NITROARGININE DOES NOT INHIBIT LYSOPHOSPHATIDYLCHOLINE (LPC)-INDUCED VASCULAR RELAXATION AND ACCUMULATION OF CYCLIC GMP

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Abstract. Lysophosphatidylcholine (LPC) is a potent endothelium-dependent vascular smooth muscle relaxant. The possibility that its action is mediated through endothelium-derived nitric oxide (EDNO), although suggestive, has not been proven. Both lysophosphatidylcholine and endothelium-derived nitric oxide relax by activating guanylate cyclase to form cyclic GMP. Based on the finding that EDNO formation is inhibited by NNA (N-omega-nitro-L-arginine), we followed cyclic GMP changes in bovine intrapulmonary arteries with LPC after incubation with NNA. Inhibition of cyclic GMP by LPC following NNA exposure would be suggestive of the production of EDNO by LPC. However, while NNA significantly inhibited accumulation of cyclic GMP after exposure to the calcium ionophore A23187 which releases EDNO, NNA failed to inhibit LPC-induced accumulation of cyclic GMP. The results indicate that LPC relaxes vascular smooth muscle through a non NO-mediated pathway.

Introduction. LPC is a potent vascular relaxant of rabbit thoracic aorta and bovine intrapulmonary arteries and veins (1,2). Its action is mediated through the release of an endothelium dependent relaxing factor (EDRF) and subsequent elevation of cyclic GMP (3). One of the relaxing factors involved in vascular relaxation has been shown to be nitric oxide (NO) with arginine as its precursor (4,5). N-omega-nitro-L-arginine (NNA), a potent and specific inhibitor of the L-arginine pathway of NO production is reported to inhibit endothelium-derived relaxation produced by acetylcholine (6) and accumulation of cyclic GMP induced by calcium ionophore A23187 (7). In the present study the mechanism of LPC-induced relaxation was investigated by utilizing the stimulation of cyclic GMP accumulation by LPC and A23187 as a measure of EDNO production.

Material and Methods. Bovine lungs were obtained from a local abattoir (Shamrock Co., Vernon, CA). The third branch of the intrapulmonary artery, cleaned of perivascular tissue, was cut into 3 mm rings. The rings were opened as transverse strips under a resting tension of 2g in 20 ml organ baths filled with oxygenated Krebs-Henseleit buffer solution at 37°C. The composition of the buffer is as follows: 143 mM Na^+, 5.9 mM K^+, 2.5 mM Ca^{2+}, 1.2 mM Mg^{2+}, 1.2 mM $H_2PO_4^-$, 127.8 mM Cl^-, 25 mM HCO_3^-, 1.2 mM SO_4^{2-}, 10 mM glucose.

Tension changes were measured using isometric transducers (UL-20-Gr, Shinkoh, Minebea Co., Ltd., Tokyo, Japan) and recorded on Soltec multichannel recorders (Soltec Corp., Sun Valley, CA). After equilibration for 45 minutes the strips were preincubated with 10^{-6}M NNA for 20 minutes. The strips were precontracted with 10^{-5}M histamine (HA) in the presence of 100 units of SOD/ml (superoxide dismutase) and 10^{-5}M IND (indomethacin). The use of SOD has been recommended in experiments to assess the effect of inhibitors on cyclic GMP levels (7). Percent relaxation is calculated from the decrease in tension from histamine-induced contraction. After a steady state of contraction had been reached, 10^{-10}M A23187 or 10^{-5}M LPC was added. At maximum relaxation the strips were rapidly frozen in liquid nitrogen for cyclic GMP determination. Extraction of cyclic GMP from the tissue has been described previously (3). The powder obtained after lyophilization was dissolved in 0.3 ml of Tris buffer (pH 7.4) and 0.1 aliquot was used for the assay. All values are recorded as fmol/mg tissue.

Drugs. L-alpha lysophosphatidylcholine from egg yolk, calcium ionophore A23187, N-omega-nitro-L-arginine, histamine dihydrochloride (HA), indomethacin (IND) and superoxide dismutase (SOD) were purchased from Sigma Chemical Company, St. Louis, MO. The Amersham cyclic GMP radioimmunoassay kit (Amersham Corp., Arlington Heights, IL) was used for cyclic GMP assay. Statistical analysis was done by Students-t-test for unpaired comparison. $P<0.05$ was considered significant.

Results. Lower doses of NNA, 10^{-7}M and 10^{-6}M, did not produce any significant changes in the spontaneous relaxation of bovine pulmonary arterial strips after contraction with 10^{-5}M HA (Fig 1A). In contrast, 10^{-5}NNA caused a contractile response of about 14 ± 3%. Therefore, a concentration of 10^{-6}M NNA was used to evaluate its inhibitory effect on A23187 and LPC-induced relaxations.

The minimum dose of A23187 and LPC required to produce noticeable relaxant responses of HA precontracted bovine arterial strips were 10^{-10}M and 10^{-5}M respectively. Preincubation of the strips with 10^{-6}M had no inhibitory effect on either LPC-induced relaxation or cyclic GMP levels (Fig 1B; Table 1). Although 10^{-6}M NNA had no appreciable inhibitory effect on A23187 induced relaxation (Fig 1C), the levels of cyclic GMP were significantly reduced from 54 to 36 fmol/mg tissue ($P<0.05$; Table 1).

Discussion. Changes in cyclic GMP levels in the presence and absence of analogues of L-

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arginine, the proposed precursor of EDNO, have been employed previously to screen various compounds for EDNO release (7). In the present study we have utilized this approach to investigate the mechanism of action of LPC. It has been reported that NNA is seventy times more potent than the more commonly used analogue, N-monomethyl-L-arginine (5) and that 10^{-7} M NNA inhibited basal, as well as A23187-stimulated release of cyclic GMP and hence EDNO formation/release in cultured bovine aortic endothelial cells. An almost complete inhibition of cyclic GMP accumulation was observed with 10^{-5} M NNA (5). Similarly, acetylcholine-induced relaxation in rabbit thoracic aorta was also reduced to 10% by 10^{-5} M NNA (6). Our results indicated that NNA concentrations higher than 10^{-6} M produced an intrinsic contractile response in bovine pulmonary arterial strips. By using lower doses of NNA (10^{-6} M) which by itself did not significantly alter the magnitude of relaxant responses, we have demonstrated that NNA caused significant decreases ($P<0.05$) in A23187-stimulated cyclic GMP accumulation, but was without effect in the presence of LPC (Table 1; Fig 1C). Chemical as well as pharmacological evidence has suggested that vascular relaxation produced by A23187 is mediated via the formation of EDNO (8,9,10). The present study indicates that A23187 and LPC relax vasular smooth muscle by different mechanisms.

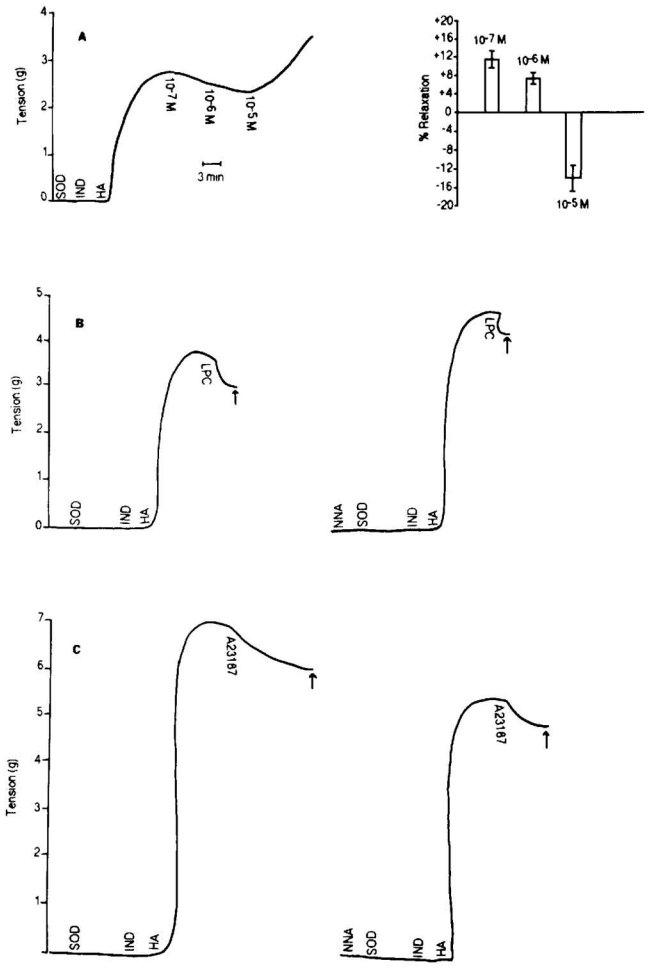

Figure 1. (A) Left panel, dose-response curve of relaxation of pulmonary artery strips with different concentrations of NNA. Right panel, percentage of relaxation obtained with these concentrations. At a higher concentration of NNA (10^{-5} *M*) an increase in tension was noted. Values are ±SEM; $n = 10$. (B) Left panel, effect of LPC on tension in the absence; right panel, in the presence of NNA. (C) Representative tracing of effect of 10^{-10} *M* A23187 in the absence (left panel) and presence (right panel) of NNA. In both B and C NNA does not interfere with relaxation by either LPC or A23187 (see also Table I). Arrow indicates collection for cyclic GMP. SOD, superoxide dismutase; IND, indomethacin; HA, histamine.

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Table I. Effect of NNA on Agonist-Stimulated Accumulation of Cyclic GMP in Bovine Pulmonary Artery[a]

	10^{-10} *M* A23187		10^{-5} *M* LPC	
	−NNA	+NNA	−NNA	+NNA
% Relaxation	15.6 ± 1.3 (18)	15.3 ± 1.8 (11)	13.8 ± 2.2 (17)	13.2 ± 2.9 (20)
Cyclic GMP	53.8 ± 1.5 (10)*	35.8 ± 3.6 (18)	34.4 ± 8.7 (6)	30.1 ± 5.3 (12)

[a] Values are mean ± SE. Numbers in parentheses indicate the number of experiments. Cyclic GMP is expressed as fmol/mg tissue. * $P < 0.05$.

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