with the penile bulb from the control group of rats. The maximum contraction (E_{max}) and the pD_2 values are given in Table 1. In the lipopolysaccharide group, the contractile response to phenylephrine was not altered, being not significantly different from the control response (Table 1).

Acetylcholine (10^{-7}–10^{-3} M) induced only a slight relaxation response in precontracted penile bulb isolated from the control group. This response remained constant on subsequent administration of acetylcholine at hourly intervals. The maximum relaxations elicited by acetylcholine in four consecutive concentration–response curves were between 11.58 ± 3.75% and 13.95 ± 3.98% ($n = 5$). The NOS inhibitor L-NMMA (10^{-4} M) completely inhibited the relaxation response to acetylcholine ($n = 4$).

L-arginine (10^{-7}–10^{-3} M) elicited a small relaxation response in precontracted penile bulb at the first concentration–response curve in the control group. However, subsequent administration of L-arginine at hourly intervals induced a time-dependent increase in the relaxation response (Fig. 1, Table 2). Furthermore, hourly exposure to L-arginine was not necessary for the time-dependence of this response, as the relaxation obtained at the time matching the fourth concentration–response curve was not significantly different in tissues that were and were not exposed to L-arginine.

In one group of experiments, the tissues were incubated with either the nonselective NOS inhibitor, L-NMMA or the selective inducible NOS inhibitor, aminoguanidine (10^{-4}

Table 1
Phenylephrine-induced maximum contraction (E_{max}) and the pD_2 values of four consecutive concentration–response curves (CR) in the penile bulb isolated from control and lipopolysaccharide group of rats ($n = 7$)

	Phenylephrine	
	pD_2	E_{max} (g)
Control group		
First CR	6.02 ± 0.09	2.86 ± 0.36
Second CR	5.81 ± 0.09	3.03 ± 0.32
Third CR	5.67 ± 0.08	2.84 ± 0.31
Fourth CR	5.63 ± 0.10	2.73 ± 0.25
Lipopolysaccharide group		
First CR	5.74 ± 0.05	3.40 ± 0.40

pD_2 values are the negative logarithm of EC_{50}, the concentration causing half-maximal contraction.

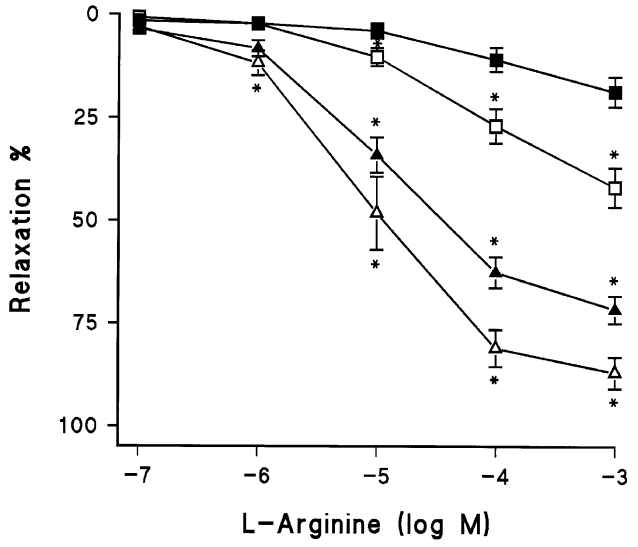

Fig. 1. First (■), second (□), third (▲) and fourth (△) consecutive concentration–relaxation curves for L-arginine (10^{-7}–10^{-3} M) made at hourly intervals with isolated rat penile bulb. The data are expressed as percentages of phenylephrine-induced contraction and are shown as means ± S.E.M. (* significantly different from the first concentration–response curve, $P<0.05$) ($n=6$).

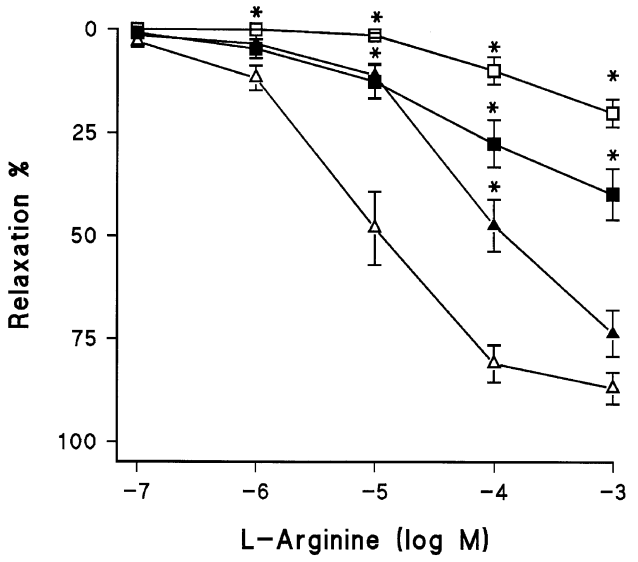

Fig. 2. The fourth concentration–response curve for L-arginine in the absence (△) and presence of either aminoguanidine (10^{-4} M) (■), L-NMMA (10^{-4} M) (▲) or ODQ (10^{-5} M) (□) in isolated rat penile bulb. The data are expressed as percentages of phenylephrine-induced contraction and are shown as means ± S.E.M. (* significantly different from control group, $P<0.05$) ($n=5$–6).

M), or the guanylate cyclase inhibitor ODQ (10^{-5} M) for 30 min before the fourth concentration–response curve to L-arginine. Aminoguanidine and ODQ significantly inhibited L-arginine-induced relaxation at the fourth concentration–response curve (Fig. 2). L-NMMA also inhibited this response without altering the maximum relaxation (Fig. 2). Incubation of the tissues with the protein synthesis inhibitor, cycloheximide (10^{-5} M), throughout the experiments inhibited L-arginine-induced relaxation and prevented its time-dependent increase. However, incubation with the LPS neutralising agent, polymyxin B (30 μg/ml), did not alter the time-dependence of L-arginine-induced relaxation (Table 2).

In the LPS-treated group, L-arginine elicited pronounced relaxation in the first concentration–response curve, which was comparable to the fourth concentration–response curve from in the control group. L-arginine-induced relaxation was not time-dependent in this group and was also significantly inhibited by aminoguanidine (Fig. 3).

Table 2
L-arginine (10^{-7}–10^{-3} M)-induced maximum relaxation response (E_{max}) from four consecutive concentration–response curves in the absence (control) and presence of cycloheximide or polymyxin B in isolated rat penile bulb

	Control E_{max} (%)	Cycloheximide E_{max} (%)	Polymyxin B E_{max} (%)
First CR	18.66 ± 3.65	5.1 ± 1.39[a]	17.25 ± 3.03
Second CR	41.84 ± 4.76	5.28 ± 1.20[a]	54.75 ± 9.59
Third CR	71.57 ± 3.32	5.35 ± 1.17[a]	67.74 ± 9.97
Fourth CR	86.97 ± 3.85	8.16 ± 0.75[a]	78.30 ± 6.63

[a] Significantly different from the matching control response, $P<0.05$ ($n=6$).

D-arginine (10^{-7}–10^{-3} M) induced a very small relaxation response and did not show time-dependence as had L-arginine in the precontracted penile bulb in the control group. The maximum relaxation responses to D-arginine in four consecutive concentration–response curves were between 7.76 ± 2.01% and 14.36 ± 0.35%.

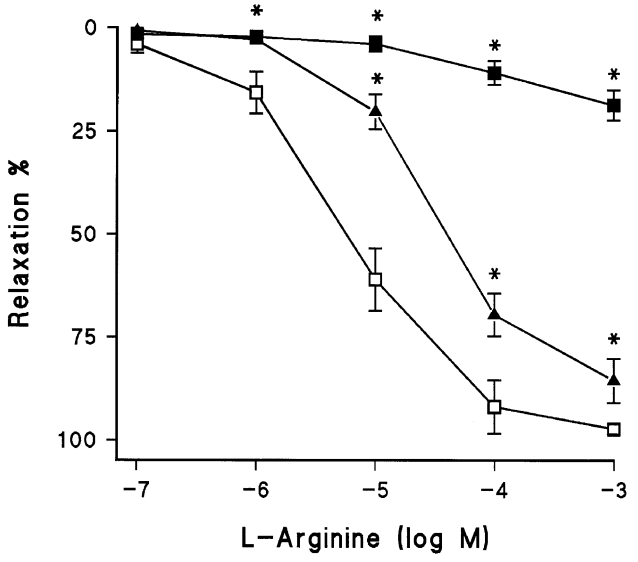

Fig. 3. The first concentration–response curve for L-arginine in penile bulb isolated from control (■) and lipopolysaccharide-treated (□) rats and the effect of incubation with aminoguanidine (10^{-4} M) (△) in the lipopolysaccharide group. The data are expressed as percentages of phenylephrine-induced contraction and are shown as means ± S.E.M. (* significantly different from control group, $P<0.05$) ($n=6$).

4. Discussion

The penile bulb is the proximal part of the corpus spongiosum penis and is composed of erectile and glandular tissues (Hayes, 1965; Hebel and Stromberg, 1986). The distribution and function of NOS-containing nerves in this tissue have been described (Hedlund et al., 1995). In the present study, we demonstrated that acetylcholine induced only a slight relaxation response that is mediated by NO in the rat penile bulb. This slight effect of acetylcholine is consistent with previous reports, which suggest that the release of NO from endothelium in corpus cavernosum of the rat does not play a crucial role for penile erection (Miller et al., 1994; Way and Reid, 1999). In addition, we investigated the direct effects of L-arginine, the precursor amino acid in the synthesis of NO in the rat isolated penile bulb. L-arginine induced a concentration-dependent relaxation response, which increased in a time-dependent manner. Furthermore, the absence of marked relaxation with D-arginine suggests the stereoselectivity of this response.

Previous studies using isolated vascular preparations, showed that L-arginine itself did not relax arteries or that it caused negligible relaxation at very high concentrations (Thomas and Rammell, 1988; Thomas et al., 1989). However, others reported controversial results, suggesting that L-arginine induced relaxation in isolated rat aorta and bovine pulmonary artery (Gold et al., 1989; Moritoki et al., 1991, 1992). Time-dependence of L-arginine response has also been described in some of the vascular studies (Schini and Vanhoutte, 1991; Moritoki et al., 1991; 1992).

In the present experimental model, the time-dependent increase in relaxation response to L-arginine was not due to the alteration in the contractility of the penile bulb because the consecutive concentration–response curves elicited by phenylephrine were not significantly altered.

The time-dependent increase in L-arginine-induced relaxation seems to be due to in vitro activation or induction of a biological pathway in the penile bulb exposed to L-arginine. We have demonstrated that the relaxation of the penile bulb was fully developed even in the absence of prior exposure to L-arginine. In accordance with the present results, it has been shown that L-arginine-induced relaxation in isolated vascular preparations increased with time or was still evident after 24-h incubation in Krebs solution without L-arginine exposure (Wood et al., 1990; Schini and Vanhoutte, 1991). However, others proposed that repeated exposure to L-arginine was necessary for the development of relaxation (Moritoki et al., 1991).

The relaxation evoked by L-arginine was inhibited by both the NOS inhibitors, L-NMMA and aminoguanidine and the guanylate cyclase inhibitor, ODQ. These data show that L-arginine-induced relaxation is due to formation of cGMP coupled to NOS and thus suggest the presence of an L-arginine-NO-cGMP pathway in the penile bulb. Incubation with the protein synthesis inhibitor, cycloheximide, prevented L-arginine-induced relaxation in the penile bulb. It has been demonstrated that cycloheximide blocks the induction of NO synthase in macrophages and vascular smooth muscle cells (Busse and Mülsh, 1990; Fleming et al., 1991; Jorens et al., 1991; Mollace et al., 1991). Furthermore, aminoguanidine has been suggested as the selective inhibitor of inducible NOS (Griffiths et al., 1993). The findings that cycloheximide prevented the development of L-arginine-induced relaxation, and that aminoguanidine inhibited the fully developed response suggest a role of inducible NOS in L-arginine-induced relaxation.

Polymyxin B has the ability to bind to LPS and neutralise its activity (Weinberg et al., 1978; Danner et al., 1989). Moritoki et al. (1992) reported that incubation with polymyxin B prevents the development of L-arginine-induced relaxation in isolated rat aorta. They suggested that lipopolysaccharide contamination of the Krebs solution led to the induction of NOS and thus, increases in L-arginine response. This was not observed in the present study, as the development of L-arginine-induced relaxation of the penile bulb was not prevented in the presence of polymyxin B. Therefore, accumulation of endotoxin as possible contaminant of the Krebs solution did not have any influence on L-arginine-induced responses in the present experimental model.

We studied the effects of in vivo lipopolysaccharide administration on the L-arginine response in the penile bulb. There was a pronounced relaxation response to L-arginine in the penile bulb isolated from lipopolysaccharide pretreated group of rats. The first concentration–response curve elicited by L-arginine in the lipopolysaccharide group was similar to the response obtained at the fourth concentration–response curve in the control group. Bacterial lipopolysaccharide is capable of triggering the inducible isoform of NOS (Busse and Mülsh, 1990; Lopez-Belmonte and Whittle, 1995; Cuzzocrea et al., 1997). Increased formation of NO by inducible NOS in experimental models of endotoxaemia has been demonstrated. (Laszlo et al., 1994; Cuzzocrea et al., 1997). A role of NO in the pathological changes associated with endotoxaemia, including the alterations of vascular reactivity has been proposed (Guthrie et al., 1984; Vallance et al., 1989). Therefore, the pronounced relaxation elicited by L-arginine in the present study reflects the inducible NOS induction in the penile bulb following lipopolysaccharide administration and thus, enhanced conversion of L-arginine to NO to produce relaxation. Furthermore, incubation with aminoguanidine partially reversed the augmented L-arginine response in the penile bulb from lipopolysaccharide-pretreated rats. This is consistent with the proposed inhibitory effect of aminoguanidine on inducible NOS.

In summary and conclusion, the present study demonstrated that L-arginine evoked a marked relaxation in the penile bulb, which was stereoselective and was a function of concentration and incubation time. As inhibitors of both the synthesis of, and biological responses to NO impaired the relaxation evoked by L-arginine, we suggest that the penile bulb possesses a biochemical pathway converting L-arginine

to NO coupled to cGMP formation. Furthermore, the NO synthesising mechanism responsible for L-arginine-evoked relaxation is an inducible type and is induced in vitro in the organ bath as well as after in vivo lipopolysaccharide pretreatment.

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