

Effect of change in oxygen tension on release pattern and nature of endothelium-derived substances in isolated rabbit aorta

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KEY WORDS rabbits; aorta; vascular endothelium; acetylcholine; oxygen; endothelium-derived relaxing factor; free radicals

ABSTRACT

AIM: To observe the effect of change in oxygen tension on the release pattern and nature of endothelium-derived substances in isolated rabbit aorta. **METHODS:** Isometric contractions and relaxations in isolated rabbit strip were observed in response to changes in oxygen tension and effect of various drugs was noted on them. **RESULTS:** Change in oxygen tension from high [$p_{O_2} = (618.9 \pm 0.4)$ mmHg; 1 mmHg = 133.3 Pa] to low [$p_{O_2} = (117.6 \pm 0.6)$ mmHg] was observed to convert the relaxant effect of acetylcholine (ACh), in rabbit aorta precontracted with phenylephrine, to a marked contractile response. As the aerating gas was changed from 100 % to 20 % oxygen, the relaxant effect to ACh, recorded every hour, gradually decreased till it gave way to a significant contraction over a period of 3.5 h. On reoxygenation the relaxant effect to ACh was irreversibly inhibited, however, the relaxant effect to SOD (40 kU/L) was not. The *per se* constrictor effect to ACh was abolished by endothelium removal and by combination of SOD (40 kU/L), catalase (1000 kU/L) and indomethacin (1×10^{-5} mol/L). SQ-030741 (1×10^{-5} mol/L) or GR-32191B (1×10^{-5} mol/L), both TXA₂-PGH₂ receptor antagonists but not OKY-046, a TXA₂-synthetase inhibitor, also attenuated the ACh-mediated contractions in combination with SOD and catalase. **CONCLUSION:** The above results implicate that some functional change occurs in the endothelium exposed to low p_{O_2} such that the stimulated release of endothelium-derived relaxing factor (EDRF) in response to ACh is

completely and irreversibly inhibited, whereas, the basally released EDRF in response to SOD is not and marked increase in prostaglandin synthesis is stimulated.

INTRODUCTION

Endothelium-derived relaxing factor (EDRF) is continuously generated and released from endothelium intact vascular preparations^[1]. This continuous basal release of EDRF can be further enhanced by stimulation of both receptor-dependent (acetylcholine, ATP, bradykinin) and receptor independent (Ca^{2+} -ionophore A23187, polyocations) mechanisms^[2]. Mild to moderate changes in oxygen tension are observed to produce endothelium-dependent contractions either through reduced basal production of EDRF^[3,4] or through release of vasoconstrictor substances from the endothelium^[5-8].

The vascular endothelium acts as a local sensor and effector system for the regulation of oxygen supply to the tissues^[9]. The nature of the endothelium-based oxygen sensor is still unknown. Hypoxia is accompanied by a reduction in the tissue content of cyclic GMP^[10]. The threshold p_{O_2} (partial pressure for oxygen) for decrease in cGMP production is around 100 mmHg (1 mmHg = 133.3 Pa) in rabbit aorta^[9]. The first demonstration that a mild to moderate decrease in oxygen content could cause endothelium-dependent contractions was reported by Rubanyi and Vanhoutte^[5]. Organ bath p_{O_2} in the range of 150 mmHg has been reported to produce isometric contractions in the rabbit aorta^[11]. Endothelial cells exposed to hypoxia synthesize prostaglandins via a specific activation of phospholipase A₂ along with a rise in the cytosolic calcium^[8]. Decrease in oxygenation (from 680 mmHg to 150 and 40 mmHg) is reported to enhance cyclo-oxygenase activity via increase in COX-I (cyclo-oxygenase I) protein synthesis in ovine neonatal pulmonary endothelial cells and acute endothelium-dependent increases in prostaglandin I₂ (PGI₂, prostacyclin) and prostaglandin E₂ (PGE₂) have been demonstrated^[12].

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The cyclo-oxygenase activity in the rabbit endothelium is very high^[13-15] and all prostanoids, including PGI₂ and PGE₂ are reported to be vasoconstrictors in rabbit^[16-19].

In the present study, the effect due to change of oxygen tension from high ($p_{O_2} = 618.9 \pm 0.4$ mmHg) to low ($p_{O_2} = 117.6 \pm 0.6$ mmHg) was observed on the release pattern and nature of endothelium-derived relaxing factor (EDRF) and endothelium-derived contracting factor (EDCF) in isolated rabbit aorta.

MATERIALS AND METHODS

Isolated rabbit aorta preparation Albino rabbits of Belgium strain (either sex), weighing about 1.5–2 kg were maintained on rabbit feed (Agro Laboratory Animal Feed, India) and tap water *ad libitum*. The rabbits were stunned, the chest cavity was opened and the thoracic aorta was removed and placed in aerated physiologic salt solution (PSS). Extreme care was taken to avoid any possible damage to the endothelial lining of the aorta. The aorta, cut into a helical strip, was mounted in a 10 mL organ bath containing Krebs Henseleit solution (mmol/L: NaCl 120; KCl 4.8; NaHCO₃ 25; KH₂PO₄ 1.2; MgSO₄ 1.2; CaCl₂ 2.5, and glucose 11.1). The preparation was bubbled either with oxygen or air and was maintained and a pH of 7.4 at a temperature of 37 °C. The helical strip was equilibrated for 90 min under 2 g tension. The PSS was prepared fresh every hour and the tissue was superfused with PSS every 5 min. The responses were recorded using isometric force transducer (UGO Basile, Italy) and a 'Gemini' two channel recorder (UGO Basile, Italy).

The rabbit aortic strips were confirmed for the presence of an intact endothelium. Superoxide dismutase (SOD, 10 kU/L) relaxed precontracted (phenylephrine, PE, 3×10^{-7} mol/L) strips upto 50 % and 42 % when the rabbit aorta was bubbled with oxygen or air respectively. Moreover pyrogallol (1×10^{-4} mol/L) and methylene blue (1×10^{-5} mol/L) produced a further contracture in rabbit aortic strips precontracted with PE 3×10^{-7} mol/L. This relaxation to SOD^[20] and contraction to pyrogallol^[21] is endothelium-dependent and confirms its functional viability. To prepare an endothelium free preparation the intimal layer of the rabbit aorta was rubbed gently with a moistened filter paper for 30 s^[20]. Loss of relaxation to 10 kU/L SOD and 1×10^{-6} mol/L acetylcholine and loss of contraction to 1×10^{-4} mol/L pyrogallol confirmed the absence of endothelium.

Experimental design The rabbit aorta was equilibrated in physiologic salt solution aerated with oxygen for 90 min. A response to ACh (1×10^{-8} – 1×10^{-4} mol/L) was observed. The aeration was then changed from oxygen to air and response to ACh was observed at regular intervals upto 3.5 h. The aeration was again changed from air to oxygen and response to ACh (1×10^{-8} – 1×10^{-4} mol/L) was observed. The effect of various drugs was then observed on the relaxant and the contractile effect of ACh.

The functional ability of the endothelium to release basal EDRF was confirmed at the end of the experiment by exposing the precontracted rabbit aorta to SOD and pyrogallol. The smooth muscle relaxing viability of the preparation was tested with sodium nitroprusside at the end of each experiment.

Estimation of dissolved oxygen in physiological salt solution in organ bath Isolated aortic preparations of rabbit were exposed to PSS containing low oxygen (bubbled with air) and high oxygen (bubbled with 100 % oxygen). The amount of oxygen present in the PSS after bubbling with air or 100 % oxygen for 90 min was estimated using Alsterberg Azide modification method. The dissolved oxygen present in the sample of PSS oxidises divalent manganous to its higher valency (manganese) which precipitates as a brown hydrated oxide after addition of NaOH and KI. Upon acidification, manganese reverts back to divalent state and liberates iodine from KI equivalent to the dissolved oxygen content in the sample. The liberated iodine is titrated against Na₂S₂O₃ using starch as an indicator. The amount of dissolved oxygen was converted into partial pressure using Henry's Law^[22].

Drugs used L-Phenylephrine hydrochloride, N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME), SOD (bovine liver), and catalase were from Sigma, St Louis, MO, USA. Acetylcholine hydrochloride and pyrogallol were from Loba Chemie Indoaustralian Ltd, Bombay, India. Methylene blue was from Boots Pure Drugs, Nottingham, England and sodium nitroprusside from SD Fine Chemicals, Bombay, India. SQ-030741 was a gift from Bristol-Myers Squibb Pharma Research Institute, Princeton, NJ, USA, and GR-32191B a gift from Glaxo, Middlesex, England. Indomethacin was obtained from Ranbaxy Labs Ltd, Toansa, Panjab, India, nordihydroguaretic acid (NDGA) from Aldrich, WI, USA and, OKY-046 from Ono Pharmaceuticals, Osaka, Japan.

SQ-030741 was dissolved in a 0.84/0.16 mixture by volume of ethanol and 1×10^{-3} mol/L Na_2CO_3 . Indomethacin was sonicated in 10 mL of 5×10^{-3} mol/L Na_2CO_3 . NDGA was dissolved in ethanol. Further dilutions were made with distilled water. All the rest were dissolved in distilled water and dilutions were made with physiologic salt solution.

Statistical analysis Relaxation and contraction were measured as actual decrease or increase in tension, measured in grams, respectively. Results were expressed either as $\bar{x} \pm s$ or % response ($\bar{x} \pm s$). Paired *t*-test was applied and a *P* value ≤ 0.05 was considered significant.

RESULTS

Acetylcholine (1×10^{-9} – 1×10^{-4} mol/L) evoked dose-dependent relaxation in phenylephrine precontracted (3×10^{-7} mol/L) isolated rabbit aortic strips equilibrated in Krebs Henseleit solution bubbled with oxygen (p_{O_2} in organ chamber = 618.9 ± 0.4 mmHg) (Fig 1). However same doses of ACh were observed to produce contractions in PE (3×10^{-7} mol/L) precontracted rabbit aorta equilibrated in physiologic salt solution bubbled with air

(p_{O_2} in organ chamber = 117.6 ± 0.6 mmHg) (Fig 1). The observed relaxation and contraction were attenuated by endothelium removal (Fig 1). SOD (10, 20, and 40 kU/L) produced marked relaxation in precontracted preparations (Fig 2) bubbled with oxygen and air. Pyrogallol (1×10^{-6} mol/L and 1×10^{-4} mol/L) and methylene blue (1×10^{-5} mol/L) produced contractile responses in precontracted tissues (Fig 3) bubbled with air. Both the relaxation to SOD and the contraction to pyrogallol and methylene blue were sensitive to endothelium removal (Fig 2, 3). The relaxant response to ACh was inhibited by *L*-NAME (1×10^{-5} mol/L) and pyrogallol (1×10^{-6} mol/L) (Fig 4).

In the experimental sequence when the aerating gas was changed from O_2 to air, a complete reversal from a relaxant response to a contractile response was observed to acetylcholine (1×10^{-8} – 1×10^{-4} mol/L) in isolated rabbit aorta with intact endothelium (Fig 5). This reversal from relaxation to contraction did not occur immediately and more than three hours exposure to reduced p_{O_2} was required (Fig 5). In a control experiment run for the same duration of time with the aortic strip aerated with 100 % oxygen, no tachyphylaxis of the relaxant response to ACh in the same doses was observed. SOD

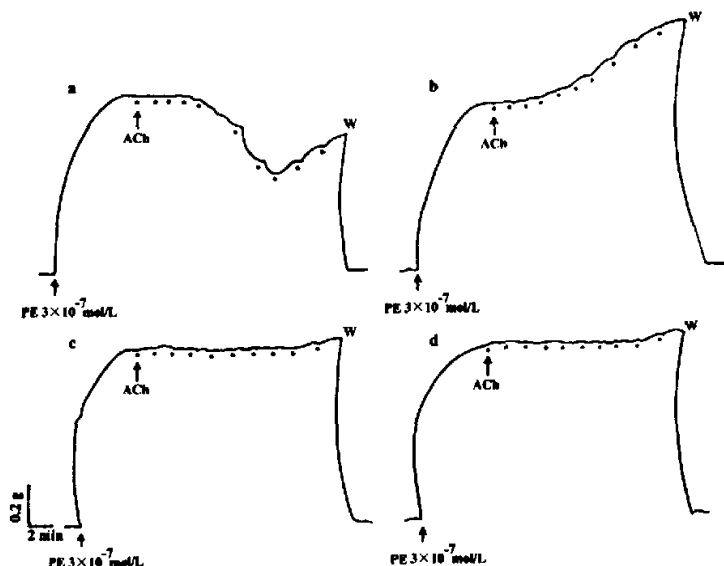


Fig 1. Acetylcholine (ACh, 1×10^{-9} – 1×10^{-4} mol/L)-evoked responses in phenylephrine (PE) pre-contracted isolated rabbit aorta, aerated with oxygen (a, c) and air (b, d) in preparations with intact endothelium (a, b) and denuded endothelium (c, d). $n = 5$ in both groups. For detailed ACh doses see Fig 6b.

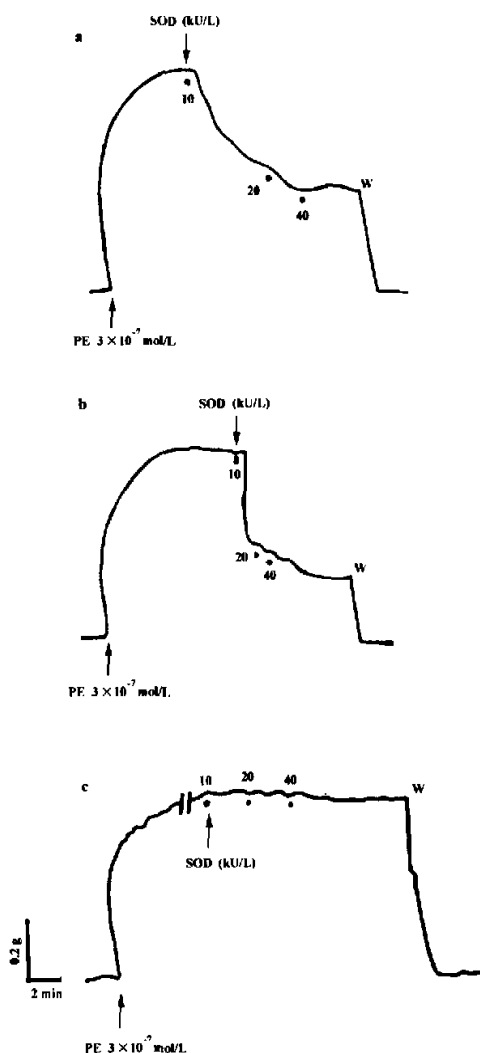


Fig 2. Superoxide dismutase (SOD; 10, 20, and 40 kU/L)-evoked responses in phenylephrine (PE) pre-contracted isolated rabbit aorta with intact endothelium and aerated with air (a) and oxygen (b) and denuded endothelium aerated with oxygen (c). $n = 5$ in both groups.

(10, 20, and 40 kU/L) and pyrogallol (1×10^{-6} mol/L and 1×10^{-4} mol/L) respectively relaxed and contracted the pre-contracted rabbit aorta at the end of the experiment (Fig 5). Sodium nitroprusside (1×10^{-8} – 1×10^{-4} mol/L), was observed to completely relax the pre-contracted rabbit aorta at the end of the experiment (result not shown).

The *per-se* contractile responses observed with ACh (1×10^{-9} – 1×10^{-4} mol/L) and contractions to ACh in

PE precontracted aorta were attenuated by a combination of SOD, catalase, and indomethacin (Fig 6a, 6b) but not by pretreatment with either SOD (40 kU/L) or catalase (1000 kU/L) alone (results not shown). To overcome the *per-se* relaxant effect of SOD, the precontracted tissue was first treated with catalase, and then SOD. Catalase is known to reduce/inhibit SOD-induced relaxation^[6]. Though the contractile effect to ACh was abolished no relaxant effect was observed after the above treatment in pre-contracted tissues as seen in Fig 6a. ACh-mediated contractions were not affected by OKY-046, a thromboxane synthetase inhibitor (result not shown) but were inhibited by SQ-030741 (1×10^{-5} mol/L) (Fig 7a) and GR-32191B (1×10^{-5} mol/L) (Fig 7b), both $\text{PGH}_2\text{-TXA}_2$ receptor antagonists. SQ-030741/GR-32191B in combination with SOD and catalase fully attenuated the ACh-evoked contractions (Fig 7a, 7b). NDGA, a lipoxygenase inhibitor had no effect on the ACh-mediated contractions (result not shown).

The direct effect of SOD (40 kU/L) on ACh-mediated relaxation could not be gauged as its *per-se* relaxation (about 70 %) masked any potentiation of ACh mediated relaxation (Fig 8a). ACh mediated relaxations were highly accentuated when treated with catalase, SOD, and indomethacin (Fig 8b). Catalase was added before SOD to prevent its *per-se* relaxation.

DISCUSSION

Acetylcholine (ACh) produced concentration-dependent relaxations in isolated rabbit aorta pre-contracted with phenylephrine and bubbled with oxygen. These relaxations were endothelium-dependent as removal of endothelium attenuated the responses. The observed relaxation to ACh was mediated through the release of nitric oxide as *L*-NAME, a specific inhibitor of nitric oxide synthase, attenuated the responses. Pyrogallol rapidly autoxidises in oxygen-containing aqueous medium and generates superoxide anions^[23] and EDRF is reported to be chemically inactivated by superoxide anions^[20]. This may be the reason of pyrogallol mediated inhibition of the endothelium-dependent relaxations to ACh.

In this study less endothelium-dependent relaxations to ACh were observed as compared to those observed by Furchgott and Zawadzki^[24]. ACh is known to elicit endothelium-dependent relaxations through both the stimulated and basal release of EDRF^[2]. In the present study

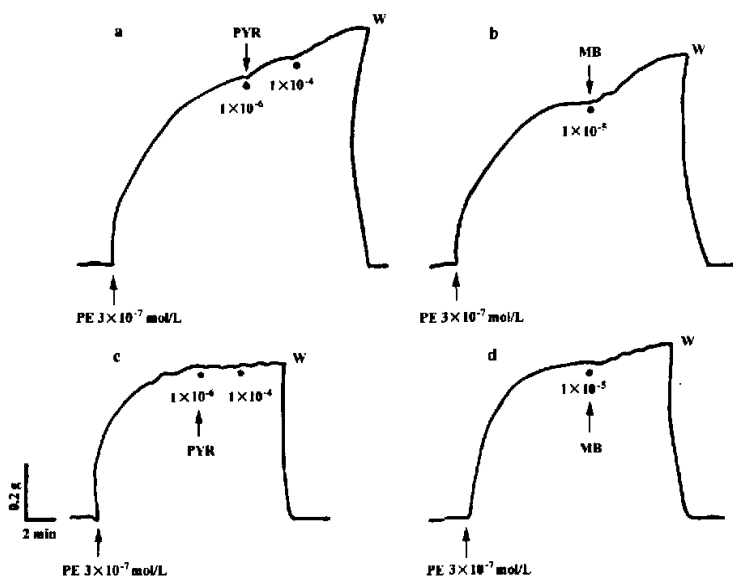


Fig 3. Effect of pyrogallol (PYR, 1×10^{-6} and 1×10^{-4} mol/L) and methylene blue (MB, 1×10^{-5} mol/L) on phenylephrine (PE) pre-contracted isolated rabbit aorta with intact endothelium (a, b) and denuded endothelium (c, d) aerated with air. $n = 5$ in each group.

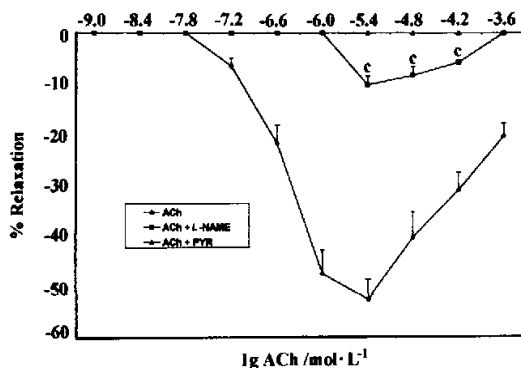


Fig 4. Effect of N^G -nitro- L -arginine methyl ester (L-NAME, 1×10^{-4} mol/L) and pyrogallol (PYR, 1×10^{-4} mol/L) on acetylcholine (ACh, 1×10^{-9} - 1×10^{-4} mol/L)-evoked endothelium-dependent relaxations in isolated rabbit aorta aerated with oxygen. $^*P < 0.01$ vs ACh mediated relaxation. $n = 5$ in both groups.

only the stimulated release of EDRF in response to ACh is evident. The basally released EDRF might be getting simultaneously destroyed by superoxide anions which are present abundantly in hyperoxic conditions^[20] produced by aeration with 100 % oxygen.

The viability of the endothelium to produce basally released EDRF is evident from the observation that SOD (40 kU/L) produced a marked relaxation (about 70 %

in isolated rabbit aorta precontracted with phenylephrine. SOD, by scavenging superoxide anions, unmasked the basally released EDRF. However, the protective effect of SOD on ACh-induced relaxation could not be observed as the marked *per-se* relaxation to SOD masked the ACh-mediated relaxation. Pyrogallol, which rapidly autoxidises in oxygen-containing aqueous medium to generate superoxide anions^[23] and methylene blue, a potent generator of superoxide anions^[25,26] were observed to further contract preparations pre-contracted with phenylephrine. This overshoot in tone is due to inhibition of the spontaneously released EDRF^[20,21]. Thus the ability of the endothelium to produce basally released EDRF is further confirmed. Removal of endothelium abolished the relaxant response to SOD and the contractile response to pyrogallol and methylene blue. The contractile response to methylene blue was partially inhibited as it is reported to elicit some direct action on the smooth muscle^[26].

As the aerating gas was changed from oxygen [$pO_2 = (618.9 \pm 0.4)$ mmHg] to air [$pO_2 = (117.6 \pm 0.6)$ mmHg] it was observed that the relaxant effect to ACh, recorded every hour, gradually decreased till it gave way to a significant contractile response. This occurred over a period of 3.5 h. When the aerating gas was changed back to oxygen, no reappearance of the relaxant effect to ACh was noticed. This implies that some functional

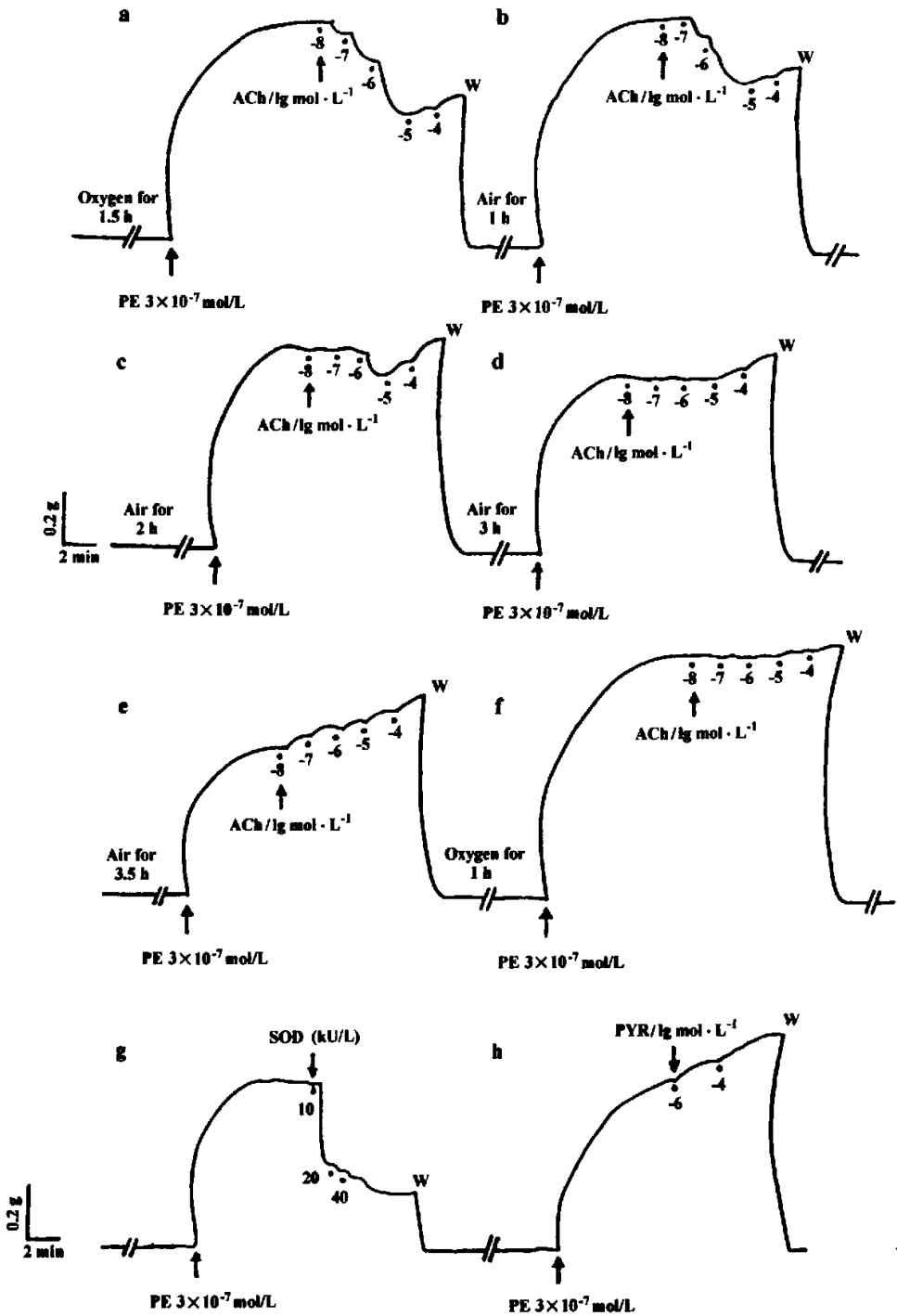


Fig 5. Effect of change of aerating gas from oxygen to air (a, b, c, d, e) and from air to oxygen (f) and effect of SOD (10, 20, and 40 kU/L , g) and pyrogallol (PYR, 1×10^{-6} and $1 \times 10^{-4} \text{ mol/L}$, h) on endothelium-dependent acetylcholine (ACh)-evoked responses in phenylephrine (PE) pre-contracted isolated rabbit aorta. $n = 4$.

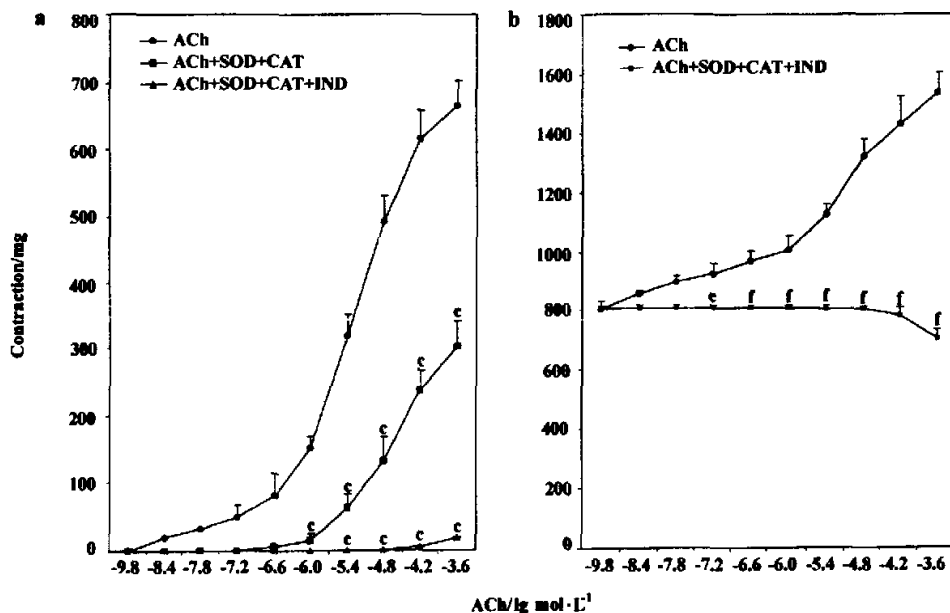


Fig 6. Effect of superoxide dismutase (SOD, 40 kU/L), catalase (CAT, 1000 kU/L) and, indomethacin (IND, 1×10^{-5} mol/L) on acetylcholine (ACh)-evoked endothelium-dependent contractions in quiescent (a) and pre-contracted (b) isolated rabbit aorta aerated with air. * $P < 0.01$ vs quiescent ACh contractions. * $P < 0.05$, † $P < 0.01$ vs ACh contractions in pre-contracted aorta. The tissue was incubated in indomethacin, catalase, and SOD for 30, 10, and 2 min respectively. $n = 5$ in both groups.

change occurs in the endothelium exposed to low pO_2 such that the stimulated release of EDRF in response to ACh is completely inhibited. However, the endothelium does not lose its ability to release basal EDRF as marked relaxation to SOD was observed at the end of the experiment. These observations tentatively suggest that a prolonged exposure to low pO_2 may have produced an irreversible change in the physiologic function of the endothelium.

The functionally compromised endothelium was observed to release some contractile factor which was sensitive to attenuation by a combination of SOD, catalase and indomethacin. This implies the role of prostaglandins and some reactive oxygen intermediates in the generation of the observed contractions. OKY-046, a thromboxane synthetase inhibitor, did not alter the ACh-evoked contractions. However, almost complete inhibition was noted when SOD and catalase were used in combination with SQ-030741 and GR-32191B. This suggests that PGH_2 , the precursor prostaglandin, or other prostaglandins (except TXA_2) and hydroxyl radicals generated simultaneously as a result of conversion of PGG_2 to PGH_2 may be responsible for the contractions evoked by ACh. It is

reported that activation of cyclo-oxygenase not only leads to enhanced production of prostaglandins but also generates reactive oxygen intermediates^[27]. SQ-030741 and GR-32191B are not selective antagonists to PGH_2/TXA_2 as antagonism at this site leads to inhibition of the production of prostaglandins such as $PGF_{2\alpha}$, PGE_2 , PGD_2 , and PGI_2 . Hypoxic endothelium is reported to enhance the release of prostanoids, especially prostacyclin^[8,11]. Basal production of prostacyclin is reported to increase in pulmonary endothelial cells when the pO_2 is decreased from 680 mmHg to 150 mmHg^[12] and also in hypoxic rabbit aorta with intact endothelium^[11]. It has also been reported to be a vasoconstrictor in rabbit aorta^[15]. Cholinergic agents have also been reported to stimulate PGI_2 synthesis in the rabbit aortic endothelium^[28]. Thus the contractions may be caused by PGI_2 in the present study seems more plausible as the low pO_2 employed may not be enough of a hypoxic stimulation to release an EDCF but is enough to produce an enhanced release of prostacyclin. The contractile effect to ACh thus may be partially due to prostaglandins other than TXA_2 , most probable of which is prostacyclin, and some free radical generated along with under low oxygen tension conditions.

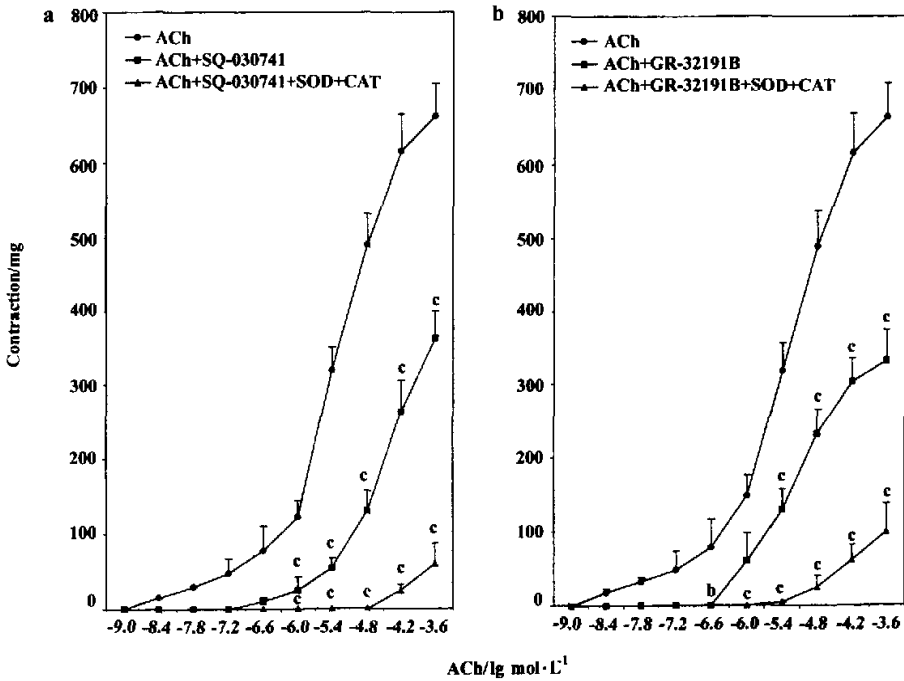


Fig 7. Effect of SQ-030741 (1×10^{-5} mol/L, a), GR-3219B (1×10^{-5} mol/L, b) and a combination of SQ-030741/GR-3219B with catalase (CAT, 1000 μ /mL) and superoxide dismutase (SOD, 40 kU/L) on acetylcholine-evoked endothelium-dependent contractions in rabbit aorta aerated with air. The tissue was incubated with SQ-030741/GR-3219B, catalase, and SOD for 10, 10, and 2 min respectively. ^b $P < 0.05$, ^c $P < 0.01$ vs ACh mediated contractions. $n = 5$ in both groups.

In conclusion, it was observed in this study that there seem to be an oxygen sensor in the endothelial cells which appears to be sensitive towards even mild changes in the oxygen tension and can differentiate between the endothelial cells sensitivity towards stimulated and basally released EDRF. Also that the functionally compromised endothelium releases some contractile factor which may be a prostanoid other than thromboxane and some oxygen free radical generated alongwith.

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REFERENCES

1 Ignarro LJ. Biological actions and properties of endothelium-

derived nitric oxide formed and released from artery and vein. *Circ Res* 1989; 65: 1-21.
 2 Newby AC, Henderson AH. Stimulus-secretion coupling in vascular endothelial cells. *Ann Rev Physiol* 1990; 52: 661-74.
 3 Rubanyi GM, Gräser T. Hypoxic contraction in rat aorta is due to inhibition of EDRF action on vascular smooth muscle. *Circulation* 1991; 84: II-655.
 4 Simmonet S, Porro de Baillencourt J, Descombes JJ, Mennecier P, Lanbie M, Verbeuren TJ. Hypoxia causes an abnormal contractile response in the arteriosclerotic rabbit aorta. Implication of reduced NO and cGMP production. *Circ Res* 1993; 72: 616-50.
 5 Rubanyi GM, Vanhoutte PM. Hypoxia releases a vasoconstrictor substance from the canine vascular endothelium. *J Physiol* 1985; 364: 45-56.
 6 Rubanyi GM, Vanhoutte PM. Oxygen-derived free radicals, endothelium and responsiveness of vascular smooth muscle. *Am J Physiol* 1996; 250: 4815-21.
 7 Kovitz KL, Aleskowitz TD, Sylvester JT, Flavahan NA. Endothelium-derived contracting and relaxing factors contribute to hypoxic responses of pulmonary arteries. *Am J Physiol* 1993; 245: 41139-48.
 8 Michiels C, Amould T, Knott I, Dieu M, Ramacle J. Stimulation of prostaglandin synthesis by human endothelial cells

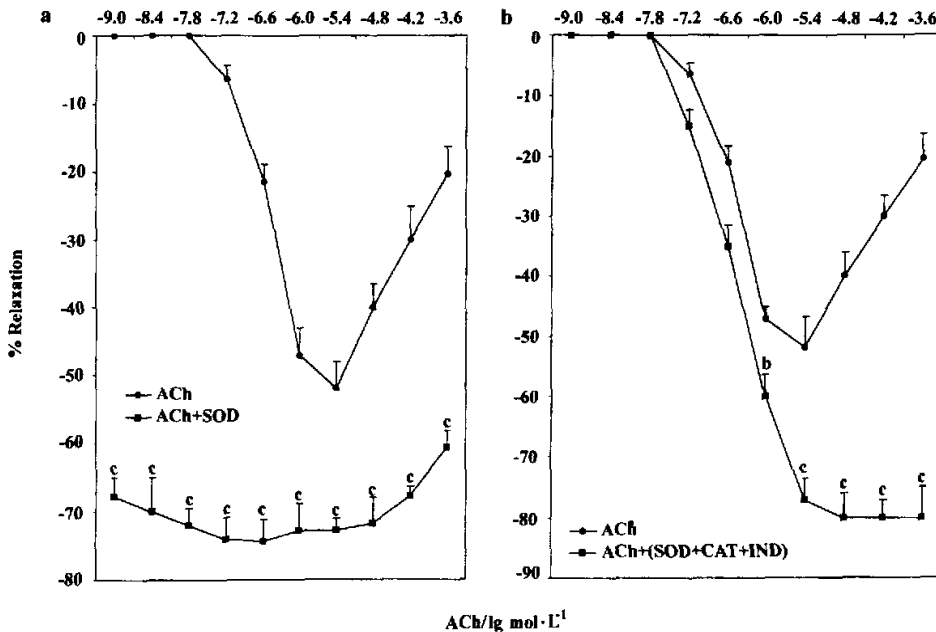


Fig 8. Effect of superoxide dismutase (SOD, 40 kU/L) and a combination of SOD (40 kU/L), catalase (CAT, 1000 kU/L) and indomethacin (IND, 1×10^{-5} mol/L) on acetylcholine (ACh)-evoked endothelium-dependent relaxations in pre-contracted isolated rabbit aorta aerated with oxygen. The tissue was incubated with indomethacin, catalase, and SOD for 30, 10, and 2 min respectively. ^b*P* < 0.05, ^c*P* < 0.01 vs ACh-mediated relaxation. *n* = 5 in both groups.

- exposed to hypoxia. *Am J Physiol* 1993; 264: C866 - C874.
- 9 Coburn RF, Eppinger R, Scott DP. Oxygen-dependent tension in vascular smooth muscle; Does the endothelium play a role? *Circ Res* 1986; 58: 341 - 7.
 - 10 Gräser T, Vanhoutte PM. Hypoxic contraction of canine coronary arteries. Role of endothelium and cGMP. *Am J Physiol* 1991; 261: H1769 - H1777.
 - 11 Pohl V, Büsse R. Hypoxia stimulates the release of endothelium-derived relaxant factor. *Am J Physiol* 1989; 256: H1595 - H1600.
 - 12 North AJ, Brannon TS, Wells LB, Campbell WB, Shaul PW. Hypoxia stimulates prostacyclin synthesis in newborn pulmonary artery endothelium by increasing cyclooxygenase-1 protein. *Circ Res* 1994; 75: 33 - 40.
 - 13 Singer HA, Peach MJ. Endothelium-dependent relaxation of rabbit aorta: 1. Relaxation stimulated by arachidonic acid. *J Pharmacol Exp Ther* 1983; 226: 790 - 5.
 - 14 Papp AC, Crowe L, Pettigrew LC, Wu KK. Production of eicosanoids by de-endothelialized rabbit aorta; Interaction between platelets and vascular wall in the synthesis of prostaglandin. *Thromb Res* 1986; 42: 549 - 56.
 - 15 Pagano PJ, Lin L, Sessa WC, Nasjletti A. Arachidonic acid elicits endothelium-dependent release from the rabbit aorta of a constrictor prostanoid resembling prostaglandin endoperoxides. *Circ Res* 1991; 69: 396 - 405.
 - 16 Borda ES, Sterin-Borda L, Gimeno MF, Lazzari MA, Gimeno AL. The stimulatory effect of prostacyclin on isolated rabbit and rat aorta is probably associated to the generation of thromboxane A₂ like material. *Arch Int Pharmacodyn Ther* 1983; 261: 79 - 89.
 - 17 Van Dam J, Maddox YT, Ramwell PW, Kot PA. Role of the vascular endothelium in the contractile response to prostacyclin in the isolated rat aorta. *J Pharmacol Exp Ther* 1986; 239: 390 - 4.
 - 18 Tesfamariam B, Jakubowski JA, Cohen RA. Contraction of diabetic rabbit aorta due to endothelium-derived PGH₂/TXA₂. *Am J Physiol* 1989; 257: H1327 - H1333.
 - 19 Cohen RA. Dysfunction of vascular endothelium in diabetes mellitus. *Circulation* 1993; 87: V67 - V76.
 - 20 Ignarro LJ, Byrns RE, Buga GM, Wood KS, Chaudhuri G. Pharmacological evidence that endothelium-derived relaxing factor is nitric oxide; Use of pyrogallol and superoxide dismutase to study endothelium-dependent and nitric-oxide elicited vascular smooth muscle relaxation. *J Pharmacol Exp Ther* 1988; 244: 181 - 9.
 - 21 Goldschmidt JE, Tallarida RJ. Pharmacological evidence that captopril possesses an endothelium-mediated component of vasodilation; Effect of sulfhydryl groups on endothelium-derived relaxing factor. *J Pharmacol Exp Ther* 1991; 257: 1136 - 45.
 - 22 Willard HH, Merritt LL, Dean JA, Settle FA. Solutions. In: *Instrumental methods of analysis*; 6th ed. USA:

Wadsworth Publishers; 1986. p 811 - 5.

23 Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 1994; 47: 469 - 74.

24 Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; 288: 373 - 6.

25 Martin W, Villani AM, Jothianandan D, Furchgott RF. Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by haemoglobin and by methylene blue in the rabbit aorta. *J Pharmacol Exp Ther* 1985; 232: 708 - 16.

26 Marczin N, Ryan US, Catravas JD. Methylene blue inhibits nitro vasodilator and endothelium-derived relaxing factor-induced cyclic GMP accumulation in cultured pulmonary arterial smooth muscle cells via generation of superoxide anion. *J Pharmacol Exp Ther* 1992; 263: 170 - 9.

27 Lüscher TF, Boulanger CM, Dohi Y, Yang ZH. Endothelium-derived contracting factors. *Hypertension* 1992; 19: 117 - 30.

28 Tesfamariam B, Brown ML, Derykin D, Cohen RA. Elevated glucose promotes generation of endothelium-derived vasoconstrictor prostanoids in rabbit aorta. *J Clin Invest* 1990; 85: 929 - 32.

氧张力变化对离体兔主动脉内皮细胞释放物质的释放模式和状态的作用

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关键词 兔; 主动脉; 血管内皮; 乙酰胆碱; 氧; 内皮细胞舒血管因子; 自由基

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The American Physiological Society will host the following two conferences in 2001:

Conference 1: "Cellular and Molecular Physiology of Sodium-Calcium Exchange"
2001 October 10 - 14, Banff, Alberta, Canada

Conference 2: "Genome & Hormones: An Integrative Approach to Gender Differences in Physiology"
2001 October 18 - 20 Pittsburgh, Pennsylvania

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