Inhibition of NO Synthase Does Not Affect Exercised-Induced Pulmonary Capillary Hypertension or the Incidence of EIPH

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1. Introduction
The incidence of exercise-induced pulmonary hemorrhage (EIPH) in racehorses is rather high. Airway endoscopic observations indicate that >75% of racing Thoroughbred and Standardbred horses experience EIPH.1,2 Exercising horses develop significant pulmonary capillary hypertension,3–6 and the resulting high transmural (intracapillary minus perivascular–alveolar) pulmonary capillary pressure probably contributes to the stress failure of pulmonary capillaries, leading to the occurrence of EIPH.3–7

Nitric oxide (NO) is an endogenous vasodilator substance produced by the vascular endothelium that modulates the pulmonary vascular tone in healthy animals and man.8,9 It has been demonstrated that high shear rates (at high blood flows) result in an enhanced endothelial-dependent vasodilation by means of an increased production of NO.8–12 Thus, in galloping horses, in which the cardiac output increases enormously, enhanced NO production by the pulmonary vascular endothelium (in response to high shear rates) may help limit the extent of exercise-induced pulmonary hypertension. A recent report in submaximally exercised horses13 suggested that a reduction in the NO production or action of NO during near-maximal exercise may contribute to the high incidence of EIPH in racehorses.13 In that research,13 basal measurements in resting horses were not made; nor were the pulmonary venous and capillary blood pressures determined. Also, exercise was performed only at a submaximal workload approaching 55% VO₂ max, and attention was not paid to the occurrence of EIPH.13 Thus, it cannot be determined whether the conclusions of Mills et al.13 apply to high-intensity exercise that elicits a maximal heart rate and VO₂ max. Therefore, our objective in this study was to examine the effects of NO synthase inhibition on the right atrial and pulmonary arterial, capillary, and venous blood pressures of Thoroughbred horses at...
rest and during strenuous exercise performed at maximal heart rate. The goal was to ascertain whether endogenous NO production modulates the pulmonary vascular tone in standing and strenuously exercising horses. In this study, the inhibition of NO synthase was accomplished with intravenously administered Nω-nitro-L-arginine methyl ester (L-NAME).8–13

2. Materials and Methods

Experiments were carried out on seven healthy, sound, exercise-trained Thoroughbred horses. Two sets of experiments, namely control (no medications) and NO synthase inhibition (L-NAME) studies, were carried out on all horses in random order, 7 days apart. In the NO synthase inhibition studies, an administration of L-NAME 20 mg/kg IV was given 10 min before exercise. Pilot trials had revealed this dose to be effective in blocking NO synthase in horses. Hemodynamic measurements were made in both experiments at rest and during treadmill exercise performed at 14.2 m/s on a 5.0% uphill grade. Galloping at 14.2 m/s on a 5% uphill grade elicited maximal heart rate in horses and could not be sustained for >90 s. Right heart and pulmonary arterial, capillary, and wedge (venous) pressures were determined by using previously described standard procedures.3–6 The data were analyzed by using a repeated measures analysis of variance, followed by the use of the least-squares significant difference method. An airway endoscopic examination was performed 50–55 min postexercise, and the presence of fresh blood in the trachea was regarded as evidence for the occurrence of EIPH.

3. Results

A. Control Study

From the resting values of 36 ± 1 beats/min and 7 ± 1, 31 ± 1, 27 ± 1, and 23 ± 1 Torr, heart rate, mean right atrial pressure, mean pulmonary artery pressure, mean pulmonary capillary pressure, and mean pulmonary artery wedge pressure increased significantly (p < 0.05) to reach 212 ± 2 beats/min and 69 ± 4, 118 ± 3, 99 ± 3, and 80 ± 3 Torr, respectively, during exercise performed at 14.2 m/s on a 5% uphill grade. All horses sweated profusely in the control experiments and were found to have experienced EIPH.

B. Effects of NO Synthase Inhibition in Standing Horses

The hemodynamic effects of L-NAME were very rapid in onset. Within 60 s after the L-NAME injection was completed, the heart rate had decreased significantly (p < 0.05) because of the development or exaggeration of second-degree atrioventricular block. The right atrial as well as pulmonary vascular pressures had also increased significantly (p < 0.05). Significant changes in these variables were not observed during the 10 min following L-NAME injection. At 10 min following the injection of L-NAME, the respective values of heart rate and mean right atrial, mean pulmonary artery, mean pulmonary capillary, and mean pulmonary artery wedge pressures were 26 ± 2 beats/min and 13 ± 1, 37 ± 1, 31 ± 1, and 27 ± 1 Torr. It was also readily evident following L-NAME administration that the horses had a calmer and more submissive demeanor.

C. Exercise After L-NAME Administration

During exercise performed at 14.2 m/s on a 5% uphill grade after the administration of L-NAME, heart rate increased to the same value as observed in the control study. Also, exercise in the L-NAME experiments was attended by significant (p < 0.05) increments in the right atrial as well as pulmonary arterial, capillary, and wedge pressures, but the values recorded in the L-NAME study were not found to be significantly different from those in the control experiments. Also, it was consistently observed in all horses that sweating in response to the same treadmill exercise protocol was dramatically diminished in comparison with exercise in the control experiments. All horses in the L-NAME trials were also found to have experienced EIPH.

4. Discussion

Our data in the control study confirmed earlier findings3–6 that exercising horses develop significant right atrial as well as pulmonary arterial, capillary, and venous hypertension. In addition to observations that L-NAME administration altered the demeanor of horses and dramatically diminished sweating in response to strenuous exercise, the new findings in the present study include the following: First, it was demonstrated that NO synthase inhibition with L-NAME in healthy resting horses causes marked bradycardia, and significant (p < 0.05) increments in the right atrial, pulmonary vascular, and systemic pressures. The latter is similar to observations in other species10–12 and it indicates that endogenous NO modulates the pulmonary vascular tone in quietly standing horses. Second, L-NAME administration did not affect the pulmonary arterial, capillary, and venous hypertension of horses during exercise performed at maximal heart rate, suggesting that the pulmonary hemodynamic response of strenuously exercising Thoroughbred horses is not modified by NO synthase inhibition. Thus, it was concluded that endogenous NO production modulates the equine pulmonary vascular tone at rest, but it does not play a significant role in modulating the pulmonary vascular tone in strenuously exercising Thoroughbred horses.

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