NO inhalation reduces pulmonary arterial pressure but not hemorrhage in maximally exercising horses

CASEY A. KINDIG,1 PAUL MCDONOUGH,1 MELISSA R. FINLEY,1 BRAD J. BEHNKE,1 TROY E. RICHARDSON,1 DAVID J. MARLIN,2 HOWARD H. ERICKSON,1 AND DAVID C. POOLE1

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Kindig, Casey A., Paul McDonough, Melissa R. Finley, Brad J. Behnke, Troy E. Richardson, David J. Marlin, Howard H. Erickson, and David C. Poole. NO inhalation reduces pulmonary arterial pressure but not hemorrhage in maximally exercising horses. J Appl Physiol 91: 2674–2678, 2001.—In horses, the exercise-induced elevation of pulmonary arterial pressure (Ppa) is thought to play a deterministic role in exercise-induced pulmonary hemorrhage (EIPH), and thus treatment designed to lower Ppa might reasonably be expected to reduce EIPH. Five Thoroughbred horses were run on a treadmill to volitional fatigue (incremental step test) under nitric oxide (NO; inhaled 80 ppm) and control (N2, same flow rate as per NO run) conditions (2 wk between trials; order randomized) to test the hypothesis that NO inhalation would reduce maximal Ppa but that this reduction may not necessarily reduce EIPH. Before each investigation, a microtipped pressure transducer was placed in the pulmonary artery 8 cm past the pulmonic valve to monitor Ppa. EIPH severity was assessed via bronchoalveolar lavage (BAL) 30 min postrun. Exercise time did not differ between the two trials (P > 0.05). NO administration resulted in a small but consistent and significant reduction in peak Ppa (N2, 102.3 ± 4.4; NO, 98.6 ± 4.3 mmHg, P < 0.05). In the face of lowered Ppa, EIPH severity was significantly higher in the NO trial (N2, 22.4 ± 6.8; NO, 42.6 ± 15.4 × 106 red blood cells/ml BAL fluid, P < 0.05). These findings support the notion that extremely high Ppa may reflect, in part, an arteriolar vasoconstriction that serves to protect the capillary bed from the extraordinarily high Ppa evoked during maximal exercise in the Thoroughbred horse. Furthermore, these data suggest that exogenous NO treatment during exercise in horses may not be poor prophylaxis but may actually exacerbate the severity of EIPH.

Bronchoalveolar lavage; exercise-induced pulmonary hemorrhage; nitric oxide

CONSTITUTIVE NITRIC OXIDE (NO), synthesized from L-arginine and O2 in a reaction catalyzed by NO synthase, is critical for basal blood flow and pressure regulation across many organs and species. Therapeutic NO inhalation designed to lower pulmonary hypertension in human patients with both primary and secondary pulmonary hypertension has been successful (24). In horses, mean pulmonary arterial pressure (Ppa) can exceed 120 mmHg during high-intensity running, which is thought to contribute substantially to pulmonary capillary stress failure-induced pulmonary hemorrhage (22, 27, 28). In this regard, Mills et al. (15, 16) demonstrated that NO inhalation reduced mean Ppa significantly in Thoroughbred horses performing a short-duration, single bout of high-intensity treadmill exercise; however, exercise-induced pulmonary hemorrhage (EIPH) was not measured. Under the presumption that lowered Ppa would reduce EIPH, Mills (14) concluded that prophylactic treatment with an exogenous NO source may be beneficial in constraining the severity of EIPH in horses.

The Ppa-to-cardiac output ratio (normalized to body weight) is elevated in the exercising horse compared with humans (18). Regardless of the mechanism, this relationship is consistent with the notion that equine pulmonary arterioles do not vasodilate to the extent seen in the human and furthermore that arterioles set the majority of total transpulmonary vascular resistance in the horse. In concert with low venomous resistance, it has been suggested that the arteriolar bed provides a protective vasomotor tone to constrain the exercise-induced rise in downstream vascular pressures (i.e., capillary, venular), thereby helping to preserve capillary structural integrity (25). Experimental support for this rationale comes from the observation that systemic nitro-L-arginine methyl ester (NO synthase inhibitor) administration reduces peak Ppa concomitant with elevated EIPH severity in maximally exercising horses (5, 6).

From the above, although the mechanism remains unclear, an elevated Ppa-to-cardiac output ratio may actually be beneficial for preserving capillary integrity during exercising conditions. Consequently, treatment designed to reduce Ppa in the face of unchanged cardiac output (15) may evoke deleterious rather than therapeutic results. Hence, the present investigation
was designed to test the hypothesis that NO inhalation during maximal exercise in horses would reduce peak mean Ppa but that such a reduction may not necessarily be associated with a mitigation in the severity of EIPH.

METHODS

Five healthy Thoroughbred geldings (age 7 ± 1 yr) trained twice weekly were used in this study. The animals were housed in a dry lot with free access to water and were fed twice daily. Food and water were withheld for 4 h before exercise testing. All procedures were approved by the Kansas State University Institutional Animal Care and Use Committee.

Animal preparation and data acquisition. Before each experiment, each horse had two 7-Fr introducer catheters placed in the right jugular vein and one catheter placed in either a previously elevated left carotid artery (18 gauge, 2 in., Abbocath) or transverse facial artery (20 gauge, 1.5 in., Abbocath) by aseptic techniques. The jugular vein catheters were emplaced under local anesthesia (2% lidocaine). A 7-Fr microtip pressure transducer (model SPC-471A, Millar Instruments, Houston, TX) was advanced through one jugular introducer catheter 8 cm past the pulmonic valve in the pulmonary artery as determined by visual inspection of the pressure waveforms viewed on a monitor via a computer-based data acquisition system (DATAQ, Akron, OH). A thermistor catheter (Columbus Instruments, Columbus, OH) was placed into the right ventricle for blood temperature measurement. On the arterial side, a cannula (polyethylene; 1.6 mm ID, 3.2 mm OD) served as the site for withdrawal of arterial blood samples. The pressure transducer was calibrated before and after each run with a mercury manometer. No baseline transducer drift or change in gain was detected in any of the runs. In addition, a bilateral nostril gas infusion device was secured to each horse, thereby facilitating either N2 or NO inhalation (10–20 μg/kg iv) to facilitate the BAL as a means of quantifying EIPH severity. A BAL tube (Bivona Medical Technology, Gary, IN) was passed through the right naris until it wedged in a subsegmental bronchus of the dorsal caudal portion of the lung (12). Next, a total of 300 ml (in 50-ml aliquots) of lactated Ringer solution was infused. After two breaths, the fluid (a percentage of the entire 300 ml) was aspirated. Fluid recovery averaged 181 ml (60%); no significant differences in recovery existed between trials. The BAL fluid was analyzed via an automated particle counter (Beckman-Coulter, Coulter Counter model Z2, Fullerton, CA). Data are presented as red blood cells per milliliter of recovered BAL fluid minus tube dead space.

Statistical analysis. Data are presented as means ± SE. Differences in blood gases, pH, Ppa, and EIPH between control and NO conditions were tested via a paired t-test. Statistical significance was accepted at P < 0.05.

RESULTS

Time to exhaustion during the final stage at 15 m/s was not different between control (N2) and NO runs (N2, 115 ± 3; NO, 150 ± 23 s; P > 0.05, β = 0.27). Furthermore, at end exercise, no differences existed between control and NO runs (all P > 0.05) for arterial PO2 (N2, 64.5 ± 1.1; NO, 64.2 ± 0.8 Torr), Pco2 (N2, 56.6 ± 4.4; NO, 61.3 ± 1.9 Torr), or pH (N2, 7.223 ± 0.033; NO, 7.254 ± 0.007). As shown in Fig. 1, NO inhalation significantly reduced peak Ppa (N2, 102.3 ± 4.4; NO, 98.6 ± 4.3 mmHg, P < 0.05). Despite the reduction in Ppa, EIPH severity was higher in four of five horses after NO administration (N2, 22.4 ± 6.8; NO, 42.6 ± 15.4 × 10⁶ red blood cells/ml BAL fluid, P < 0.05; Fig. 2).

DISCUSSION

Contrary to the presumption that a reduction in Ppa is always beneficial in reducing the severity of EIPH, this investigation demonstrates that although NO in-
Inhalation produces a small reduction in peak Ppa, EIPH severity is increased in the maximally exercising Thoroughbred horse. This finding supports the contention that the pulmonary arteriolar bed is not fully vasodilated in maximally exercising horses under control conditions. As discussed below, this lack of maximal vasodilation may serve a protective function in the maintenance of capillary integrity.

**Efficacy of NO inhalation.** The inhaled NO concentration of 80 ppm was selected on the basis of the work of Mills et al. (15, 16). As demonstrated therein, those studies significantly reduced Ppa in Thoroughbred horses running at maximal O2 uptake (11 m/s, 5° incline) by inhalation of intranasally delivered NO. The NO-induced reduction in Ppa seen in the present investigation (i.e., ~4 mmHg) was less than that seen by Mills and colleagues (i.e., ~10 mmHg). However, in that investigation a more rapid acceleration to high speeds was utilized, which has been shown to elevate Ppa above that attained during a more gradual ramp protocol (9) and which may explain, in part, the difference in NO inhalation efficacy between investigations. However, although between-horse variability certainly exists, peak Ppa values reported herein are higher than those reported by Mills et al. (15), and thus the less rapid ramp acceleration utilized in our investigation may not have had a large bearing on the slight difference in efficacy of inhaled NO on peak Ppa seen between the two investigations.

**Agreement with current literature.** In the present investigation, inhaled NO attenuated the exercise-induced increase in Ppa, which supports an important role for NO in the regulation of Ppa. Indeed, it has been shown previously that increased NO availability reduces Ppa (15, 16) and NO synthase inhibition increases Ppa, at least during submaximal running (6, 15), in the exercising horse. Furthermore, inhaled NO has also been shown to be effective for treating pulmonary hypertension in resting neonatal foals (8) as well as in many human pulmonary disease conditions (24). It is important to note that the concentration of NO and route of delivery used in the present study is such that the hemodynamic effects will be limited primarily to the pulmonary circulation (20). This may explain, in part, the apparent conflict between this investigation and others in which exogenous NO sources were administered systemically. Specifically, Manohar and Goetz (10) reported reductions in resting pulmonary vascular pressures after intravenous nitroglycerin infusion; however, no differences were seen during treadmill running. Similarly, Hackett et al. (4) reported no reduction in exercise-induced pulmonary hypertension in horses after oral nitroglycerin paste application. The site of action of nitroglycerin is not specific to the pulmonary vasculature. Thus, to the extent that pressures and blood flows in other organs were affected by systemic NO delivery, the effect of increased NO availability in the pulmonary circulation and on pulmonary vascular pressures may be confounded. However, one interesting finding from Hackett et al. was that, at rest, nitroglycerin elicited a significant reduction in pulmonary arterial pressure; however, pulmonary capillary pressure was not reduced. As discussed below, this may lend insight into the mechanism by which EIPH can be increased in the face of reduced pulmonary arterial pressure.

**Mechanistic interpretation.** Stress failure of pulmonary capillaries resulting from exercise-induced pulmonary hypertension during high-intensity exercise has been implicated in the pathogenesis of EIPH (27, 28). Furthermore, from the ex vivo morphometric analysis of excised horse lungs, it appears that a threshold exists such that transmural capillary pressure increases above 75 mmHg, a significant increase in endothelial breaks occurs (2). Indeed, our laboratory demonstrated recently that furosemide administration (4 h before high-intensity treadmill running) was associated with ~7-mmHg reduction in mean Ppa and ~90% reduction in EIPH severity (7). However, it should be noted that the mechanism by which furosemide reduces EIPH is thought to be based on its diuretic properties that result in a reduced blood volume. Furosemide acts to reduce Ppa in the face of unchanged pulmonary vascular conductance. Thus, in contrast, inhaled NO reduces Ppa in the face of unaltered cardiac output (15) and must therefore increase pulmonary vascular conductance, possibly exposing those regions of the pulmonary capillary bed most prone to EIPH to higher intraluminal pressures. Thus, as discussed below, it may be that the normally elevated Ppa-to-cardiac output ratio (shown not to be the consequence of pulmonary hypoxic vasoconstriction; Refs. 11, 18) seen in horses compared with humans (18) is functionally beneficial rather than detrimental with respect to the severity of EIPH.

The fact that Ppa can be reduced via exogenous NO supports the notion that the pulmonary vasculature is not completely vasodilated at maximal exercise in the horse (15). A likely reason for this may be to “protect” pulmonary capillaries from damaging vascular pressures (25). Thus, at a given cardiac output, active vasoconstriction upstream of the pulmonary capillaries would serve to reduce capillary intraluminal (and

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**Fig. 2.** Severity of exercise-induced pulmonary hemorrhage (EIPH), quantified via bronchoalveolar lavage (BAL), was significantly increased (\(*P < 0.05\) during nitric oxide inhalation vs. the control trial. rbc, Red blood cell. Small circles, individual data points; large circles, group means ± SE.
transmural) pressures and constrain the incidence of capillary stress failure. In the present investigation, the NO-induced reduction of Ppa likely reflects greater pulmonary arteriolar vasodilation (the primary site of inhaled NO action; Ref. 23) and thus a lessening of the protective vasoconstrictor tone at this location. The consequence of this vasodilation would be the more effective transduction of the (albeit slightly reduced) Ppa to the pulmonary capillary bed, resulting in augmented capillary stress failure and EIPH.

Bernard et al. (1) demonstrated that, with exercise, the majority of lung blood flow redistribution was directed toward the dorsal region. Ex vivo examination of equine pulmonary arteries has also revealed significant regional differences in endothelial function between the ventral and dorsal lung regions (19). Specifically, in response to methacholine challenge (endothelium-dependent vasodilator), dorsal (top) region vessels relaxed whereas arteries from the ventral (bottom) region demonstrated a transient dilation followed by a profound constriction. Furthermore, these authors (19) showed that vessels from the ventral and dorsal regions responded similarly to sodium nitroprusside (an exogenous, endothelium-independent NO source). We do not know exactly how or to what extent inhaled NO affected either blood flow distribution or regional arteriolar resistance within the lung. However, it has been established that EIPH occurs primarily within the dorsal caudal region of the lung (17), the same region in which EIPH severity is quantified via BAL (12), and thus it seems likely that this region was impacted by NO inhalation. Therefore, the possibility exists that an NO inhalation-mediated redistribution of blood flow from ventral to dorsal aspects of the lung may have occurred. However, given that cardiac output (15) and arterial blood gases (present data) were unchanged during NO inhalation, it seems unlikely that any substantial pulmonary blood flow redistribution or ventilation-perfusion mismatch was manifest.

Inhaled NO has been shown to be effective in decreasing airway resistance; however, the full magnitude of the response may be obscured by the reductions in nasal cavity volume consequent to an NO-mediated increase of nasal mucosal blood flow (21). Regarding the horse, Sweeney et al. (26) demonstrated that inhaled NO had a modest yet significant effect in reducing airway resistance after histamine-induced bronchoconstriction in standing horses. Furthermore, Hackett et al. (4) demonstrated less negative esophageal pressure swings (indicative of less negative intrapleural pressure swings during inspiration) in exercising horses after nitroglycerin paste administration. In contrast to the effects demonstrated in the present investigation, reduced esophageal negative pressure swings might be expected to reduce EIPH by mitigating the negative extravascular pressures that increase capillary transmural pressures. However, with respect to the severity of EIPH, any potential benefit arising from airway dilation was overshadowed by intravascular mechanisms.

In conclusion, NO inhalation at 80 ppm significantly decreases Ppa concomitant with an augmented EIPH in maximally exercising Thoroughbred horses. This finding is consistent with the hypothesis that a mechanism exists that prevents maximal vasodilation of pulmonary arterioles, thereby providing a protective precapillary vasoconstriction that reduces or limits the transduction of high intravascular pressures to the fragile pulmonary capillary bed. Thus, although inhaled NO-mediated pulmonary arteriolar vasodilation induces a mild decrease in Ppa, one likely consequence of this is an elevated pulmonary capillary intraluminal pressure that exacerbates capillary stress failure and EIPH. These data suggest that exogenous NO therapy designed to target specifically airways and pulmonary vessels in exercising horses may not only offer little benefit but also may actually exacerbate EIPH.

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