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Evidence for the differential sensitivity to hypoxia of basal and agonist-induced nitric oxide release

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Abstract

Rat pulmonary arterial rings (phenylephrine pre-contracted), were relaxed by carbachol or thapsigargin, or were contracted by *N*ω-nitro-L-arginine methyl ester (L-NAME). Mild hypoxia (41 mm Hg) attenuated the carbachol-induced relaxation, whereas the relaxant and contractile effects produced by thapsigargin and L-NAME were unaffected. More severe hypoxia (20 mm Hg) abolished thapsigargin-induced relaxation, with no further change in responses to carbachol or L-NAME. At 7 mm Hg, carbachol-induced relaxation was completely inhibited, and the L-NAME-induced contraction was attenuated but not abolished. The present data is consistent with the conclusion that nitric oxide (NO) synthase activity is less susceptible to oxygen deprivation under basal conditions than during activation.

Keywords: Hypoxia; Endothelium-dependent relaxation; Carbachol

Constitutive nitric oxide (NO) synthase is a Ca^{2+}/calmodulin dependent enzyme; consequently, stimulation of NO formation depends on increasing the intracellular Ca^{2+}. Hypoxia decreased Ca^{2+} influx into bovine pulmonary arterial endothelial cells (Stevens et al., 1993), and thus low PO_2 might be expected to limit NO formation, depending on the oxygen sensitivity of the Ca^{2+} mobilisation process used by different agonists. Thus, acetylcholine-induced vascular relaxation is inhibited by hypoxia (Furchgott and Zawadzki, 1980). Moreover, in pulmonary artery rings, the inhibition of NO production by hypoxia causes hypoxia-induced constriction (Rodman et al., 1990). It was reported that basal Ca^{2+} influx occurs into unstimulated endothelial cells (Johns et al., 1987) which is consistent with the spontaneous release of NO from vascular rings under basal conditions. However, it is not known whether hypoxia affects basal and simulated NO release equally. Inhibition of NO synthase by hypoxia may also occur because of the requirement for molecular oxygen as a substrate for this enzyme (Rengasamy and Johns, 1991).

The present study was designed to evaluate the sensitivity of NO release by different stimuli to graded hypoxia in rat pulmonary arteries in vitro. The contraction induced by *N*ω-nitro-L-arginine methyl ester (L-NAME) was taken to be an indicator of basal NO release, while relaxations produced by carbachol or thapsigargin were used as measures related to agonist-induced NO formation. These responses were investigated in normoxic conditions (PO_2 = 152 mm Hg), and three different levels of hypoxia (PO_2 = 7, 20 and 41 mm Hg).

Rings (3 mm length) of left and right pulmonary artery were removed from male Sprague–Dawley (200 ± 25 g) rats (BK Universal, Hull). The rings were mounted on L-shaped wires (resting force = 1 g) in Krebs–Henseleit physiological solution of composition (mM) NaCl 118, KCl 4.7, D-(+)-glucose 11, $NaHCO_3$ 25, $MgSO_4$ 1.18, KH_2PO_4 1.18, $CaCl_2$ 2.5. Functional endothelium was confirmed in all preparations by obtaining a relaxation to carbachol 10^{-6} M. Rat pulmonary artery rings were precontracted with phenylephrine at its EC_{50} which was determined to be 10^{-7} M. Concentration–response curves were obtained to carbachol (10^{-8} to 10^{-5} M), thapsigargin (10^{-9} to 3×10^{-7} M) or L-NAME (10^{-5} to 3×10^{-4} M). Four pairs of rings were subjected to four different gas mixtures containing 20, 5, 2 or 0% O_2 (each gas mixture contained 5% CO_2, balance N_2). The total duration of the experiment was 5 h. In each pair of rings one was used to

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determine the relaxant/contractile responses of carbachol or thapsigargin or L-NAME, while the other was used to determine the control response of phenylephrine (for thapsigargin the relevant control rings received the solvent, 30 μl of 10% dimethyl sulphoxide (DMSO) in absolute alcohol). These parallel control experiments showed that phenylephrine-induced contraction remained stable during the experimental period. The *n* refers to the number of animals used. Previous studies in the laboratory (Karamsetty et al., 1996) showed that profuse bubbling of the buffer in the organ bath with these gas mixtures for 20 min produces a stable pO_2 in the bath (152, 41, 20 and 7 mm Hg, respectively. None of the levels of hypoxia altered the basal force of rat pulmonary arterial rings.

Hypoxia depressed the concentration–response curves to carbachol, thapsigargin and L-NAME throughout their entire concentration range; thus Fig. 1 shows the effects of hypoxia on the maximal concentration of each. Carbachol-induced relaxation of rat pulmonary artery rings was significantly reduced at $pO_2 = 41$ mm Hg. No further inhibition occurred at 20 mm Hg, but $pO_2 = 7$ mm Hg completely abolished carbachol-induced relaxation (Fig. 1). Thapsigargin-induced relaxation was not affected by reduction of pO_2 to 41 mm Hg, but was abolished at $pO_2 = 20$ or 7 mm Hg (Fig. 1). Under normoxic conditions L-NAME produced further contraction of pre-contracted rings by $238 \pm 33\%$ of the phenylephrine-induced tone, and this was unaltered in $pO_2 = 41$ or 20 mm Hg. At

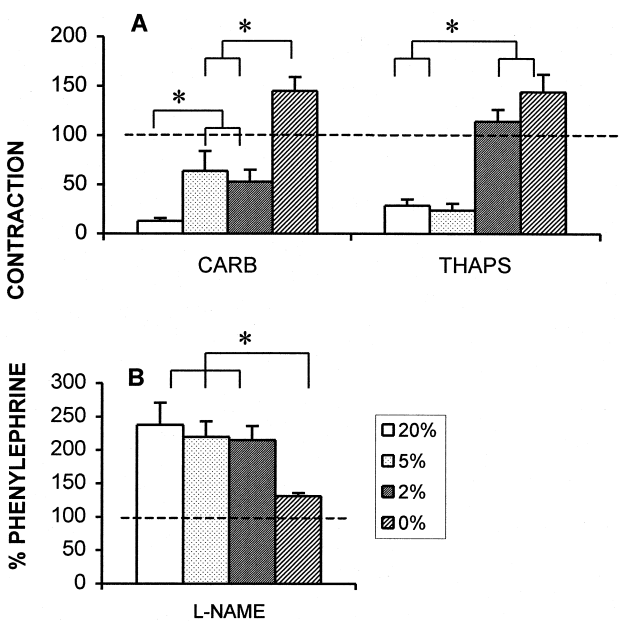

Fig. 1. Rat pulmonary artery rings, precontracted with phenylephrine, and (A) relaxed by addition of carbachol 10^{-5} M or thapsigargin 3×10^{-7} M or (B) contracted by addition of L-NAME 3×10^{-4} M. Parallel rings were equilibrated throughout the experiment with 20%, 5%, 2% or 0% O_2. Mean ± S.E.M., $n = 5$. Concentration–response curves significantly different by two-way ANOVA with Newman–Keuls range test at $P < 0.05$.

$pO_2 = 7$ mm Hg the L-NAME contractile response was significantly attenuated, although a significant L-NAME-induced contraction remained ($131 \pm 5\%$ of the maximum contraction), indicating that some basal release of NO was able to continue (Fig. 1).

The present data shows that, in rat pulmonary artery rings, relaxation produced by carbachol or thapsigargin is inhibited by mild hypoxia ($pO_2 = 41$ or 20 mm Hg) whereas the contraction produced by L-NAME is attenuated only with severe hypoxia ($pO_2 = 7$ mm Hg). Thus, the present study suggests that agonist-induced release of NO is more susceptible to hypoxia than is basal NO formation. This data may be compared with Shaul et al. (1993), who reported that reducing pO_2 from 150 to 40 mm Hg in pulmonary arterial segments caused a 52% reduction in basal cyclic GMP production with no significant change in acetylcholine or calcimycin-stimulated cGMP production. Presumably a greater level of hypoxia below 40 mm Hg would have further elevated basal, and inhibited stimulated cyclic GMP formation, consistent with the changes in endothelium-dependent contraction and relaxation observed in the present study. Shaul et al. (1993) found a slight but not significant decrease in the acetylcholine-induced cyclic GMP production; however, a small change in cyclic GMP might be sufficient to account for the diminution in carbachol-induced relaxation observed in the present study at the same level of hypoxia.

Agonists elevate the intracellular Ca^{2+} of the endothelial cells through different mechanisms. Carbachol and thapsigargin share the property of releasing Ca^{2+} from the intracellular stores. In addition, carbachol causes Ca^{2+} influx and prolongs the activation of NO synthase (Himmel et al., 1993). It is likely that hypoxia alters the endothelium-dependent relaxant responses by interfering with the intracellular Ca^{2+} distribution in the vascular endothelial cells because the sensitivity of this response to hypoxia was found to be different with different agonists. In the present study, the milder level of hypoxia ($pO_2 = 41$ mm Hg) significantly attenuated carbachol-induced relaxation only, whereas the intermediate level of hypoxia ($pO_2 = 20$ mm Hg) was required to inhibit the thapsigargin response. Since thapsigargin acts by preventing Ca^{2+} accumulation into the endoplasmic reticulum, it may give a strong Ca^{2+} signal that occurs even with moderate oxygen depletion. Since the carbachol response is only partly blocked at intermediate levels of hypoxia, the calcium influx component of carbachol's action may be preserved at this pO_2.

Our data suggests that hypoxia initially affects the activation of NO synthase, rather than basal release of NO. Calcium mobilisation in pulmonary arterial endothelial cells is modulated by pO_2 (Stevens et al., 1993) and certain steps in the calcium release process may be very sensitive to hypoxia. Thus, it is likely that relatively mild hypoxia impairs agonist-induced Ca^{2+} mobilisation and thence activation of NO synthase. At the most severe level of

hypoxia, the lack of oxygen as a substrate for NO synthase would prevent the formation of NO regardless of the Ca^{2+} availability.

In conclusion, the present results are consistent with hypoxia acting through Ca^{2+} mobilisation to inhibit activation of NO synthase. With extreme hypoxia, it is probable that the lack of substrate molecular oxygen prevents basal NO formation.

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