L-NAME does not affect exercise-induced pulmonary hypertension in Thoroughbred horses

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Manohar, Murl, and Thomas E. Goetz. L-NAME does not affect exercise-induced pulmonary hypertension in Thoroughbred horses. J. Appl. Physiol. 84(6): 1902–1908, 1998.—The present study was carried out to examine the effects of nitric oxide synthase inhibition with N\(^{-}\)-nitro-L-arginine methyl ester (L-NAME) on the right atrial as well as on the pulmonary arterial, capillary, and venous blood pressures of horses during rest and exercise performed at maximal heart rate (HR\(_{\text{max}}\)). Experiments were carried out on seven healthy, sound, exercise-trained Thoroughbred horses. Using catheter-tip manometers, with signals referenced at the point of the shoulder, we determined phasic and mean right atrial and pulmonary vascular pressures in two sets of experiments [control (no medications) and L-NAME (20 mg/kg iv given 10 min before exercise studies)]. The studies were carried out in random order 7 days apart. Measurements were made at rest and during treadmill exercise performed on a variable uphill grade at 6, 8, and 14.2 m/s. Exercise on a 5% uphill grade at 14.2 m/s elicited HR\(_{\text{max}}\) and could not be sustained for >90 s. In quietly standing horses, L-NAME administration caused a significant reduction in right atrial, as well as pulmonary arterial, capillary, and venous pressures. This indicates that nitric oxide synthase inhibition modifies the basal pulmonary vascular tone. In both treatments, exercise caused progressive significant increments in right atrial and pulmonary vascular pressures, but the values recorded in the L-NAME study were not different from those in the control study. The extent of exercise-induced tachycardia was significantly decreased in the L-NAME study at 6 and 8 m/s but not at 14.2 m/s. Thus, L-NAME administration may not modify the equine pulmonary vascular tone during exercise at HR\(_{\text{max}}\). However, as indicated by a significant reduction in heart rate, L-NAME seems to modify the sympathoneurohumoral response to submaximal exercise.

Endogenous nitric oxide (NO) has been reported to play a role in modulating the pulmonary vascular tone in resting animals (1, 3, 4, 6), but its role in exercising animals (7–9) and humans is less well understood. Because it is known that high shear rates result in enhanced endothelial-dependent vasodilation (6), it is possible that NO may also play a role in the exercise-induced pulmonary vasodilation (7–9), although concrete evidence, at least during strenuous exercise, is lacking. N\(^{-}\)-nitro-L-arginine methyl ester (L-NAME) has been widely used as a competitive inhibitor of NO synthase in experimental animals (1, 3, 5, 7–9). It was recently reported in submaximally exercised horses that competitive inhibition of NO synthase with intravenously administered L-NAME (20 mg/kg) resulted in accentuation of the exercise-induced pulmonary arterial hypertension, which was partially reversed on administration of L-arginine (15). On the basis of these observations, it was concluded that NO plays a role in modulating the pulmonary vascular tone in exercising horses, and it was 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 (15). Although the findings of Mills et al. (15) are interesting, it should also be noted that there were several deficiencies in their report. 1) The effects of L-NAME on the pulmonary vascular pressures under basal resting conditions were not examined. Thus, it is unknown whether endogenous NO plays a role in modulating the equine pulmonary vascular tone at rest. 2) The measurements of left atrial/pulmonary venous pressure were not made. Therefore, estimates of pulmonary capillary blood pressure, an important variable for stress failure of pulmonary capillaries leading to EIPH (21), could not be ascertained. 3) In the report of Mills et al. (15), the catheter-tip transducer used for measurement of pulmonary arterial pressure was not referenced in any manner. Thus, the true atmospheric zero for the pressure measurement was not known, nor could the direction and/or magnitude of the catheter-manometer’s drift be determined and accounted for (22). 4) Furthermore, in the previous experiments (15), it is unclear whether the findings apply to horses exercising at high workloads because exercise was performed at a submaximal workload (55% maximal O\(_2\) uptake (V\(_{\text{O}_2\text{max}}\))). Therefore, in the present study, our objective was to examine the effects of the inhibition of NO synthase by L-NAME on the right atrial and pulmonary arterial, capillary, and venous blood pressures of Thoroughbred horses during quiet rest as well as during exercise performed at maximal heart rate (HR\(_{\text{max}}\)). The goal was to ascertain whether NO synthase inhibition would modify pulmonary hemodynamics; right heart pressures; N\(^{-}\)-nitro-L-arginine methyl ester; nitric oxide synthase inhibition; catheterization; exercise-induced pulmonary hemorrhage; exercise-induced pulmonary hypertension.

THE INCIDENCE of exercise-induced pulmonary hemorrhage (EIPH) in racehorses is quite high. Based on airway endoscopic examination, it is reported that >75% of racing Thoroughbreds experience EIPH (19). Exercising horses develop significant pulmonary arterial, capillary, and venous hypertension (10–12), and it is increasingly recognized that high transmural (intracapillary–perivascular/alveolar) pulmonary capillary pressure in galloping horses probably contributes to the stress failure of pulmonary capillaries, leading to the occurrence of EIPH (13, 21). The magnitude of exercise-induced pulmonary arterial, capillary, and venous hypertension in Thoroughbred racehorses probably exceeds that in other species (10, 13, 21).
the equine pulmonary vascular tone at rest and during strenuous exercise. For our experiments, the dosing and/or administration regimens of L-NAME and L-arginine (to reverse the effects of L-NAME) were adopted from previous reports (5, 8, 14, 15) and our pilot work.

**MATERIALS AND METHODS**

**Horses**

Experiments were carried out on seven healthy, sound Thoroughbred horses (1 filly and 6 geldings; 2.5–6 yr old; weight, 402–488 kg). They were exercised trained for a period of 7 wk before hemodynamic studies were undertaken. 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 strangies vaccine. The protocols and procedures were approved by the Institutional Laboratory Animal Care and Use Committees.

**Exercise Training**

After the horses were familiarized with walking, trotting, cantering, and galloping on the high-speed treadmill for 1 wk, all horses were exercised 3 days/wk in the following manner, with the treadmill set on the flat (i.e., 0% grade). Beginning with a walk at 2 m/s for 60 s, belt speed was increased at the rate of 1 m/s every 60 s until the horse had trotted at 6 m/s for 60 s. Treadmill speed was then raised to 8 m/s, and the horses were cantered for 60 s. This was followed by galloping at 10 m/s for 60 s and at 14.2–14.5 m/s for 120 s. Belt speed was then decreased, first to 5 m/s for 60 s and then to 2 m/s for 5 min before the treadmill was stopped. After initial exercise training was completed in this manner for 4 wk, this incrementally exercise regimen was performed 3 days/wk for the next 3 wk, with the treadmill set at a 3.5% uphill grade.

**Work Intensity Eliciting Maximal HR**

After 7 wk of exercise training were completed (see Exercise Training), trials were undertaken to ascertain the work intensity needed to elicit maximal HR of the horses. It was observed that the HR of horses during exercise at 14.2 m/s on a 3.5% uphill grade was not different from the HR during exertion at 14.2 m/s on a 5.0% uphill grade (212 ± 2 beats/min). Thus, for the present study, the latter workload was selected, because it represented a more strenuous effort performed at maximal HR. In these trials, it was also found that horses could not perform galloping at 14.2 m/s on a 3.5% uphill grade for >90 s, despite vigorous but humane encouragement.

**Pilot Trials with L-NAME in Standing Horses**

Using previous studies (5, 8, 14, 15) as a guide, pilot trials were carried out on five horses to determine the effects of intravenous L-NAME administration, at cumulative doses of 10 and 20 mg/kg, on pulmonary arterial and aortic pressures and on HR. It was observed that the hemodynamic effects of L-NAME were rapid in onset and that the administration of the first dose of L-NAME (10 mg/kg dissolved in 125 ml of physiological saline, iv) significantly (P < 0.05) increased mean pulmonary arterial and aortic blood pressure to 119 and 123%, respectively, of the baseline values before L-NAME (31 ± 1 and 99 ± 2 mmHg, respectively). HR decreased significantly (P < 0.05) by 28% because of development and/or exaggeration of the second-degree atrioventricular (AV) block. The second dose of L-NAME (10 mg/kg iv; cumulative dose, 20 mg/kg), given 10 min after the first dose, did not cause further changes in HR or mean pulmonary artery blood pressure. Although mean aortic pressure increased to 130% of the baseline value before L-NAME was administered, this change was found to be statistically insignificant compared with values recorded after administration of the first 10 mg/kg dose. These effects of L-NAME were observed to persist for the next 60 min, at which time L-arginine was administered (200 mg/kg iv, in 500 ml of physiological saline). We monitored horses for 60 min after 20 mg/kg L-NAME, because this duration was deemed adequate for completion of our L-NAME exercise experiments (see Experimental Design and Protocol). After L-arginine was administered, mean pulmonary arterial and aortic blood pressures were 110 and 117%, respectively, of the baseline values before L-NAME, and HR was not different from the baseline values before L-NAME.

These pilot trials thus demonstrated that, when the L-NAME dose was doubled (from 10 to 20 mg/kg), mean pulmonary artery pressure and HR of standing horses did not change. Although mean aortic pressure increased further by 7% when L-NAME dose was increased from 10 to 20 mg/kg, this change was found not to be statistically significant. Also, it was demonstrated that the effects of L-NAME persisted for 60 min after it was administered, at which time L-arginine was administered to the horses.

**Experimental Procedures**

Our hemodynamic procedures have been described in detail previously (10–13). Therefore, only a brief description is given here. On the day of the study, four cardiac catheters (8-Fr) equipped with tip manometers and fluid-filled lumens (Millar Instruments, Houston, TX) were advanced via the left jugular vein so as to simultaneously record phasic right atrial, right ventricular, pulmonary arterial, and pulmonary artery wedge pressures. The in vivo catheter manometer signals were matched with corresponding fluid-filled pressure signals obtained with conventional transducers (Statham-Gould, Oxnard, CA) zeroed at the level of the point of the left shoulder. The data were continuously displayed on an oscillographic recorder (Marquette Medical Systems, Jupiter, FL), and mean pressures were obtained by electronic integration of the phasic pressure signals.

**Experimental Design and Protocol**

Two sets of experiments, the control study and the L-NAME study, were carried out on all horses. The sequence of the two treatments was randomized for each horse, and we allowed 7 days between experiments on each horse. Control study. In these trials, horses received no medication(s). Measurements were first made on quietly standing horses (hereafter referred to as control rest) when HR and pulmonary vascular pressures had been stable for 10–15 min. Then exercise was performed in the following manner on the high-speed treadmill set at a 5% uphill grade. Exercise began with a walk at 2 m/s for 60 s, and then belt speed was increased in increments of 1 m/s every 60 s until the speed was 6 m/s. After the horses had trotted for 60 s at 6 m/s, belt speed was raised to 8 m/s (canter) for 60 s and then to 14.2 m/s. Horses galloped for 90 s at 14.2 m/s on a 5% uphill grade. Thereafter, the belt speed was reduced to 5 m/s, and the horses were trotted for 60 s. Then belt speed was decreased to 2 m/s, and the horses were walked for 5 min before the treadmill was stopped.

L-NAME study. In these trials, measurements were first made on quietly standing horses (without any drugs) when...
HR and pulmonary vascular pressures had been stable for 10–15 min (see control rest, in Control Study). Then, a freshly prepared solution of L-NAME (Sigma Chemical, St. Louis, MO) in physiological saline was administered (20 mg/kg iv). For each trial, the dose of L-NAME was dissolved in 250 ml of physiological saline. This dose of L-NAME was used based on our pilot work and previous reports in the literature (5, 8, 15). Hemodynamic measurements were then made during steady conditions in quietly standing horses at 1, 5, and 10 min after administration of L-NAME (rest after L-NAME). In the 11th min after intravenous administration of L-NAME, exercise was initiated and was performed on the high-speed treadmill set at a 5% uphill grade in exactly the same manner as described above for the control study. As soon as the treadmill was stopped, L-arginine (Sigma Chemical) was administered (200 mg/kg iv) to reverse the effects of L-NAME, as suggested by Mills et al. (15). For each experiment, this dose of L-arginine was dissolved in 500 ml of physiological saline.

Postexercise Airway Endoscopic Examination

In the control as well as in the L-NAME trials, careful endoscopic examination of the nasopharynx, larynx, and trachea (up to the carina) was undertaken at 45–50 min postexercise with the use of a flexible fiber-optic endoscope (Pentax fibrescopes, Orangeburg, NY). The presence of fresh blood in the airway was regarded as indicative of the occurrence of EIPH (19).

Measurements and Data Analysis

Hemodynamic data were obtained at rest and during exercise on a 5% uphill grade at 6, 8, and 14.2 m/s. During exercise at 6 and 8 m/s, measurements were made on all consecutive cardiac cycles recorded during 20–45 s; during exertion at 14.2 m/s, measurements were made on all consecutive cardiac cycles recorded between 30 and 75 s. On the basis of the work of Bhattacharya et al. (2) and as suggested by West et al. (21), mean pulmonary capillary blood pressure was estimated as (mean pulmonary artery pressure + mean pulmonary artery wedge pressure)/2, and the HR was determined from the continuously recorded phasic right ventricular pressure.

The data from both treatments were subjected to repeated measures, split-plot design analysis of variance (18), using the SAS statistical software package (SAS Institute, Cary, NC), and comparisons were made using the least-squares significant difference method (18). Data for the control and the L-NAME experiments were also individually subjected to analysis of variance, followed by Newman-Keuls multiple-range test (18) to determine the significant effects of work intensity within each treatment. For all statistical analyses, the level of significance was set at P < 0.05, and the data are presented as means ± SE.

RESULTS

Control Study (Figs. 1–5)

As expected, exercise in the control experiments was attended by significant progressive increments in HR, as well as right atrial and pulmonary arterial, capillary, and wedge pressures. From their resting values HR (36 ± 1 beats/min) and mean right atrial pressure, mean pulmonary artery pressure, mean pulmonary capillary pressure, and mean pulmonary artery wedge pressure (7.3 ± 1.1, 31.3 ± 1.2, 27.4 ± 1.1, and 23.7 ± 1.1 mmHg, respectively) increased significantly during exercise performed at 14.2 m/s on a 5% uphill grade (to reach a HR of 212 ± 2 beats/min and and mean right atrial pressure, mean pulmonary artery pressure, mean pulmonary capillary pressure, and mean pulmonary artery wedge pressure of 69.3 ± 4.0, 118.1 ± 2.5, 99.3 ± 2.6, and 80.4 ± 2.9 mmHg, respectively). These data are similar to those in our previous study (11) in which
a similar exercise protocol was used for Thoroughbred horses.

Exercise in the control experiments caused all the horses to sweat profusely, and airway endoscopy revealed that all the horses had experienced EIPH.

Effects of L-NAME Administration in Standing Horses (Figs. 1–5)

In these experiments, HR, as well as right atrial and pulmonary vascular pressures recorded in quietly standing horses before administration of L-NAME (see control rest in L-NAME Study) were similar to values for resting horses in the control study. The cardiopulmonary effects of L-NAME were very rapid in onset. Within 60 s after the L-NAME injection was completed, it was obvious that the HR of horses had decreased and that the right atrial as well as the pulmonary vascular pressures had increased significantly. Significant further changes in these variables did not occur during the 10 min after completion of the L-NAME injection. The decrease in HR was caused by development and/or exaggeration of second degree AV block. At 10 min after injection of L-NAME in quietly standing horses, HR was 26 ± 2 beats/min, and mean right atrial, mean pulmonary artery, mean pulmonary capillary, and mean pulmonary artery wedge pressures were 12.6 ± 0.9, 36.8 ± 1.0, 31.4 ± 1.0, and 26.6 ± 1.1 mmHg, respectively. It was also readily evident that the horses had a calmer, more submissive demeanor after administration of L-NAME.

Exercise After Administration of L-NAME

During incremental exercise carried out after administration of L-NAME, we consistently observed that, unlike during the control study, the horses were not aggressive in charging at the front bar of the treadmill, and the HR during exercise on a 5% uphill grade at 6 m/s (141 ± 4 beats/min) as well as at 8 m/s (169 ± 6 beats/min) was significantly (P < 0.001) less than corresponding values in the control study (166 ± 5 and 189 ± 5 beats/min, respectively). However, during exercise at 14.2 m/s on a 5% uphill grade, HR in the L-NAME experiments increased to reach the same values as observed in the control study (Fig. 1).
As in the control study, incremental exercise in the L-NAME experiments was attended by significant progressive increments in the right atrial as well as pulmonary vascular pressures, and the values recorded in the L-NAME study were not found to be significantly different from those in the control experiments at any of the three workloads examined (Figs. 2–5). Also, it was consistently observed in all horses during the L-NAME experiments that sweating in response to the same exercise protocol was dramatically diminished compared with sweating during the control exercise experiments. All horses in the L-NAME trials were also found to have experienced EIPH.

**DISCUSSION**

Our objective in the present study was to examine whether L-NAME administration would accentuate the right atrial and/or pulmonary arterial, capillary, and venous hypertension in Thoroughbred horses performing exercise at maximal HR. Our results in the control study confirmed earlier findings (10–13) that exercising horses develop significant work-intensity-related increments in right heart and pulmonary vascular pressures. New findings in this study include the following. 1) NO synthase inhibition with L-NAME caused significant bradycardia and increased the right atrial and pulmonary vascular pressures of quietly standing horses, indicating that endogenous NO probably plays a role in modulating the pulmonary vascular tone of resting horses. 2) In Thoroughbred horses performing exercise at maximal HR (14.2 m/s on a 5% uphill grade), administration of L-NAME did not accentuate the pulmonary arterial, capillary, and venous hypertension, indicating that NO synthase inhibition failed to modify the equine pulmonary vascular tone during strenuous exercise. 3) Intravenously administered L-NAME caused a significant reduction in HR during submaximal workloads, but the right atrial or pulmonary vascular pressures did not exceed those recorded during control exercise at 6 and 8 m/s. 4) Intravenously administered L-NAME altered the general outlook/demeanor of the horses at rest as well as during exercise at 6 and 8 m/s. 5) L-NAME administration dramatically diminished sweating by our horses in response to strenuous exercise.

**L-NAME Administration to Resting Horses**

In a previous study that used L-NAME in horses (15), drug effects were not assessed at rest. Spontaneously occurring second-degree AV block is often observed in quietly standing, relaxed horses and is regarded as a manifestation of their high parasympathetic tone (17). Our observations regarding bradycardia (Fig. 1) and development and/or exaggeration of the second-degree AV block in standing horses after intravenous administration of L-NAME are consistent with reports in other species that L-NAME administration is attended by cardiac parasympathomimetic effects (5). The latter is probably mediated via the baroreceptor reflex in response to arterial hypertension resulting from inhibition of NO synthase in the systemic vascular bed (5, 6). That this effect of L-NAME in horses was probably secondary to an increase in arterial blood pressure was demonstrated in separate experiments (n = 5 horses) in which mean aortic blood pressure increased by 36 ± 6 mmHg after intravenous L-NAME administration and the HR decreased by 8–12 beats/min because of development and/or exaggeration of second-degree AV block (unpublished data).

Several investigators have suggested that NO plays a role in modulating the pulmonary vascular resistance in resting animals (1, 3, 4, 7–9). Our observations on increased right atrial as well as pulmonary arterial, capillary, and wedge pressures (Figs. 2–5) after intravenous administration of L-NAME in quietly standing horses are consistent with the known effects of the drug as a competitive inhibitor of NO synthase (5–9). In resting sheep, L-NAME administration also resulted in significant increments in pulmonary arterial and left atrial pressures as pulmonary vascular resistance increased (7, 8). Our observation that the pulmonary arterial, capillary, and venous pressures increased significantly after L-NAME administration (Figs. 3–5) supports the contention that endogenous NO modulates the pulmonary vascular tone in resting horses. It has been reported that L-NAME crosses the blood-brain barrier and modifies the central sympathetic discharge as well as the behavior of animals (16, 20). Behavioral changes have also been observed after administration of L-NAME to conscious rats (5). It is likely that the calmer, more submissive demeanor observed in our horses after L-NAME was administered, both while they stood quietly as well as during exercise at lower work intensities, may be related to the effects of the drug on the central nervous system tissues. In separate trials on two horses, when L-arginine administration (to reverse the effects of L-NAME) was delayed by 24 h, we observed that horses became severely depressed and lost interest in eating and drinking, but the administration of L-arginine quickly restored normalcy. Thus, it appears that intravenous L-NAME administration, in addition to exhibiting vascular effects, also alters the general outlook or demeanor of the horses, possibly through effects on the central nervous system tissues.

**Exercise After Administration of L-NAME**

In the present study, L-NAME administration dramatically diminished the exercise-induced sweating compared with sweating in the control study. This is similar to observations during submaximal exercise in a previous report (14) and is thought to be mediated, at least in part, via diminished vasodilation at the sweat glands upon competitive inhibition of NO synthase with L-NAME (14).

Our observations regarding the effects of L-NAME on pulmonary artery blood pressure of exercising horses (Fig. 3) do not concur with those of Mills et al. (15) in horses exercised at ~55% $\dot{V}O_{2max}$. During exercise performed at 14.2 m/s on a 5% uphill grade in our L-NAME experiments, HR (Fig. 1) and mean right
atrial as well as pulmonary arterial, capillary, and venous pressures (Figs. 2–5) were not different from values recorded in the control study, indicating that L-NAME-induced inhibition of NO synthase failed to significantly modify the pulmonary vascular tone during exercise performed at maximal HR.

In the previous report examining the effects of L-NAME in submaximally exercised horses (15), HR was not measured. In the present study, after L-NAME administration, the workloads of 6 and 8 m/s on a 5% uphill grade elicited a lower HR (mean Δ = 25 and 20 beats/min, respectively) than in the control experiments (Fig. 1) because the horses appeared calmer and more submissive, and they were notinclined to charge at the front bar of the treadmill. However, the observed HRs of 141 ± 4 and 169 ± 6 beats/min during exertion on a 5% uphill grade at 6 and 8 m/s, respectively, in the L-NAME experiments were well above the known values of the intrinsic rate of the equine sinoatrial node (80–100 beats/min) elicited on bilateral vagotomy and/or parasympathetic blockade with atropine (17). During exercise, cardiac parasympathetic tone is withdrawn, and the increments in HR above the intrinsic rate of the sinoatrial node are probably brought about via sympathetic neurohumoral influences (17). Thus, the lower HR during exertion at 6 and 8 m/s in the L-NAME experiments is indicative of a less vigorous cardiac sympathetic response. In the present study, although cardiac output was not measured, it should be noted that preload for the right and left ventricles [as indicated by mean right atrial (Fig. 2) and pulmonary artery wedge (Fig. 4) pressures, respectively] during exercise at 6 and 8 m/s was similar in the control and L-NAME experiments. At similar ventricular preload during exercise, a mean reduction in HR of 20–25 beats/min caused by intravenously administered L-NAME (Fig. 1) is likely to reduce the cardiac output. Thus, it appears that during exercise at 6 and 8 m/s in the L-NAME experiments, pulmonary vascular pressures similar to the control study were likely achieved at a lower cardiac output. The inference is that the pulmonary vascular resistance was likely higher than in the control study. This is similar to observations in submaximally exercising sheep, in which administration of L-NAME increased pulmonary vascular resistance compared with control trials (7). It appears therefore, that intravenously administered L-NAME may affect the equine pulmonary vascular tone during exercise at submaximal workloads. It remains to be determined whether this is a direct consequence of NO synthase inhibition with L-NAME or is somehow related to the altered sympathoneurohumoral response during submaximal exercise performed after L-NAME administration (as indicated by the lower HRs at 6 and 8 m/s, Fig. 1).

In contrast with our observations of horses performing exercise at 6 and 8 m/s that L-NAME administration did not accentuate the exercise-induced right atrial or pulmonary arterial, capillary, and venous hypertension (Figs. 2–5), Mills et al. (15) reported that mean pulmonary artery pressure of horses exercised at ~55% V̇O₂max increased significantly after administration of L-NAME. We used the same doses of L-NAME and L-arginine as Mills et al. (15), so it is difficult to explain the divergent findings. However, it should be pointed out that there were several significant differences between the two studies.

First, in the experiments of Mills et al. (15), the pulmonary artery catheter-tip transducer was not referenced in any manner; therefore, the true atmospheric zero for their pressure measurements was not known. Also, because there was no reference signal for comparison, the direction and magnitude of the drift of the catheter manometer could not be determined and accounted for. In this context, Yang et al. (22) have noted, "shortcomings of the catheter-tip transducers are their delicate zeroing and calibration procedures, which are best carried out in-vivo against a classic catheter-tansducer setup." For these reasons, in our experiments, careful attention was directed at proper referencing of the catheter-tip transducer signals against pressure signals obtained via conventional transducers connected to the fluid-filled lumens of the cardiac catheters, and all pressure signals were addressed at the point of the shoulder.

Second, the exercise protocol of Mills et al. (15) was quite different from that used in our experiments. In their experiments, horses performed prolonged submaximal exercise lasting ~85 min, during which treadmill speed was incrementally raised and lowered three times, and L-NAME was administered immediately after the first stage of the exercise test was completed. Thus, observations could not be made under quiet resting and/or basal conditions after L-NAME administration. It is common experience that, immediately after completing treadmill exercise, fit Thoroughbred horses are excited or agitated. It is not known whether administration of L-NAME to excited or agitated horses has similar effects to those observed when L-NAME is administered to quietly standing, relaxed horses. Incidentally, it is also interesting to note that Mills et al. (15) did not report any change(s) in the HR and/or demeanor of horses after intravenous administration of L-NAME; however, these changes were obvious in our experiments.

In summary, our experiments demonstrated that intravenous L-NAME administration at 20 mg/kg to quietly standing Thoroughbred horses resulted in bradycardia because of development and/or exaggeration of second-degree AV block. This was attended by a significant increase in the pulmonary arterial, capillary, and venous blood pressures that indicates that NO synthase inhibition with L-NAME probably affects the pulmonary vascular tone in quietly standing horses. Exercise in both treatments caused progressive significant (P < 0.05) increments in right atrial as well as pulmonary vascular pressures, but the values recorded in the L-NAME study were not different from those in the control study. The extent of exercise-induced tachycardia at 6 and 8 m/s was significantly diminished in the L-NAME study but not during exertion at 14.2 m/s on a 5% uphill grade. Thus, while it appears that
L-NAME administration may not affect the equine pulmonary vascular tone during exercise performed at maximal HR, its effects during exercise at lower workloads need further investigation, because L-NAME may also modify the sympathoneurohumoral response as indicated by the significant attenuation of the exercise-induced tachycardia. Administration of L-NAME to horses also diminished the extent of exercise-induced sweating.

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