Functional Study on Nitroxidergic Nerve in Isolated Dog Pulmonary Arteries and Veins

Kazuhide Ayajiki¹, Tomio Okamura¹, Kumiko Noda² and Noboru Toda¹,²,*

¹Department of Pharmacology, Shiga University of Medical Science, Seta, Ohtsu 520-2192, Japan
²Research Laboratories, Nippon Shinyaku Co., Kisshou-in, Minami-ku, Kyoto 601-8550, Japan

Received February 1, 2002 Accepted April 9, 2002

ABSTRACT—In dog pulmonary arterial and venous strips without endothelium under treatment with prazosin, nicotine induced relaxation that was abolished by N⁶-nitro-L-arginine, hexamethonium and methylene blue. L-Arginine antagonized the N⁶-nitro-L-arginine action. Neurogenic relaxations tended to be more evident in the vein. Nitric oxide (NO)-induced relaxations were greater in the veins than in the arteries. Concentrations of NO to induce the same magnitude of relaxation as that to nicotine were higher in the arteries. In conclusion, dog pulmonary arteries and veins are innervated by nitroxidergic (nitrergic) nerves, and NO is released by nerve stimulation with nicotine in a larger amount in the artery than the vein.

Keywords: Nicotine, Nitroxidergic (nitrergic) nerve, Pulmonary vasculature

Inhalation of nitric oxide (NO) is recognized as a useful therapy for primary pulmonary hypertension, persistent pulmonary hypertension of the neonate and adult respiratory distress syndrome (1). The effect is considered to be due mainly to reduced arterial resistance and also to bronchiolar smooth muscle relaxation. Endogenous NO derived from the endothelium (2) or autonomic efferent nerve (3, 4) of pulmonary arteries is expected to play an important role in the regulation of vascular resistance.

Inhaled NO acts mainly on pulmonary arterioles via alveoli, thus resulting in therapeutic effectiveness locally. Intravenously applied NO donors, such as nitroglycerin, nitroprusside, Sin 1, etc., and endothelium-derived NO-releasing substances also act on pulmonary veins and systemic vasculature. Some studies have indicated the different responsiveness to chemical stimuli of pulmonary arteries and veins (2, 5, 6). However, no information is available concerning quantitative analyses of the heterogeneous response to nitroxidergic nerve stimulation and NO in the arteries and veins.

There is a discrepancy in mechanisms of neurogenic vasodilatation in pulmonary arteries. It has been reported that NO is involved in vasodilation of the guinea pig pulmonary arteries (3, 4), whereas CGRP acts as a neurotransmitter in the same arteries (7, 8).

The aim of the present study was to clarify the mechanism underlying neurogenic relaxation and to determine different responsiveness to chemical perivascular nerve stimulation by nicotine and exogenously applied NO of canine pulmonary arteries and veins. The nicotine-induced response is considered to be caused by nerve stimulation from the functional and histological studies in canine penile veins (9).

Beagle dogs of either sex, weighing 9 to 13 kg, were used for the present study. The Animal Care and Use Committee at our University approved the use of dog blood vessels. The dogs were anesthetized with thiopental sodium (20 mg/kg, i.v.) and killed by bleeding from carotid arteries. Lungs were rapidly removed. Second or third branches of intrapulmonary arteries and accompanying veins (0.7 – 0.9 mm diameter) were isolated and helically cut into strips of approx. 20-mm length. The endothelium was removed by gently rubbing the intimal surface by a cotton ball. The specimen was vertically fixed between hooks in a muscle bath (20-ml capacity) containing the modified Ringer-Locke solution of the following composition: 120 mM NaCl, 5.4 mM KCl, 2.2 mM CaCl₂, 1.0 mM MgCl₂, 25.0 mM NaHCO₃ and 5.6 mM dextrose. The bathing media were maintained at 37 ± 0.3°C and aerated with a mixture of 95% O₂ and 5% CO₂; the pH of the solution was 7.37 – 7.44. The hook fixing the upper end of the strips was connected to the lever of a force-displacement transducer, and the resting tension was adjusted to 1.5 g for the artery and 0.5 g for the vein, which are optimal for inducing the maximal contraction. Before the start of experiments, the
arterial strips were allowed to equilibrate for 60–90 min in the bathing media, during which time the bathing media were replaced three times at about every 10 min. Isometric mechanical responses were displayed on an ink-writing oscillograph. The arteries and veins were treated with prazosin (10⁻⁵ M) and partially contracted with prostaglandin (PG) F₂α to obtain relaxations induced by nicotine (10⁻⁴ M) or NO (10⁻⁴ to 10⁻⁶ M). The relaxations by nicotine and NO did not differ between endothelium-intact and -denuded pulmonary arteries and veins (K. Ayajiki et al., unpublished observation). Concentrations of NO to produce the same magnitude of relaxation as that elicited by nicotine were estimated. In order to examine the effects of the agents, the strips were treated with blocking agents for 20 min or longer before the response to agonists was obtained. Papaverine (10⁻⁴ M) was added at the end of experiments to attain the maximal relaxation, and relaxations induced by the agonists used were presented as relative values to those due to papaverine.

The results shown in the text and figures are expressed as mean values ± S.E.M. Statistical analyses were made by Student’s unpaired t-test for two groups and ANOVA followed by Tukey’s test for three or more groups. Drugs used were nicotine (base), hexamethonium bromide, methylene blue, l-arginine (Nacalai Tesque, Kyoto); N⁵-nitro-l-arginine (l-NA), N⁵-nitro-D-arginine (D-NA) (Peptide Institute, Minoh); prazosin hydrochloride (Wako Pure Chemical Industries Ltd., Osaka); timolol maleate (Banyu Co., Tokyo); prostaglandin F₂α (Pharmacia-Upjohn, Tokyo); and papaverine hydrochloride (Dainippon Co., Osaka). Responses to NO were obtained by adding the NaNO₂ solution adjusted at pH 2, and concentrations of acidified NaNO₂ in the bathing media were expressed as those of NO.

In endothelium-denuded arterial strips treated with 10⁻⁵ M prazosin and partially contracted with PGF₂α, nicotine (10⁻⁴ M) and NO (10⁻⁷ and 10⁻⁶ M) produced relaxations, which were abolished by 10⁻⁵ M methylene blue (n = 4), but were unaffected by timolol (10⁻⁷ M, n = 5). The response to nicotine was abolished by 10⁻⁵ M l-NA, but not d-NA, and the inhibition was reversed by 10⁻³ M l-arginine (Fig. 1). The data are summarized in Fig. 2. NO-induced relaxations were not influenced by l-NA. Hexamethonium (10⁻⁵ M) abolished the nicotine-induced relaxation (n = 5). Concentrations of NO to induce the same magnitude of relaxation as that caused by nicotine were obtained by applying nicotine and two doses of NO in the same strips, as shown in Fig. 1. The mean value was 1.55 × 10⁻⁷ M

---

**Fig. 1.** Tracings of the response to nicotine (N, 10⁻⁴ M) and exogenous NO (10⁻⁷–10⁻⁵ M) in pulmonary arterial strips contracted with PGF₂α before and after treatment with l-NA, d-NA (10⁻⁵ M), l-arginine (l-Arg., 10⁻³ M) and methylene blue (10⁻³ M). Two strips were obtained from the same dog. PA represents 10⁻⁶ M papaverine that produced the maximal relaxation.
In endothelium-denuded venous strips treated with \(10^{-5}\) M prazosin and partially contracted with PGF\(_{2\alpha}\), relaxations induced by nicotine \(\left(10^{-4}\right)\) M were abolished by \(10^{-5}\) M L-NA, and \(10^{-3}\) M L-arginine restored the response (Fig. 2). Nicotine-induced relaxations were also abolished by hexamethonium \(\left(10^{-5}\right)\) M, n = 5) and methylene blue \(\left(10^{-5}\right)\) M, n = 5) but were unaffected by timolol \(\left(10^{-7}\right)\) M, n = 5). Relaxant responses to NO were not influenced by L-NA and hexamethonium, but were abolished by methylene blue (n = 4). The concentration of NO to elicit the same extent of relaxation as that obtained with nicotine averaged \(3.36 \pm 1.45\) \(\times 10^{-6}\) M, which was 1/4.6 of that obtained in the arterial strips (Table 1).

Relaxations induced by NO \(\left(10^{-8}\right)\) to \(10^{-6}\) M were compared in the arterial and venous strips (Table 1). Significantly greater relaxation was induced by \(10^{-7}\) M NO in the venous strip than in the artery.

This is the first report comparing the neurogenic relaxations between pulmonary artery and vein. The nicotine-induced relaxation in canine pulmonary arteries and veins denuded of the endothelium was abolished by hexamethonium, L-NA and methylene blue, and the inhibitory effect of L-NA was reversed by high concentrations of L-arginine. The relaxation was not affected by timolol, a \(\beta\)-adrenoceptor antagonist. Similar findings have also been demonstrated in cerebral arteries from dogs, pigs, monkeys and humans (reviewed by Toda and Okamura, 10). Histochernical studies have demonstrated the presence of neurons containing NO synthase in rat and guinea pig pulmonary arteries (11). Therefore, the neurogenic vasodilatation appears to be not mediated by noradrenaline but instead,

![Fig. 2](image.jpg)

**Table 1.** Relaxations induced by NO and concentrations of NO sufficient to elicit the same magnitude of relaxation as that due to \(10^{-4}\) M nicotine (equivalent conc.) in pulmonary arterial and venous strips contracted with prostaglandin F\(_{2\alpha}\).

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Relaxation (%) induced by NO (M) at (10^{-4}) M</th>
<th>10(^{-7}) M</th>
<th>10(^{-6}) M</th>
<th>Equivalent conc. of NO ((\times 10^{-7}) M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artery</td>
<td>7</td>
<td>0 ± 0</td>
<td>5.6 ± 1.6</td>
<td>42.6 ± 2.8</td>
<td>1.55 ± 0.35 (n = 6)</td>
</tr>
<tr>
<td>Vein</td>
<td>13</td>
<td>2.6 ± 1.5</td>
<td>31.2 ± 3.5*</td>
<td>50.3 ± 6.1</td>
<td>0.34 ± 0.15 (n = 7)\†</td>
</tr>
</tbody>
</table>

Significantly different from the value obtained from arteries, \*\(P<0.001\), \(P<0.01\) (unpaired t-test). ‘n’ in the first row represents the number of strips from separate dogs; ‘n’ in parentheses is the number of strips used to obtain equivalent concentrations of NO.
solely mediated by NO synthesized in nerve terminals in the canine pulmonary vein as in the pulmonary artery from dogs (present study) and guinea pigs (4). Cyclic GMP would be involved in the NO-induced relaxation.

Relaxations induced by nicotine tended to be less in the arteries than in the veins and appreciably greater responses to NO were seen in venous strips. The vasodilative effect of NO is more evident in pulmonary veins than in the arteries also in other mammals (2, 5). In contrast, in the other vasculature, such as mesenteric and femoral, there is no difference in the vasodilative response to NO or NO donors, such as nitroglycerin and sodium nitroprusside, between arteries and veins (12, 13). However, more marked dilatation in veins than in arterioles has long been recognized (14). Our unpublished data indicate that NO-induced dilatation is greater in the mesenteric vein than in resistance vessels in the perfused canine mesentery. The reason why pulmonary veins are more sensitive to NO than the accompanying arteries remains undetermined. According to Bina et al. (6), higher activity of cyclic GMP-phosphodiesterase in the porcine pulmonary artery than in the vein might be responsible for the greater sensitivity to exogenous NO of venous rings.

Concentrations of NO released from nitrooxidergic nerves by nicotinic stimulation, estimated from the responses to nicotine and exogenously applied NO in the same preparations, were significantly greater in the arteries than in the veins (1.55 vs 0.34 \times 10^{-7} M). In contrast, more NO synthase in the endothelium of the pulmonary vein than in that of the artery is reported (6). There is evidence indicating that the relaxing factor or NO derived from the endothelium is less in the canine femoral, pulmonary, saphenous, and splenic veins than in the arteries (12, 15), whereas the opposite has been observed in lamb and porcine pulmonary vasculature (5, 6). Exogenously applied NO donors appear to affect the venous side to a greater extent than the arterial side. These findings suggest that NO from the nerve or endothelium and NO donors applied as therapeutics differently modulate pulmonary circulation. Such a characteristic feature may be important in analyzing the pathogenesis of pulmonary hypertension and understanding mechanisms of drug action on the circulation of lungs.

NO derived from vasodilator nerves appears to play a major role in regulating the tone of pulmonary venous smooth muscle as well as that of the artery. Further analytical study is required to clarify the physiological control of pulmonary circulation by NO from the nerve and endothelium in reference to different responsiveness of arteries and veins of the lung.

REFERENCES