Nitric oxide synthase inhibition does not affect the exercise-induced arterial hypoxemia in Thoroughbred horses

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Manohar, Murli, Thomas E. Goetz, and Aslam S. Hassan. Nitric oxide synthase inhibition does not affect the exercise-induced arterial hypoxemia in Thoroughbred horses. J Appl Physiol 91: 1105–1112, 2001.—Because sensitivity of equine pulmonary vasculature to endogenous as well as exogenous nitric oxide (NO) has been demonstrated, we examined whether endogenous NO production plays a role in exercise-induced arterial hypoxemia. We hypothesized that inhibition of NO synthase may alter the distribution of ventilation-perfusion mismatching, which may affect the exercise-induced arterial hypoxemia. Arterial blood-gas variables were examined in seven healthy, sound Thoroughbred horses at rest and during incremental exercise protocol leading to galloping at maximal heart rate without (control; placebo = saline) and with N\textsuperscript{-}nitro-l-arginine methyl ester (L-NAME) administration (20 mg/kg iv). The experiments were carried out in random order, 7 days apart. At rest, L-NAME administration caused systemic hypertension, pulmonary hypertension, and bradycardia. During 120 s of galloping at maximal heart rate, significant arterial hypoxemia, desaturation of hemoglobin, hypercapnia, hyperthermia, and acidosis occurred in the control as well as in NO synthase inhibition experiments. However, statistically significant differences between the treatments were not found. In both treatments, exercise caused a significant rise in hemoglobin concentration, but the increment was significantly attenuated in the NO synthase inhibition experiments, and, therefore, arterial O\textsubscript{2} content (C\textsubscript{aO\textsubscript{2}}) increased to significantly lower values. These data suggest that, whereas L-NAME administration does not affect pulmonary gas exchange in exercising horses, it may affect splenic contraction, which via an attenuation of the rise in hemoglobin concentration and C\textsubscript{aO\textsubscript{2}} may limit performance at higher workloads.

artrial blood-gas tensions in exercise; N\textsuperscript{-}nitro-l-arginine methyl ester; exertion

THE OCCURRENCE OF ARTERIAL hypoxemia in strenuously exercising horses has been well documented (1, 2, 4, 30, 32), and it is reported that the occurrence of arterial hypoxemia poses a limit to exercise performance (9, 31). Although “relative” alveolar hypoventilation, as evidenced by the increasing arterial CO\textsubscript{2} tension (P\textsubscript{aCO\textsubscript{2}}) during strenuous exercise, contributes to the observed reduction in arterial O\textsubscript{2} tension (P\textsubscript{aO\textsubscript{2}}; 2), this mechanism usually does not account for the entire decrease in P\textsubscript{aO\textsubscript{2}} observed in exercising horses (12). Thus it is often suggested that diffusion limitation probably related to the dramatic shortening of the pulmonary capillary transit time as well as ventilation-perfusion inhomogeneity play a role in the exercise-induced arterial hypoxemia in horses (1, 2, 4, 9, 12, 30–32). Exercising horses exhibit significant pulmonary arterial, capillary, and venous hypertension (13–17), and the ensuing high transmural pulmonary capillary pressures contribute to the stress failure of pulmonary capillaries, resulting in exercise-induced pulmonary hemorrhage (EIPH; 33). Although the role of exercise-induced structural changes in the blood-gas barrier (8, 25, 33) in causing exercise-induced arterial hypoxemia continues to be a contentious issue, it has been reported in healthy human subjects (3, 7, 27) and Thoroughbred horses (18) that a successive bout of high-intensity exercise, performed soon after the first high-intensity exercise bout, did not accentuate the arterial hypoxemia. Inference from these reports (3, 7, 18, 27) is that the exercise-induced structural changes in the blood-gas barrier (8, 25, 33) may not contribute to the exercise-induced arterial hypoxemia. Thus, despite considerable interest, uncertainty exists as to the precise mechanism(s) responsible for the exercise-induced arterial hypoxemia in the horse.

Nitric oxide (NO) is an endogenous vasodilator substance that is reported to play a significant role in modulating the pulmonary vascular tone in resting healthy animals and humans (5). Although the sensitivity of equine pulmonary vasculature to endogenous as well as exogenous NO has been demonstrated at rest (15, 16), the role of NO in modulating the equine pulmonary vascular tone during exercise is uncertain. However, it has been reported that NO synthase inhibition with N\textsuperscript{-}nitro-l-arginine methyl ester (L-NAME) caused an intensification of the exercise-induced pulmonary arterial hypertension in horses (22) and that exogenous NO inhalation caused a significant reduction in the severity of exercise-induced pulmonary hypertension in horses (21). Despite the possibility that NO may modulate the equine pulmonary vascular tone...
at rest (15, 16) and during exercise (21, 22), only recently has attention been directed at examining the effects of NO on arterial blood-gas tensions during exercise (6, 10, 26). In horses, L-NAME administration to cause NO synthase inhibition significantly decreased $\text{PaCO}_2$ during exercise at 50 and 80% maximal $\text{O}_2$ consumption ($\text{VO}_2\max$, whereas $\text{PaO}_2$ remained unchanged (10), indicating that endogenous NO production plays a role in pulmonary gas exchange of exercising horses. Recently, it was also reported that increased endogenous NO production (through administration of L-arginine) enhances aerobic exercise capacity (20). In view of the above reports (10, 20), our objective in the present study was to ascertain whether endogenous NO production plays a role in exercise-induced arterial hypoxemia in horses. We hypothesized that inhibition of NO synthase with L-NAME may alter the distribution of ventilation-perfusion mismatching within the lungs, which may affect the exercise-induced arterial hypoxemia. Toward this goal, we examined changes in arterial blood-gas tensions in healthy horses performing an incremental exercise protocol leading to galloping at maximal heart rate without (control) and with L-NAME administration. In the present study, L-NAME was infused intravenously at 20 mg/kg, a dose known to cause significant inhibition of NO synthase in healthy horses (10, 15, 22, 23).

MATERIALS AND METHODS

Horses

Experiments were carried out on seven healthy, sound Thoroughbred horses (2 fillies, 5 geldings), 2.5–5 yr old and weighing 466 ± 14 kg. The horses were housed in an air-conditioned building and were accustomed to being handled by people. They were fed a diet of alfalfa hay and oats, and free access to water was provided. The horses were dewormed periodically and were inoculated with tetanus toxoid and strangles vaccine. Our protocols and procedures were approved by the Institutional Laboratory Animal Care and Use Committees.

Exercise Training

All horses were exercise trained for a period of 7 wk before undertaking the blood-gas studies. Our exercise training regimen has been described in detail previously (14–18).

Work Intensity Eliciting Maximal Heart Rate

Trials to ascertain work intensity needed to elicit maximal heart rate were undertaken on completion of exercise training (see above). It was observed that galloping at 14 m/s on a 3.5% uphill grade not only elicited maximal heart rate (217 ± 2 beats/min) but also induced EIPH in all horses, as demonstrated by the presence of fresh blood in the trachea on airway endoscopic examination (11, 29). These trials also revealed that our horses could not sustain galloping at 14 m/s on a 3.5% uphill grade for >120 s despite vigorous humane encouragement. Thus, for the present study, this workload, i.e., 14 m/s on a 3.5% uphill grade, was selected because it represented a strenuous effort capable of eliciting maximal heart rate and inducing EIPH consistently.

Experimental Procedures

Our procedures for hemodynamic and pulmonary gas-exchange studies have been described in detail previously (13–19, 24); therefore, only a brief description is given here. On the day of the study, after local infiltration of 2% lidocaine HCl in the 17th intercostal space, the abdominal aorta was percutaneously catheterized as described previously (19, 24). Thereafter, using local infiltration of 2% lidocaine HCl, cardiac catheters (8F) equipped with tip manometer (Millar Instruments, Houston, TX), fluid-filled lumen, and a thermistor (Edward Laboratories, Santa Clara, CA) were advanced into the pulmonary artery via introducers inserted into the left jugular vein. The location of various catheters was confirmed by monitoring the characteristic phasic blood pressure waveforms on an oscillographic recorder (E for M, Lanexa, KS), and all pressure signals were referenced at the point of the left shoulder. These catheters permitted sampling of the aortic blood and continuous monitoring of the pulmonary arterial blood (core) temperature during the experiments. After catheter placement, horses stood quietly on the treadmill for ~45–50 min before blood-gas studies were undertaken.

Blood-gas tensions, arterial pH (pH$_a$), hemoglobin concentration, arterial hemoglobin $O_2$ saturation (SaO$_2$), and $O_2$ content (CaO$_2$) were determined using a carefully calibrated blood-gas analyzer/CO-oximeter (ABL520 system, Radiometer, Copenhagen, Denmark), and all blood-gas tensions/pH data were corrected to the simultaneously measured pulmonary artery blood temperature. The calibration of our blood-gas/pH analyzer/CO-oximeter was checked frequently (at 30-min intervals) and was verified using tonometered solutions of known blood-gas tensions, pH, hemoglobin concentration, and $O_2$ saturation.

Experimental Design and Protocol

All horses were studied in the control (placebo) as well as the L-NAME experiments. The sequence of these treatments was randomized for all horses, and 7 days were allowed between experiments. All experimentation was carried out in an air-conditioned laboratory, where ambient temperature was maintained at 20°C.

Control (placebo) study. Measurements were first made on quietly standing horses when heart rate and the aortic and pulmonary arterial blood pressures had been stable for ~10 min (rest 1). Thereafter, physiological saline (250 ml of 0.9% NaCl) was infused intravenously over a period of 5 min via the side port of the introducer used for advancing the pulmonary arterial blood (core) temperature during the experiments. After catheter placement, horses stood quietly on the treadmill for ~45–50 min before blood-gas studies were undertaken.

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L-NAME study. In these experiments, measurements were first made on quietly standing horses (without any drugs) when heart rate and the aortic and pulmonary arterial blood pressures had been stable for ~10 min (rest 1). Thereafter, a freshly prepared solution of L-NAME (20 mg/kg; Sigma Chemical, St. Louis, MO) was infused intravenously in 250 ml of physiological saline over 5 min in exactly the same manner as described above for the saline injection in the control study. Several reports have documented the efficacy of this dosage in inhibiting NO synthase in horses (10, 15, 22, 23). Postinfusion resting measurements (rest 2) were then made in quietly standing horses during the 9th and 10th min after administration of L-NAME. Exercise was initiated in the 11th min after L-NAME administration and was performed on the high-speed treadmill set at a 3.5% uphill grade exactly as described above for the control study. Along with continuous measurement of core temperature, $P_{A_{CO_2}}$, $P_{A_{CO_2}}$, pH, hemoglobin concentration, $S_A_{O_2}$, and $C_A_{O_2}$ were determined at exactly the same intervals as described above for the control experiments.

Immediately after the treadmill was stopped, L-arginine (Sigma Chemical) was administered intravenously at 200 mg/kg to reverse the effects of L-NAME (15, 22, 23). For each experiment, this dose of L-arginine was dissolved in 500 ml of physiological saline.

Postexercise Airway Endoscopic Examination

In all experiments, using a flexible fiber-optic endoscope (Pentax Fiberscopes, Orangeburg, NY), careful endoscopic examination of the nasopharynx, larynx, and trachea (up to the carina) was undertaken 45–50 min postexercise, and the presence of fresh blood in the airway(s) was regarded as indicative of the occurrence of EIPH (11, 29).

Data Analysis

The data were first subjected to repeated measures, split-plot design analysis of variance (28) using the SAS statistical software package (SAS version 6.12, SAS Institute, Cary, NC), and the treatment comparisons were made using the least squares significant difference method (28). Data for the control as well as L-NAME experiments were also subjected to Newman-Keuls multiple-range test (28) to determine the significant effects of work intensity/duration within each treatment. For all statistical analyses, the level of significance was set at $P < 0.05$ and the data are presented as means $\pm$ SE.

RESULTS

General Observations

In keeping with our previous work (15), the cardiovascular effects of L-NAME were very rapid in onset. Within 60 s of completion of the L-NAME injection, heart rate had decreased significantly ($P < 0.0001$) to 27 $\pm$ 2 beats/min (from 36 $\pm$ 1 beats/min pre-L-NAME administration), and consistent with the effects of NO synthase inhibition, mean aortic (128 $\pm$ 7 mmHg) and pulmonary artery (37.2 $\pm$ 1.0 mmHg) blood pressures of standing horses had increased significantly ($P < 0.01$) from pre-L-NAME resting values of 97 $\pm$ 2 and 30.1 $\pm$ 1.2 mmHg, respectively. Further significant changes in these variables did not occur during the 10 min after completion of L-NAME injection. The bradycardia after L-NAME injection was due to the development of an atrioventricular block. Also, in agreement with our previous observations (15), it was readily apparent that the horses had a much calmer and more submissive demeanor after L-NAME administration. Similar to our previous work (15), after L-NAME administration, it was a consistent observation that, unlike in the control study, the horses were not aggressive in charging at the front bar of the treadmill during submaximal exercise, and the heart rates recorded during submaximal exercise at 6 as well as 8 m/s on a 3.5% uphill grade (142 $\pm$ 4 and 170 $\pm$ 5 beats/min, respectively) were significantly ($P < 0.001$) less than corresponding values in the control study (168 $\pm$ 5 and 192 $\pm$ 4 beats/min, respectively). However, in the L-NAME experiments, during galloping at 14 m/s on a 3.5% uphill grade, heart rate increased to its maximal value (217 $\pm$ 2 beats/min) as observed in the control study. These changes in heart rate are consistent with our previous observations with L-NAME administration to galloping thoroughbreds (15).

Changes in Core Temperature and Sweating

From its resting value (37.3 $\pm$ 0.08 and 37.4 $\pm$ 0.1°C in the control and L-NAME trials, respectively), core temperature of horses increased progressively with increasing work intensity to reach peak values of 40.6 $\pm$ 0.2 and 40.7 $\pm$ 0.2°C at 120 s of galloping at 14 m/s on a 3.5% uphill grade in the control and L-NAME experiments, respectively, and statistically significant differences between the two treatments were not found. Whereas exercise in the control experiments was attended by profuse sweating in all horses, in the L-NAME experiments little to no sweating was evident in response to the same exercise protocol.

Changes in $P_{A_{O_2}}$ and $S_A_{O_2}$

Preinfusion resting values of $P_{A_{O_2}}$ and $S_A_{O_2}$ were similar in the control and the L-NAME experiments, and the administration of L-NAME to standing horses did not cause significant changes (Fig. 1). During submaximal exercise at 6 and 8 m/s, $P_{A_{O_2}}$ and $S_A_{O_2}$ were well maintained in both treatments. In both experiments, during galloping at 14 m/s on a 3.5% uphill grade, a statistically significant ($P < 0.0001$) decrease in $P_{A_{O_2}}$ was evident at 30 s, but further significant changes in $P_{A_{O_2}}$ did not occur as exercise duration increased to 120 s. The $P_{A_{O_2}}$ values at 120 s of galloping at 14 m/s on a 3.5% uphill grade in the control and the L-NAME experiments were 72.9 $\pm$ 1.6 and 76.4 $\pm$ 1.6 Torr, respectively. Statistically significant differences between the treatments were not discerned at any point during the exercise protocol.

In both treatments, galloping at 14 m/s on a 3.5% uphill grade also caused statistically significant desaturation of hemoglobin in the arterial blood at 30 s. As exercise duration increased to 120 s, the desaturation of hemoglobin intensified in both treatments, but statistically significant differences were not found between the control and the L-NAME experiments. The
increasing desaturation of arterial hemoglobin observed in going from 30 to 120 s of galloping at 14 m/s on a 3.5% uphill grade probably resulted from the rightward shift of the hemoglobin-O2 dissociation curve as hypercapnia (Fig. 2), acidosis (Fig. 3), and hyperthermia (from core temperature of 39.2 ± 0.2 and 39.1 ± 0.2°C at 30 s in the control and L-NAME experiments, respectively, to 40.6 ± 0.2 and 40.7 ± 0.2°C at 120 s in the control and L-NAME experiments, respectively) intensified with increasing exercise duration. SaO2 values at 120 s of galloping at 14 m/s on a 3.5% uphill grade in the control and the L-NAME experiments were 85.0 ± 1.7 and 87.5 ± 1.9%, respectively, and statistically significant differences between the treatments were not discerned at any point during the exercise protocol.

Changes in PaCO2

In standing horses, PaCO2 values were unaffected by inhibition of NO synthase with L-NAME (Fig. 2). Whereas submaximal exercise at 6 and 8 m/s in both experiments was attended by hyperventilation, during galloping at 14 m/s on a 3.5% uphill grade a significant hypercapnia developed. The extent of exercise-induced hypercapnia in galloping Thoroughbreds was similar between the control and the L-NAME experiments. PaCO2 values at 120 s of galloping at 14 m/s on a 3.5% uphill grade in the control and the L-NAME experiments were 50.8 ± 1.8 and 49.3 ± 2.1 Torr, respectively.

Changes in pH

Preinfusion and postinfusion values of pH were similar between the control and the L-NAME experiments (Fig. 3). Exercise at 6 and 8 m/s was not attended by significant changes in pH in either treatment. Differences in pH between the treatments were not discerned at any point during the exercise protocol.

Fig. 1. Administration of Nω-nitro-L-arginine methyl ester (L-NAME) to standing horses did not affect either arterial O2 tension (PaO2) or arterial hemoglobin O2 saturation (SaO2). Rest 1, preinfusion data; rest 2, data obtained 9–10 min after saline (control study) or L-NAME administration. Whereas during exercise at 6 and 8 m/s, PaO2 and SaO2 were well maintained in both treatments, galloping at 14 m/s on a 3.5% uphill grade caused a significant decrease in PaO2 as well as SaO2 in all horses in both treatments. However, statistically significant differences between the control and the L-NAME experiments were not found at any step of the protocol. Although PaO2 did not change significantly as exercise duration increased from 30 to 120 s, a progressive reduction in SaO2 was observed in both treatments. aStatistically significant difference from rest 1 in the same study, P < 0.05. bStatistically significant difference from rest 2 in the same treatment, P < 0.05. cStatistically significant difference from data for exercise at 6 m/s in the same study, P < 0.05. dStatistically significant difference from values at 30 s of galloping at 14 m/s in the same study, P < 0.05. eStatistically significant difference from values at 60 s of galloping at 14 m/s in the same study, P < 0.05. fStatistically significant difference from rest as well as all exercise data in the same study, P < 0.05.

Fig. 2. After L-NAME administration, statistically significant changes in arterial CO2 tension (PaCO2) were not observed either at rest or during exercise. In both treatments, whereas submaximal exercise caused hyperventilation, PaCO2 increased during galloping at 14 m/s on a 3.5% uphill grade. gStatistically significant difference from rest 1 in the same study, P < 0.05. hStatistically significant difference from rest 2 in the same treatment, P < 0.05. iStatistically significant difference from data for exercise at 6 m/s in the same study, P < 0.05. jStatistically significant difference from values at 30 s of galloping at 14 m/s in the same study, P < 0.05. kStatistically significant difference from values at 60 s of galloping at 14 m/s in the same study, P < 0.05. lStatistically significant difference from rest as well as all exercise data in the same study, P < 0.05.
ment. During galloping at 14 m/s on a 3.5% uphill grade, a progressive, significant acidosis of a similar magnitude developed in both treatments. The \( \text{pH}_a \) values at 120 s of galloping at 14 m/s on a 3.5% uphill grade in the control and the L-NAME experiments approached 7.125 ± 0.031 and 7.143 ± 0.036, respectively.

**Changes in Arterial Blood Hemoglobin Concentration**

Preinfusion values of arterial hemoglobin concentration in standing horses were similar in the control and the L-NAME experiments, and L-NAME administration did not cause significant changes (Fig. 4). In both treatments, exercise caused significant (\( P < 0.0001 \)) increments in arterial hemoglobin concentration, but the increment in hemoglobin concentration in the L-NAME experiments was found to be significantly (\( P < 0.01 \)) attenuated at all work intensities. Arterial hemoglobin concentration in the control study at 6 and 8 m/s and at 60 s of galloping at 14 m/s on 3.5% uphill grade was 19.5 ± 0.4, 20.7 ± 0.3, and 21.5 ± 0.2 g/dl, respectively. Corresponding values in the L-NAME study were significantly less, being 16.3 ± 0.3, 17.6 ± 0.5, and 19.5 ± 0.2 g/dl, respectively.

**Changes in \( \text{CaO}_2 \)**

The \( \text{CaO}_2 \) of standing horses was unaffected by L-NAME administration (Fig. 5). In both experiments, a large significant (\( P < 0.0001 \)) increment in \( \text{CaO}_2 \) was observed during exercise as hemoglobin concentration increased significantly (Fig. 4). However, for all work intensities, the values of \( \text{CaO}_2 \) in the L-NAME experiments remained significantly less (\( P < 0.05 \)) than in the control experiments. \( \text{CaO}_2 \) values in the control study at 6 and 8 m/s and at 60 s of galloping at 14 m/s...
on a 3.5% uphill grade were 26.5 ± 0.5, 27.6 ± 0.4, and 26.6 ± 0.4 ml O₂/dl blood, respectively. Corresponding values in the L-NAME study were significantly (P < 0.05) lower, being 22.3 ± 0.4, 23.8 ± 0.7, and 24.9 ± 0.6 ml O₂/dl blood, respectively.

Airway Endoscopy

It was observed that all horses had experienced EIPH in the control as well as the L-NAME experiments, as demonstrated by the presence of fresh blood in the trachea (11, 29).

DISCUSSION

Our findings regarding arterial hypoxemia, desaturation of hemoglobin, hypercapnia, acidosis, increased hemoglobin concentration, and CaO₂, as well as significant hyperthermia during high-intensity short-term exercise in the control study (Figs. 1–5), are similar to those reported previously (1, 2, 4, 9, 18, 30–32). Also, our observations regarding the effects of L-NAME administration in resting and exercising horses on heart rate, peak core temperature, and sweating mirror those reported previously (10, 15). Regarding the primary objective of the present study, i.e., to examine the effects of NO synthase inhibition on exercise-induced arterial hypoxemia, the new findings in the present study were as follows. 1) L-NAME administration to horses did not significantly affect the significant reductions in PaO₂ and SaO₂ (Fig. 1) or the rise in PaCO₂ (Fig. 2) observed during high-intensity short-term exercise. Thus our data indicate that NO synthase inhibition did not significantly affect pulmonary gas exchange in exercising horses. 2) L-NAME administration to horses caused a significant reduction in hemoglobin concentration at all work intensities (Fig. 4), and as a consequence, CaO₂ values remained significantly less than corresponding values in the control study (Fig. 5). The significant attenuation of the increment in arterial hemoglobin concentration throughout the exercise protocol in the L-NAME experiments suggests that the drug may have deleterious effects on equine splenic contraction. In view of the significant reduction in CaO₂ in horses exercising after L-NAME administration (Fig. 5), it is appropriate to ask, “How were the metabolic needs being met during strenuous exercise?” Because the extent of metabolic acidosis in our L-NAME experiments was not significantly different from that in the control study (Fig. 3), it is likely that NO synthase inhibition did not cause a further increase in anaerobic metabolism in exercising horses. This is in agreement with the report by Kindig et al. (10), wherein lactate production did not increase in horses performing peak exercise after L-NAME administration, although an increase in O₂ extraction was observed (10). Because mixed venous blood samples were not obtained in the present study, we cannot provide direct evidence for increased O₂ extraction in our NO synthase inhibition experiments.

In the present study, in both treatments, arterial hypoxemia occurred rapidly, being readily evident at 30 s of galloping at 14 m/s on a 3.5 uphill grade (Fig. 1). The extremely rapid development of arterial hypoxemia with onset of high-intensity exercise and the fact that its magnitude was unaffected by the intensifying hypercapnia (Fig. 2) suggest that the exercise-induced arterial hypoxemia in horses may not have a structural basis related to the changes in the thickness/integrity of the blood-gas barrier (25, 33) brought about via stress failure of pulmonary capillaries resulting from the high transmural pulmonary capillary pressures (13–17). This is because, whereas the structural changes in the blood-gas barrier would be expected to intensify with increasing exercise duration and should, therefore, cause an intensification of the arterial hypoxemia in exercising horses, this was not the case in the present study (Fig. 1; Ref. 18). Thus our data are more consistent with the thesis that the exercise-induced arterial hypoxemia in strenuously exercising horses has a functional basis, probably related to the significant shortening of the transit time for blood in the pulmonary capillaries as cardiac output increases dramatically (18). A similar conclusion was reached by St. Croix et al. (27) in exercising human subjects.

As shown in Figs. 1 and 2, inhibition of endogenous NO production with L-NAME did not significantly affect pulmonary gas exchange in resting or exercising horses. In this context, in contrast with the findings of Mills et al. (21, 22), we have demonstrated that neither L-NAME administration to inhibit endogenous NO production (15) nor inundation of the pulmonary circulation with exogenous NO (with intravenous nitroglycerin infusion at 20 μg·kg⁻¹·min⁻¹, a dose fourfold of that required to cause maximal pulmonary vasodilation in healthy resting horses; 16) were effective in modifying the pulmonary arterial, capillary, or venous blood pressures in horses performing strenuous exercise. The inability of exogenous NO to modify pulmonary vascular tone in exercising horses (16) was attributed to the fact that pulmonary vascular resistance of exercising horses reaches its minimal value (achieved at maximal pulmonary vasodilation) during moderate exercise (performed at 8 m/s) and becomes a fixed quantity, such that further reductions in pulmonary vascular resistance do not occur as workload is increased to maximal exercise (17).

Our observations that PaO₂, SaO₂, and PaCO₂ of horses were not significantly affected by L-NAME administration at rest or at any of the work intensities examined (Figs. 1 and 2) are, however, in conflict with those of Kindig et al. (10). These investigators (10) reported that, after L-NAME administration, PaCO₂ of horses decreased significantly during exercise performed at 50 and 80% VO₂ max but that PaO₂ remained unchanged. Although the reasons for divergent findings are difficult to discern, it is important to note that in the experiments of Kindig et al. (10), even during exercise performed at VO₂ max, horses did not exhibit the usual exercise-induced arterial hypoxemia and/or hypercapnia. This is not only in contrast with the data in the present study (Figs. 1 and 2), but also with several previous reports (1, 2, 4, 9, 12, 18, 30–33).
Furthermore, the findings of Kindig et al. regarding O2 consumption and lactate production after L-NAME administration also conflict with other data in the literature (23). For example, whereas Mills et al. (23) reported that after L-NAME administration, O2 consumption of exercising horses remains unaffected but their anaerobic metabolism (as indicated by lactate production) increases significantly, Kindig et al. (10) reported \( \overline{V}O_2 \text{max} \) and lactate production to decrease significantly after L-NAME administration.

An alternate strategy to elucidate the role of NO in pulmonary gas exchange has been to study the effects of exogenous NO added to the inhaled gas (6, 26). However, these studies did not yield unequivocal results. Whereas NO inhalation at 15 parts/million was reported to cause a significant reduction in \( P_{aO_2} \) of highly trained athletes at rest and during exercise performed at 50 and 80\% \( \overline{V}O_2 \text{max} \) (6), these findings were not confirmed in a recent study wherein NO inhalation at 20 parts/million failed to significantly affect the pulmonary gas exchange of highly trained cyclists at rest as well as during exercise performed during normoxia and hypoxia (26).

Consistent with previous reports that splenic contraction dramatically augments the O2-carrying capacity of blood in exercising horses (1, 2, 4, 9, 12, 18, 30–32), in the present study, arterial hemoglobin concentration (and, therefore, \( C_{aO_2} \); Fig. 5) increased significantly in both treatments with exertion (Fig. 4). However, throughout the exercise protocol, the increment was significantly attenuated after L-NAME administration (Fig. 4). This is suggestive of a direct or an indirect effect of L-NAME on the equine splenic contraction during exercise. The attenuated increment in \( C_{aO_2} \) by way of limiting the available O2 supply is likely to adversely affect performance at high workloads.

Our observations regarding bradycardia and systemic and pulmonary hypertension in standing horses after administration of L-NAME (cf. RESULTS) are similar to those reported previously (10, 15). The systemic and pulmonary hypertension observed after L-NAME administration to standing horses probably results from vasoconstriction ensuing on inhibition of NO synthase (15, 16), and the bradycardia is thought to be mediated via the baroreceptor reflex (15). The very pronounced changes in the attitude and demeanor of horses observed after L-NAME administration (10, 15) may be related to the inhibition of NO synthase in the cerebral tissues, whereas the diminished sweating response to exercise may in part be due to vasoconstriction at the sweat glands caused with inhibition of NO synthase (15, 22, 23). However, it is unclear why the significantly diminished sweating in our NO synthase inhibition experiments was not attended by an exaggeration of the exercise-induced hyperthermia.

The lower values of heart rate observed at 6 and 8 m/s in the L-NAME experiments are suggestive of a less vigorous sympathoadrenal discharge during submaximal exercise, which may have also contributed to the limited rise in hemoglobin concentration observed at these workloads (Fig. 4). However, it is difficult to reconcile the fact that, although in the L-NAME experiments maximal heart rate (217 ± 2 beats/min; as observed in the control study) was achieved during galloping at 14 m/s on 3.5\% uphill grade (indicating that cardiac sympathetic effects were probably similar in the 2 treatments), the hemoglobin concentration remained significantly less than in the control study (Fig. 4). These observations lead us to suggest that L-NAME administration may have direct or indirect effects on the splenic contraction.

In summary, our data demonstrated that L-NAME administration to horses did not significantly affect pulmonary gas exchange at rest or during exercise. Thus endogenous NO production is unlikely to play a role in mediating the exercise-induced arterial hypoxemia and hypercapnia in horses. However, because L-NAME administration caused a significant attenuation of the exercise-induced rise in arterial hemoglobin concentration, the \( C_{aO_2} \) of exercising horses decreased significantly; the latter may diminish exercise performance at high workloads.

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REFERENCES


10. Kindig CA, Gallatin LL, Erickson HH, Fedde MR, and Poole DC. Cardiorespiratory impact of the nitric oxide synthase...