Pferdeheilkunde 12 (1996) 4 (Juli August) 551–556

Nitric oxide during exercise and pulmonary disease in the horse

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Summary

The production rate of nitric oxide (NO) in exhaled air ($\dot{V}_{NO}$) during exercise was measured in five healthy horses and one horse with subclinical chronic obstructive pulmonary disease (COPD). Cardiovascular and respiratory parameters were also measured. In the five healthy horses, exercise induced a linear increase in $\dot{V}_{NO}$ to a maximum (428.1±31.6 pmol min-1 kg-1) which coincided with the maximal oxygen uptake for the horses (138.3±11.7 ml min^{-1} kg^{-1}), although a further increase in $\dot{V}_{NO}$ (779.3±38.4 pmol min^{-1} kg^{-1}) occurred immediately after exercise. The changes in $\dot{V}_{NO}$ correlated well with the tidal volume ($r = 0.968$; $P < 0.05$) and the haematocrit ($r = 0.855$; $P < 0.05$). In the horse with COPD, $\dot{V}_{NO}$ also increased linearly with exercise intensity but the values were significantly higher at each collection time compared to the healthy horses.

The exercise protocol was repeated and NO was administered to the horses. Inhaled NO (80 ppm) significantly reduced the pulmonary artery pressure in the five healthy horses, compared to control exercise ($P < 0.05$), during the first (83.2±7.6 mmHg compared to 94.4±6.3 mmHg) and second (84.4±7.1 mmHg compared to 98.4±4.7 mmHg) minutes of high intensity exercise. Inhaled NO did not affect the PAP in the horse with COPD. There were no other significant changes in cardiovascular or respiratory indices, including cardiac output, measured during exercise between control and inhaled NO tests. The results show that NO may contribute to the regulation of pulmonary vascular tone during exercise in the horse.

Keywords: nitric oxide, horse, exercise, lung

Stickstoffmonoxidproduktion während Belastung und bei Lungenerkrankungen des Pferdes

In dieser Studie wurde während Belastung der Anteil an Stickstoffmonoxid (NO) in der ausgeatmeten Luft ($\dot{V}_{NO}$) von Pferden bestimmt. Um mögliche Unterschiede in der $\dot{V}_{NO}$-Produktion zwischen gesunden Pferden und Patienten mit chronisch obstruktiven Lungenleiden (COPD) zu erfassen, wurden 5 gesunde Pferde und ein chronischer Bronchitiker getestet.

V_{NO} besitzt sowohl positive als auch negative Effekte auf bestimmte Körperfunktionen des Pferdes wie die Neurotransmission, die Ciliarfunktion, die Bronchien oder die Immunantwort. Es inhibiert unter anderem die Plättchenaggregation im Blut und kann zur Bronchodilatation führen. Ein signifikanter Anstieg des NO-Gehalts in der Exspirationsluft wurde bei Lungenerkrankungen wie Asthma bronchiale oder entzündlichen Erkrankungen der Atemwege beobachtet. Nach körperlichen Anstrengungen wurde ebenfalls ein Anstieg des NO-Gehalts in der ausgeatmeten Luft des Pferdes gemessen. NO reguliert den Gefäßtonus sowohl im systemischen als auch im pulmonaren Bereich.

Zweck dieser Studie war die Messung der Stickoxidproduktion des Pferdes zur Klärung der möglichen Effekte des $\dot{V}_{NO}$ auf eine Lungenhämorrhagie des Pferdes (EIPH) bzw. zur Erforschung seiner potentiellen Rolle bei der Erhöhung des Blutdrucks in der Pulmonararterie (PAP).

Die 6 Testpferde wurden über einen Zeitraum von 2 Monaten hinweg an die Belastung auf dem Laufband gewöhnt, bis sie am eigentlichen Experiment teilnahmen. Die Pferde wurden im Trab und Galopp bei 5 % Steigung belastet und erhielten Erholungsphasen im Schritt. Während des Experiments wurde die ausgeatmete Luft in bestimmten Abständen gesammelt und der NO-Gehalt gemessen.

Im 2.Teil der Studie wurde den Pferden ein erhöhter Anteil an NO (80 ppm) im Luftgemisch über die Nüstern zugeleitet. Durch die Messung einer Anzahl von kardiovaskulären und respiratorischen Parametern wurde der Einfluß des Stickoxids getestet.

Die Autoren ermittelten folgende Ergebnisse: der NO-Gehalt in der ausgeatmeten Luft stand in linearer Beziehung zur jeweiligen Belastungsintensität. Dies bedeutete steigende NO-Werte der Expirationsluft bei steigendem Lauftempo. Das Pferd mit subklinischer COPD besaß höhere NO-Konzentrationen in der Expirationsluft als die gesunden Pferde.

Das inhalierte Stickoxid induzierte bei den gesunden Pferden einen deutlichen Abfall des Pulmonararteriendrucks (PAP) während der ersten und zweiten Minute der Galopp-Phase, wie Vergleiche zwischen den Daten des 1. und 2. Teilabschnitts der Studie bewiesen. Das Pferd mit COPD zeigte keinen NO-induzierten Abfall des PAP, welcher gleiche Werte wie in der ersten Studie annahm. Die Autoren entdeckten keine weiteren Differenzen zwischen den kardiovaskulären und respiratorischen Parametern beim Vergleich der beiden Testabschnitte.

Aus dieser Studie wurde ersichtlich, daß eine lineare Beziehung zwischen dem Anstieg der NO-Produktion und der Belastungsintensität der Pferde bestand. Die vermehrte Inhalation von NO senkte den Pulmonararteriendruck bei den gesunden Pferden erheblich, nicht jedoch beim Pferd mit chronisch obstruktivem Lungenleiden. Die Resultate lassen vermuten, daß Stickstoffmonoxid (NO) bei der Regulierung des Gefäßtonus der Pferdelunge während anstrengender Arbeit eine Rolle spielt.

Schlüsselwörter: Stickstoffmonoxid, Pferd, Training, Lunge

Introduction

Furchgott and *Zawadski* (1980) first described an endothelium-derived relaxing factor (EDRF) which has been subsequently discovered to be nitric oxide (NO). Since these observations, it has become evident that NO has a range of physiological and pathological functions in the body, including host defence, ciliary function, neurotransmission, inhibition of platelet activity and bronchodilation (reviewed by *Moncada* et al. 1991).

NO is produced from L-arginine by one of three isoforms of the enzyme nitric oxide synthase (NOS). Most cells can produce NO but there are certain types of cells which generally contain one or more isoforms of NOS (*Nathan* and *Xie,* 1994). Two NOS isoforms, vascular endothelial (ecNOS or NOS III) and neuronal (ncNOS or NOS I) are dependent on calcium and calmodulin. An inducible isoform of NOS (iNOS or NOS II) is expressed in most cells types and its expression is enhanced by endotoxin and some cytokines (*Nathan* and *Xie,* 1994). A significant increase in exhaled NO has been measured during inflammatory lung diseases, such as asthma (*Alving* et al. 1993; *Kharitonov* et al. 1994) which is thought to represent upregulation of iNOS as part of the inflammatory response.

NO regulates vascular tone in both the systemic and pulmonary circulations and it is one of the major vasodilators released from vascular endothelial cells (*Moncada* et al. 1991). A deficiency in the activity of ecNOS may be responsible, at least in part, for pulmonary hypertension in man while inhaled NO in low concentrations (up to 80 ppm) reduces pulmonary hypertension in man (*Pepke-Zaba* et al. 1991). Exercise will increase pulmonary artery pressure (PAP), despite inducing a reduction in the pulmonary vascular resistance (PVR) (*West* et al. 1993; *Manohar,* 1994; *Kane* et al. 1994; *West* and *Mathieu-Costello,* 1995). The contribution of endogenous NO to the modulation of increased pulmonary vascular flow during exercise is uncertain. In sheep, inhaled NO does not appear to affect pulmonary vascular tone, although N^{w}-nitro-L-arginine methyl ester (L-NAME), a competitive inhibitor of NOS, induced vasoconstriction and an increase in PAP both at rest and during exercise in sheep (*Kane* et al. 1994; *Koizumi* et al. 1994).

Several studies have reported an increase in the production rate of NO ($\dot{V}_{NO}$) in exhaled air during exercise (*Persson* et al. 1993; *Bauer* et al. 1994). The exercise-induced increase in PAP in the horse (>80 mmHg; *Erickson* et al. 1990), is significantly higher than in other species, including man (approximately 30 mmHg; *West* et al. 1993). The incidence of exercise-induced pulmonary haemorrhage (EIPH), a major problem in the racehorse (*Erickson* et al. 1990; *West* et al. 1993), has been associated with a sudden and marked increase in PAP (*Erickson* et al. 1990; *West* and *Mathieu-Costello,* 1995). Little is known about the role of NO in the control of vascular tone in the horse, although *Manohar* (1995) reported a reduction in pulmonary vascular pressures in the resting horse after administration of glyceryl trinitrate, a NO donor (*Moncada* et al. 1991).

In the present study, the objective was to investigate the contribution of NO to regulation of PAP in the horse during exercise. Endogenous NO was measured in exhaled air during graded exercise and the cardiovascular and respiratory responses of the horse to inhaled NO, a selective pulmonary vasodilator, were investigated.

Materials and methods

Animals

Five clinically healthy Thoroughbred horses (2 geldings, 3 mares) and one gelding (*Rupert*) with clinically diagnosed COPD, aged between 5 and 9 years and weighing 427–550 kg, underwent treadmill training for at least eight weeks prior to commencement of the study. The horses were trained six days a week on the treadmill by trotting (4 m s^{-1} for 20 min) or cantering (9 m s^{-1} for 10 min) on alternative days with the treadmill set at a 5° incline. The experimental protocols were approved by the Ethics Committee of the Animal Health Trust.

Exercise protocol

Study 1 – Effect of exercise on the respiratory production rate of NO ($\dot{V}_{NO}$)

The horses were exercised on the treadmill set at 5°, according to the following protocol: walk (1.7 m s^{-1}) for 8 min, trot (3.7 m s^{-1}) for 8 min, gallop (11 m s^{-1}) for 2 min and recovery/walk (1.7 m s^{-1}) for 5 min. The treadmill was lowered to 0° for the recovery phase of the test. Exhaled air was collected by suction at 20 l min^{-1} for 1.0 min at the following times: (1) – at rest; (2) – after 5 min walk; (3) – after 5 min trot; (4) – from 0-1 min of gallop; (5) – from 1–2 min of gallop; (6) -from 0–1 min of recovery; (7) – from 4–5 min of recovery.

Study 2 – Effect of inhaled NO on cardiovascular and respiratory parameters

The horses repeated the exercise protocol used in study 1 and NO was administered continuously from the mid-point (4.0 min) of the trot until the completion of exercise. The NO (1000 ppm in nitrogen, BOC Special Gases, Surrey, UK) was delivered to the opening of the left nostril of the horse via inert teflon tubing attached to a mask (described below) and mixed freely with the inhaled air. The administered dose of NO for each horse was regulated by adjusting the output from stored NO by a flowmeter (model no. 846024, British Oxygen Company Special Gases, Surrey, UK) based on the minute ventilation calculated in study 1. The aim was to maintain a mean concentration of NO in inhaled air of approximately 80 ppm. NO is relatively stable under the described conditions with minimal production of nitrogen dioxide (NO_2) during the short exposure to the atmosphere (*Cremona* et al. 1995).

A large fan (model no. PBB4-630-32K, Solar and Palau, Barcelona, UK) at the front of the horse forced air over the horse at a speed equivalent to the treadmill speed, simulating airflow expected during field exercise.

Cardiovascular and respiratory measurements

A lightweight fibreglass mask, which housed two flow tubes, one per nostril, was fastened over the external nares of the horse. Ultrasonic flow transducers (BRDL Ltd, Birmingham, UK) within the flow tubes measured flow velocity while a respiratory mass spectrometer (MGA 2000, Case, Biggen Hill, UK) monitored instantaneous respiratory gas concentrations via a flexible capillary positioned in the left flow tube (*Butler* et al. 1993). The following variables were determined as described by *Butler* et al. (1993): respiratory minute volume ($\dot{V}_E$), tidal volume $\dot{V}_T$), rate of O_2 consumption ($\dot{V}_{O_2}$), rate of CO_2 production ($\dot{V}_{CO_2}$) and respiratory frequency (f_R). Values for $\dot{V}_E$ and $\dot{V}_T$ were corrected to BTPS and V_{O_2} and V_{CO_2} were corrected to STPD. Cardiac output (Q) was calculated from $\dot{V}_{O_2}$ and the arterio-venous difference in oxygen content (C_aO_2-C_vO_2) using the Fick equation. Heart rate (f_C) was recorded telemetrically (PEH 100 Hippocard, Isler Bioengineering, Zurich, Switzerland) from electrodes on the mid and lateral thorax. Two

catheter introducers (8 Fr, Arrow, Reading, PA, USA) were placed into the left jugular vein using local anaesthesia (xylocaine 2 %, Astra Pharmaceutical, UK) and both a thermodilution catheter (7 Fr Criticath, Spectramed Inc., USA) and a transducer-tipped catheter (6 Fr, Millar Mikro-tip, Millar Instruments, USA) were advanced into the pulmonary artery to measure central body temperature or pulmonary artery pressure (PAP), respectively (*Butler* et al. 1993). Arterial blood was withdrawn from a catheter (20 G x 32 mm, Intraflon, Vygon, Ecouen, France) placed in either the left or right transverse facial artery under local anaesthesia. Venous blood was collected via the side-port of the catheter introducer. Blood (5 ml) was placed into tubes containing ethylenediamine tetracetic acid (EDTA), stored on ice and the haematocrit (Hct) and haemoglobin content was measured using a Coulter STKR haematology analyser (Coulter Electronics Ltd, Luton, UK) within 1 h of blood collection. Blood gas samples were collected into 1 ml pre-heparinised syringes (QS50, Radiometer, Denmark) and analysed as described previously (*Butler* et al. 1993).
Cardiovascular and respiratory measurements were recorded at the following times: (1) – at rest; (2) – from 3–4 min of walk; (3) – from 3–4 min of trot; (4) – from 0–1 min of gallop; (5) – from 1–2 min of gallop; (6) – from 0–1 min of recovery; (7) – from 4–5 min of recovery, and the following times during experiment 2: (1) – at rest; (2) – from 3–4 min of walk; (3) – from 7–8 min of walk; (4) – from 3–4 min of trot; (5) – from 7–8 min of trot; (6) – from 0–1 min of gallop; (7) – from 1–2 min of gallop; (8) – from 0–1 min of recovery.

Collection of exhaled air and measurement of NO

A vacuum pump (model E2M5, Edwards High Vacuum Int., Crawley, UK) was used to collect exhaled air through a polypropylene tube (2 m x 12 mm id) attached to the mask on the horse. The suction was regulated by a valve which had been calibrated for a flow rate of 20.0 (±0.3) l min^{-1}. The end of the collection tube was fixed perpendicular to the airflow of the left nostril during study 1. To account for a dilution effect of inhaled air, NO concentrations measured in exhaled air were doubled. A one-way valve system was not feasible during exercise because this induced unacceptable resistance to respiration in the horse.
The exhaled air was collected into evacuated 50 l polypropylene douglas bags (PK Morgan Ltd, Rainham, UK) and the [NO] in each collection was measured within 10 min using a sensitive chemiluminescent analyser (Model 42, Thermoelectron, Warrington, UK) with a sampling rate of 700 ml min^{-1}, a response time of 20 s and a detection limit of 0.25 ppb. The analyser was calibrated before each experiment by using a gas mixture of NO in N_2 (130 ppb; Spectraseal, British Oxygen Company, Guildford, UK), which was verified by reference to a national standard (Department of Trade and Industry, Warren Spring Laboratory, Stevenage, UK). The NO analyser was located beside the treadmill during the study and the ambient [NO] during each collection was noted and later subtracted from the average [NO] for the corresponding sample. $\dot{V}_{NO}$ was calculated by the formula:

$$\dot{V}_{NO}\ (\text{pmol min}^{-1}\ \text{kg}^{-1}) = [NO]\ (\text{ppm}) \times \dot{V}_E\ (\text{ml min}^{-1}\ \text{kg}^{-1})$$

Maximum oxygen uptake ($\dot{V}O_{2,max}$)

The $\dot{V}O_{2,max}$ for each horse was calculated by measuring $\dot{V}O_2$ during a step-wise incremental exercise test. This consisted of walking (1.7 m s^{-1}) for 10 min, cantering (6 m s^{-1}) for 1 min, then speed increments of 1 m s^{-1} each min from 8 m s^{-1} until fatigue (approximately 13 m s^{-1}) on the treadmill at a 5° incline. The $\dot{V}O_{2,max}$ was measured at the plateau of $\dot{V}O_2$.

Statistical analysis

Data are presented as mean±sem. Significant differences between cardiovascular and respiratory parameters were calculated using two-way analysis of variance. The Student's *t*-test was applied as a *post hoc* test ($P < 0.05$).

Results

Exercise intensity

A comparison of $\dot{V}O_2$ at each stage of exercise revealed the horses approached their $\dot{V}O_{2,max}$ (138.3±11.7 ml min^{-1} kg^{-1}), reaching a peak of 139.3±16.2 ml min^{-1} kg^{-1} in study 1 and 144.9±5.3 ml min^{-1} kg^{-1} in study 2 during the second min of galloping.

NO in exhaled air during graded exercise (Study 1)

$\dot{V}_{NO}$ showed a significant and linear increase with exercise intensity (Fig. 1) from rest (2.5±0.9 pmol min^{-1} kg^{-1}) until during the second min of gallop (428.1±31.6 pmol min^{-1} kg^{-1}). However, a further increase in $\dot{V}_{NO}$ (779.3±38.4 pmol min^{-1} kg^{-1}) was found during the first min of recovery. The horse with COPD (*Rupert*) had a higher $\dot{V}_{NO}$ throughout exercise (Fig. 1), particularly in the second min of canter (942.8 pmol min^{-1} kg^{-1}) and the first min of recovery (1916.6 pmol min^{-1} kg^{-1}).

Effect of inhaled NO on PAP during exercise (Study 2)

NO (80 ppm) induced a significant ($P < 0.05$) decrease in PAP during the first (83.2±7.6 mmHg) and second (84.4±7.1 mmHg) minutes of the gallop, compared to study 1 of 94.4±6.3 and 98.4±4.7 mmHg, respectively, for the five healthy horses (Fig. 2). Inhaled NO did not alter the PAP for *Rupert* at any stage of exercise, although his PAP was within the range of the other five horses during study 1.

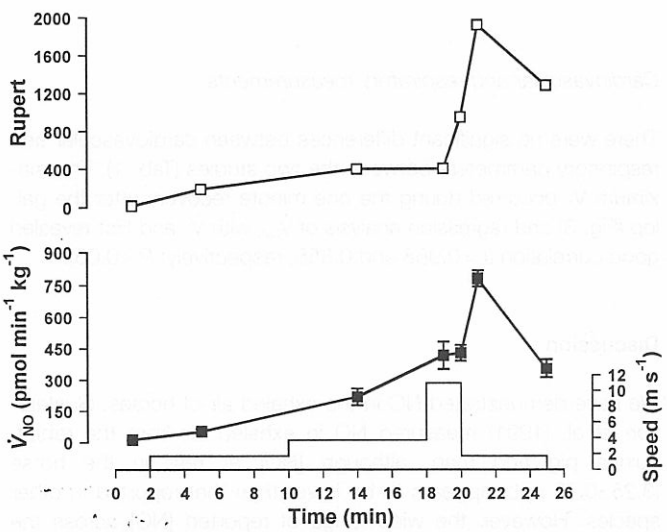

Fig. 1: NO production rate ($\dot{V}_{NO}$) in five healthy horses (mean±se) and in one horse with COPD (Rupert) at different exercise intensities.

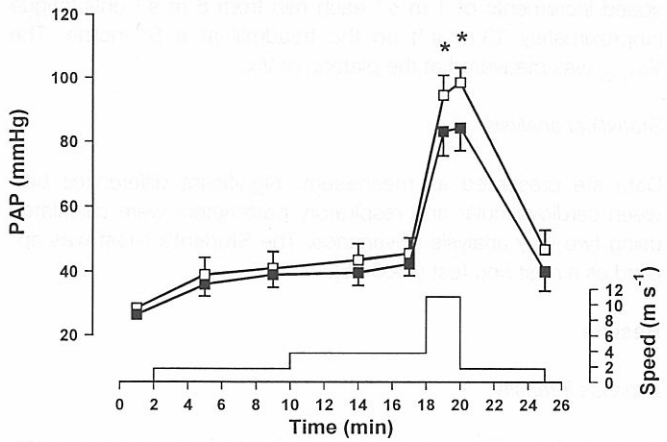

Fig. 2: Pulmonary artery pressure (PAP) in five horses (mean ±se) during study 1(□) and study 2 (inhalation of NO (■). NO administration (80 ppm) commenced 13 min into the exercise protocol. * P <0.05.

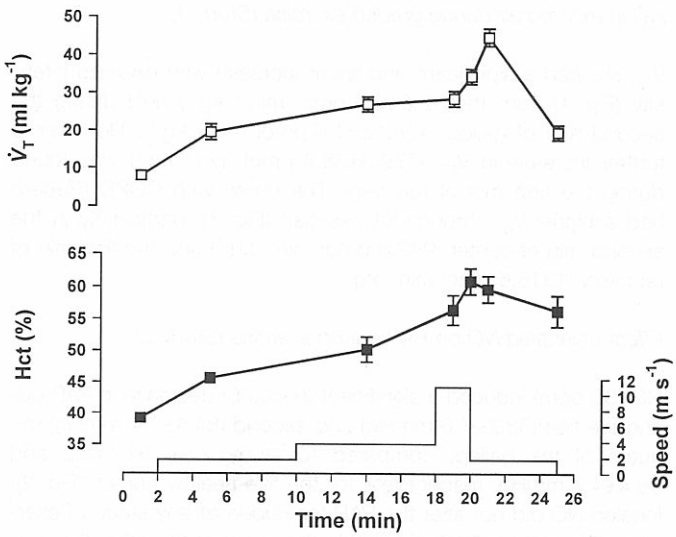

Fig. 3: Tidal volume ($\dot{V}_T$, □) and haematocrit (Hct, ■) during exercise in six horses (mean±se).

Cardiovascular and respiratory measurements

There were no significant differences between cardiovascular and respiratory parameters between the two studies (Tab. 1). The maximum $\dot{V}_T$ occurred during the one minute recovery after the gallop (Fig. 3) and regression analysis of $\dot{V}_{NO}$ with $\dot{V}_T$ and Hct revealed good correlation (r = 0.968 and 0.855, respectively; P <0.05).

Discussion

We have demonstrated NO in the exhaled air of horses. *Gustafsson* et al. (1991) measured NO in exhaled air from the rabbit, guinea pig and man, although [NO] at rest in the horse (3.25±0.75 ppb) appears to be lower than that reported in other species. However, the wide range of reported [NO] across the species (3.4–26.3 ppb) suggests that variation in experimental protocol could influence the measurement of [NO]. Exhaled air was collected for endogenous NO measurement at either perpendicular to the airflow ([NO] was doubled to account for dilution from inhaled air while assuming that inhaled and exhaled volumes will be approximately equal, even allowing for differences in temperature and humidity) or from directly over the nasal mucosa. It was not feasible to use the one-way valve apparatus during exercise because of the unacceptably high resistance at high flow rates. NO is stable in air for the conditions described (*Cremona* et al. 1995) and the fan at the front of the horse rapidly removed residual exhaled air.

Exercise increased $\dot{V}_{NO}$ in man (*Persson* et al. 1993; *Bauer* et al. 1994) and in the horses in the present study. Persson et al. (1993) found that [NO] actually decreased during exercise but $\dot{V}_{NO}$, which accounts for changes in $\dot{V}_E$, correlated with exercise intensity. Similarly, *Bauer* et al. (1994) reported an increase in $\dot{V}_{NO}$ during exercise in man. In our study, we measured a linear increase in $\dot{V}_{NO}$ with exercise intensity, although $\dot{V}_{NO}$ in the horse (428.1± 31.6 pmol min^{-1} kg^{-1} during the second min of the gallop) is substantially higher than that reported in man (e.g. 135.6 pmol min^{-1} kg^{-1}; *Bauer* et al. 1994). This species difference may be expected if, indeed, NO contributes to the regulation of pulmonary vascular tone in the horse, considering that pulmonary vascular pressures are significantly higher during exercise than other species, including man (*Erickson* et al. 1990; *West* et al. 1993; *West* and *Mathieu-Costello,* 1995).

Several studies have suggested that NO in exhaled air could originate from the lower airways, particularly the terminal and respiratory bronchioles (*Gustafsson* et al. 1991; *Persson* et al. 1993; *Cremona* et al. 1995). *Persson* et al. (1993) found a correlation between [NO] and peak CO_2 levels during a single exhalation in man and suggested that the vasculature lining the respiratory and terminal bronchioles contributed to NO in exhaled air. The vascular endothelium is in close apposition to the abluminal surface of the alveoli and a proportion of endogenous NO will escape from the vasculature and can be detected in the exhaled air (*Moncada* et al. 1991; *Cremona* et al. 1995). Infectious or inflammatory conditions of the lung, such as asthma, have been reported to increase NO in exhaled air by induction of iNOS (*Alving* et al. 1993; *Kharitonov* et al. 1994). *Rupert* had a marked increase in $\dot{V}_{NO}$ throughout the exercise protocol which was interpreted to reflect inflammatory changes associated with COPD. Further studies are necessary to ascertain the effects of lung pathology and NO release in the equine lung. It should be noted that high activity of iNOS will downregulate the activity and reponse of ecNOS (*Moncada* et al. 1991; *Nathan* and *Xie,* 1994). Inhaled NO did not affect the PAP of Rupert during intense exercise which may reflect this downregulation of ecNOS during inflammatory conditions of the lung, restricting the expected vascular dilation during exercise.

The vascular endothelial cells have a high capacity for NO production and can respond promptly to changes in blood flow (*Pohl* et al. 1993). Increased shear stress and vascular flow, such as will occur during exercise, have been reported to enhance NO release from vascular endothelial cells (*Pohl* et al. 1993) which could explain the enhanced $\dot{V}_{NO}$ during exercise. A reasonable correlation was found in the horses between $\dot{V}_{NO}$ and both f_C and Hct during exercise, yet a marked increase in $\dot{V}_{NO}$ occurred immediately after exercise and corresponded to an increased $\dot{V}_T$. Similarly, *Persson* et al. (1993) reported that hyperventilation in man acutely increased [NO]. These results suggest that the release of endogenous NO could be influenced by respiratory effort. *Persson* et al. (1995) reported that activation of stretch receptors in the lung by positive end-expiratory pressure ventilation and in-

Tab. 1: A comparison of the effect of inhaled NO (80 ppm; experiment 3) on cardiovascular and respiratory parameters in five Thoroughbred horses (mean±sem) collected between 7-8 of walk (1.7 m s-1), 7–8 min of trot (3.7 m s-1), 0–1 min of gallop (11 m s-1), 1–2 min of gallop (11 m s^{-1}) and 0–1 min of recovery (1.7 m s^{-1}).

		Stage of exercise				
Parameter	test	walk	trot	gallop 1	gallop 2	recovery
$\dot{V}_T$ (ml kg^{-1})	study 1	21.6±2.6	25.7±1.7	28.9±1.6	33.1±1.9	46.5±2.1
	study 2	21.1±2.8	28.0±2.4	31.5±1.5	37.6±3.3	47.2±2.2
$\dot{V}_E$ (ml min^{-1} kg^{-1})	study 1	1044±69	2053±138	3500±187	3813±173	2901±140
	study 2	1204±114	1919±160	3413±321	3820±356	2980±165
$\dot{V}_{O_2}$ (ml min^{-1} kg^{-1})	study 1	24.6±1.9	52.4±2.1	112.5±6.5	132.7±5.9	80.5±3.2
	study 2	25.0±3.3	52.9±1.7	121.6±8.5	144.9±5.3	86.2±3.7
$\dot{V}_{CO_2}$ (ml min^{-1} kg^{-1})	study 1	24.9±2.1	52.9±1.7	115.9±6.9	146.3±7.0	109.1±4.6
	study 2	24.4±3.9	0.8±3.0	119.9±9.7	155.6±4.1	115.6±4.8
P_aO_2 (mmHg)	study 1	102.3±3.6	105.3±2.8	63.8±3.8	60.4±3.1	120.9±5.6
	study 2	110.9±3.1	102.5±2.5	70.9±5.4	65.6±3.7	118.9±5.5
P_aCO_2 (mmHg)	study 1	45.9±2.1	44.5±2.1	60.3±3.5	66.7±4.6	36.2±2.3
	study 2	44.1±3.2	45.9±1.9	58.9±2.6	61.3±2.1	35.4±3.8
P_vO_2 (mmHg)	study 1	29.3±1.2	22.7±1.1	15.2±1.0	13.4±0.8	36.6±5.6
	study 2	31.7±1.6	23.9±0.7	19.1±1.0	15.1±0.7	40.5±2.2
P_vCO_2 (mmHg)	study 1	60.3±1.8	64.1±2.2	110.7±5.5	136.2±9.1	68.4±5.6
	study 2	56.9±4.9	65.1±2.4	100.7±4.9	125.9±9.2	54.1±2.6
f_R (breaths min^{-1})	study 1	51.7±3.1	82.3±8.3	121.4±0.6	118.1±1.3	68.5±2.6
	study 2	61.6±5.6	75.2±10.9	119.6±0.9	118.5±0.8	66.2±3.2
f_C (beats min^{-1})	study 1	89.2±5.1	125.8±4.5	193.0±5.9	196.5±6.3	121.8±2.9
	study 2	89.0±4.7	127.7±5.9	193.7±5.9	195.3±5.4	119.8±3.1
Q (ml min^{-1} kg^{-1})	study 1	142.0±10.3	194.5±12.1	271.5±18.4	285.5±21.8	89.7±7.5
	study 2	157.6±23.4	199.1±10.8	293.9±16.8	306.5±19.6	91.2±6.3

creased vagal tone could enhance NO release. However, isolated lung preparations and cultured endothelial cells will release NO in response to a variety of chemical stimuli without innervation (*Cremona* et al. 1995).

A deficiency of ecNOS activity has been implicated in pulmonary hypertension (*Pepke-Zaba* et al. 1991). Exercise will increase pulmonary vascular tone (*West* and *Mathieu-Costello,* 1995) which is compensated for by a rapid decrease in pulmonary vascular resistance via recruitment of the microvasculature (*Johnson* and *Hsia,* 1994; *Kane* et al., 1994). This functional recruitment of pulmonary capillaries is probably mediated by vasodilation of previously low-flowing capillaries and not by inducing flow in 'dormant' capillaries (*Johnson* and *Hsia,* 1994). Since NO is one of the major vasodilators released from vascular endothelial cells to maintain basal dilator tone and to oppose pressor substances in pulmonary vasculature (*Moncada* et al. 1991; *Pohl* et al. 1993), it could also contribute to the reduction of PVR during exercise.

Low doses of inhaled NO have been shown to reverse an increased PAP and *Va/Q* inequalities during severe pulmonary diseases of man (*Zapol* and *Hurford,* 1994). Inhaled NO (80 ppm) induced a small but significant decrease in PAP during exercise in the horses of this study. *Manohar* (1995) recently reported a decrease in pulmonary vascular pressures in the resting horse after the infusion of glyceryl trinitrate, a NO donor (*Moncada* et al. 1991). However, systemic hypotension is a disadvantage of commonly used anti-hypertensive agents, such as glyceryl trinitrate (*Moncada* et al. 1991). The effects of inhaled NO will be restricted to the pulmonary circulation because its affinity for oxyhaemoglobin is so high as to remove excess NO in the alveolus by the formation of methaemoglobin and nitrite (*Wennmalm* et al. 1992).

Inhaled NO had no apparent effect on PAP in the sheep, despite a significant increase in PAP at rest and during exercise after the administration of L-NAME (*Kane* et al. 1994; *Koizumi* et al. 1994), an inhibitor of ecNOS (*Moncada* et al. 1991). From their results, *Kane* et al. (1994) and *Koizumi* et al. (1994) suggested that NO has a basal vasodilator function that persists, but is not enhanced during exercise to oppose alpha-mediated vasoconstriction. However, there are species differences in the activity of NO which preclude the extrapolation of effects between species. For example, L-NAME will increase pulmonary vascular resistance (PVR) in pigs, sheep and man, but not the dog (*Cremona* et al. 1995). Furthermore, in athletic species the lung is adapted to high-intensity exercise by an eight-fold increase in pulmonary vascular flow and a thin pulmonary blood-gas barrier to permit rapid gas exchange (*Wagner* et al. 1989; *West* and *Mathieu-Costello,* 1995). The superior $\dot{V}_{O_{2,max}}$ of the horse requires a comparatively higher cardiac output and subsequently, a higher PAP than other species which will also predispose the horse to stress failure and subsequent rupture of pulmonary capillaries during near maximal exercise (*West* et al. 1993; *Wagner* et al. 1989; *West* and *Mathieu-Costello,* 1995).

Stress failure of pulmonary capillaries, resulting from excessive pulmonary vascular pressure during high-intensity exercise has been implicated in the pathogenesis of EIPH (*West* et al. 1993; *West* and *Mathieu-Costello,* 1995), a major problem in the horse during racing (*Erickson* et al. 1990; *West* et al. 1993). The severity of pulmonary capillary damage directly correlates with increasing PAP (*West* and *Mathieu-Costello,* 1995) and blood volume (*Wagner* et al. 1989). Maximal changes in PAP are induced by the sudden onset of high intensity exercise whether on the racecourse (*Erickson* et al. 1990) or in an exercise protocol such as used in the present study. Therefore, while NO may only contribute to vasodilation of pulmonary capillaries and oppose alpha-mediated vasocontriction during exercise (*Kane* et al. 1994; *Koizumi* et al. 1994), any reduction in the basal activity of NO during peak exercise may contribute to a marginal but significant increase in PAP.

The synthesis of NO requires normoxia (*Cremona* et al. 1995) while its effect in pulmonary arteries is selectively impaired by even moderate hypoxia (*Johns* et al. 1989). Hypoxic inhibition of NO production has been suggested as one of the mechanisms of

hypoxic pulmonary vasoconstriction because acute hypoxia will depress endothelium-dependent relaxation of the vasculature (*Johns* et al. 1989). Inhaled NO will reverse hypoxic pulmonary vasoconstriction in a dose-dependent manner (*Frostell* et al. 1991) and reduce the rise in PAP in the horses of this study. Alveolar hypoxia has been reported in the horse during high-intensity exercise (*Wagner* et al. 1989; *Butler* et al. 1993) which could reduce NO-dependent relaxation of pulmonary vasculature. Arterial hypoxaemia has also been reported in the horse during near-maximal exercise (*Wagner* et al. 1989; *Butler* et al. 1993). The affinity of haemoglobin for NO is inversely proportional to its oxygen saturation (*Iwamoto* and *Morin,* 1993) and arterial hypoxaemia will enhance the inactivation of NO. Any reduction in NO activity may contribute to the incidence of EIPH in the horse during near-maximal exercise. *Pelletier* et al. (1995) recently demonstrated a difference in the regional blood flow in the equine lung and that blood flow to the caudo-dorsal aspect of the lung is endothelium-dependent. Interestingly, EIPH is usually restricted to the caudo-dorsal aspect of the lung (*Erickson* et al. 1990).

In summary, NO was detected in the exhaled air of the horse and $\dot{V}_{NO}$ increased linearly with exercise intensity. Inhaled NO significantly reduced PAP in the horse at near maximal exercise. The incidence of EIPH in the horse could be related to a small but significant reduction in NO activity during near-maximal exercise.

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