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Role of pentose phosphate pathway-derived NADPH in hypoxic pulmonary vasoconstriction

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Abstract

We have previously shown that pentose phosphate pathway (PPP) inhibitors, 6-aminonicotimade (6-AN) and epiandrosterone (EPI), markedly reduce hypoxic pulmonary vasoconstriction (HPV). Although it has been suggested that changes in the NADPH/$NADP^+$ ratio and redox status are involved in the mechanism of HPV, the role of PPP-derived NADPH in this phenomenon is not known. The aim of this study, therefore, was to investigate the role of PPP-derived NADPH in HPV using isolated rat pulmonary arteries (PA) and perfused rat lungs. The NADPH/$NADP^+$ ratio and NADPH levels in PA and lungs exposed to hypoxia increased 2-fold and 7-fold, respectively, compared to time-matched normoxic controls. Both hypoxia-induced increases in lung NADPH levels and lung perfusion pressure were inhibited by 6-AN (500 μM) or EPI (300 μM). The chemical inhibitors of PPP and hypoxia similarly decreased lung tissue NOx levels by approximately 50%. In contrast, hypoxia increased the lung soluble guanylate cyclase (sGC) activity (from 22.9 ± 6.3 to 57.1 ± 7.6 pmol/min/g), which was prevented by PPP inhibitors. ODQ, a sGC inhibitor, potentiated HPV. These results suggest that while PPP-derived NADPH may play a significant role in HPV, it may also moderate the magnitude of HPV through activation of the NO-sGC-cGMP vasodilation pathway.

Keywords: 6-Aminonicotinamide; Epiandrosterone; Nitric Oxide; Guanylate Cyclase

Pulmonary arteries (PA) constrict in response to hypoxia, low partial oxygen tension (Po_2), to help maintain a balance between perfusion and ventilation ratio in the lungs. The cellular and molecular mechanisms involved in this phenomenon, hypoxic pulmonary vasoconstriction (HPV), have been extensively investigated, but the exact mechanisms responsible for the response are not yet well understood.

In 1986, Archer and Weir proposed that mitochondria in pulmonary artery smooth muscle cells (PASMC) were the primary PO_2 sensor. Subsequently, these authors suggested that hypoxic inhibition of free radical production and changes in the ratios of cytosolic reducing co-factors, such as NADPH/$NADP^+$, inactivate voltage-gated K^+ (K_v) channels in PASMC, resulting in membrane depolarization, Ca^{2+} influx, and vasoconstriction [1,27]. Although it now appears that hypoxic inhibition of the K_v channel may contribute to the initiation of HPV, the precise sequence of events that leads to the blockade of K^+ currents is still unclear. It also remains controversial whether reactive oxygen species production is decreased or increased during hypoxia [1,16,25–28].

Recently, we have demonstrated that inhibition of the pentose phosphate pathway (PPP) by 6-aminonicotinamide (6-AN) increases K_v currents in PASMC [5], epiandrosterone (EPI) attenuates L-type Ca^{2+} channels in cardiac myocytes, and 6-AN & EPI reduce Ca^{2+} release and influx thus eliciting dilatation of coronary artery [4,8]. Furthermore, studies indicate that NAD(P)H, NAD(P^+) and glutathione regulate rabbit PASMC voltage-gated and calcium-sensitive K^+ channels [12,19,20]. These channels are opened by the oxidizing agents and are inhibited by the reducing co-factors, respectively, either by directly binding to the channel protein or by regulating the redox state of residue cysteine residue on the protein [12,27]. These studies suggest that NADPH redox regulates ion channel activity and Ca^{2+} release mechanisms in vascular smooth muscle cells.

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The PPP generates most of the major reducing co-factor NADPH and co-ordinates multiple redox reactions in cells. We have recently demonstrated that PPP inhibitors reduce lung tissue NADPH production under normoxic conditions and attenuate HPV [5]. Although it seems likely that changes in the NADPH/$NADP^+$ ratio and redox status may be important in HPV [28], the role of PPP-derived NADPH has not been well investigated. Therefore, one aim of this study was to test if PPP-derived NADPH production is increased during hypoxia and plays a role in HPV.

In addition, it is well established that NADPH is an essential co-factor for nitric oxide (NO) synthase [13]. Furthermore a NADPH-dependent reductase also appears to be required to prevent the oxidation of iron in the heme group of soluble guanylate cyclase (sGC) and the inhibition of sGC activity by oxidizing agents and oxygen in bovine PA and coronary artery [7,9]. The NO-sGC pathway is regarded as an important modulator of HPV [10]. Activity of this pathway could be impaired under hypoxic conditions due to a decrease of NO synthesis resulting from suppression of oxygen-dependent NO synthase activity [10,13]. Hence, a second objective of this study was to investigate the roles of PPP-derived NADPH on the NO level and sGC activity during hypoxia.

1. Material and methods

The Institutional Animal Use Committee of Juntendo University School of Medicine (Tokyo, Japan) approved all protocols and surgical procedures, which were in accordance with National Institutes of Health and American Physiological Society guidelines.

6-AN, EPI, angiotensin II (A-II), and other salts were purchased from Sigma (St. Louis, MO). The stock solutions of steroids were made either in ethanol (Sigma) or dimethyl sulfoxide (DMSO; Sigma), and final 1:1000 dilutions in buffered physiological salt solution were used in the study. 1H-[1,2,4] Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; Cayman Chemical, MI, USA) was dissolved in DMSO.

1.1. Animals

Experiments were performed with adult male Sprague-Dawley (SD) rats (250–350 g). The rats were housed at the ambient barometric pressure. All rats were exposed to a 12:12-h light-dark cycle and allowed free access to standard rat food and water.

1.2. Determination of pulmonary arterial pressure of isolated perfused rat lungs

Isolated lungs were prepared as previously described [17] with minor modifications. The lungs were isolated from rats after intraperitoneal administration of 30 mg of pentobarbital sodium and intracardiac injection of 100 U of heparin. Isolated lungs were ventilated with a humid mixture of 21% O_2–5% CO_2–74% N_2 at 60 breaths/min, with an inspiratory pressure of 9-cm H_2O and an end-expiratory pressure of 2.5 cm H_2O. They were perfused through a main pulmonary arterial cannula with a peristaltic pump at a constant flow of 0.04 ml/g body wt/min. The perfusate was a physiological salt solution (PSS) containing (in mM) 116.3 NaCl, 5.4 KCl, 0.83 $MgSO_4$, 19.0 $NaHCO_3$, 1.04 NaH_2PO_4, 1.8 $CaCl_2 \cdot 2H_2O$, and 5.5 D-glucose (Earle's balanced salt solution; Sigma). Ficoll (4 g/100 ml, type 70; Pharmacia, Uppsala, Sweden) was included as a colloid and meclofenamate (3.1 μM) was added to inhibit cyclooxygenase and production of vasodilator prostaglandins. After blood was flushed out of the lungs with 20 ml of the PSS, the lungs were perfused with a re-circulated volume of 30 ml. Effluent perfusate drained from a left ventricular cannula into a perfusate reservoir. Lung and perfusate temperatures were maintained at 37 °C, and the pH of the perfusate was kept between 7.3 and 7.4. The pulmonary perfusion pressure was measured continuously with a transducer (Nihon Kohden; RMP-6004, Tokyo, Japan) and a pen recorder, and the lungs were equilibrated for 20 min before vascular responses were elicited. After equilibration, the lungs were challenged four times with alternating arterial injection of 0.05 μg A-II and 5-min periods of hypoxic ventilation (0% O_2–5% CO_2–95% N_2). 6-AN, EPI, or their vehicle (ethanol + DMSO) was added to perfusate 15 min prior to the fourth injection of A-II. The maximal increase in perfusion pressure over baseline in response to a given stimulus was measured as the pressor response.

1.3. Determination of changes in force of isolated rat PA

PA (lobar branch) was prepared as previously described [5] with minor modifications. The rings were mounted on wire hooks attached to force displacement transducers (TB 611T; Nihon Kohden, Tokyo, Japan) for measurement of changes in the isometric force. Resting passive force was adjusted to a previously determined optimum (determined by maximum response to 80 mM KCl: 750 mg for PA), and vessels were equilibrated for 1 h in muscle baths containing Earle's balanced salt solution, gassed with 21% O_2/5% CO_2/74% N_2. In the endothelium-intact phenylephrine pre-constricted PA rings, acetylcholine elicited $>80\%$ relaxation. Subsequently, PA was contracted with U46619 (10–50 nM) to study hypoxic vasoconstriction. After arteries reached a stable steady state contraction with U46619, they were exposed to 5 min hypoxia and then re-oxygenated for 5–10 min. All values for vasoconstriction and vasodilation are expressed as percentage change of the precontracted force.

1.4. Determination of NADPH levels in isolated lungs

The levels of NAD(P)H in isolated lungs were determined by HPLC after slight modification of previously published methods [7]. Briefly, isolated, normoxia and hypoxia-ventilated lungs were treated with 500 μM 6-AN, 300 μM EPI, or vehicle for 30 min and then freeze-clamped in liquid nitrogen. The frozen tissues were homogenized in an extraction medium consisting of 2 ml of 0.02 N NaOH containing 0.5 mM