SHORT COMMUNICATIONS

Effects of inhaled nitric oxide 10 ppm in spontaneously breathing horses anaesthetized with halothane

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Inhaled nitric oxide, a selective pulmonary vasodilator, is known to improve arterial oxygenation after cardiopulmonary bypass and during acute respiratory distress syndrome in humans. During general anaesthesia with spontaneous ventilation, healthy adult horses develop large alveolar–arterial oxygen tension differences. In this study, we have determined the effects of inhaled nitric oxide (10 parts per million (ppm)) on venous admixture and pulmonary haemodynamics in horses anaesthetized with halothane. Seven adult horses were studied twice in random sequence. After premedication with romifidine 100 µg kg−1, anaesthesia was induced with ketamine 2.2 mg kg−1 and maintained with 1.1 MAC (0.95%) of halothane in oxygen. Horses breathed spontaneously. After 65 min, each horse had nitric oxide 10 ppm added to the inspired gas for 20 min (procedure HA1NO) or anaesthesia was continued with halothane in oxygen (procedure HA). Cardiac output, minute ventilation, arterial and mixed venous oxygen and carbon dioxide tensions, and mean pulmonary and carotid arterial pressures were measured for 100 min. Shunt fraction and pulmonary and systemic vascular resistances were calculated. Shunt fraction (SF) and mean pulmonary artery pressure (P\text{PA mean}) were not different between the two groups after 65 min of general anaesthesia (HA: SF 0.20 (SD 0.06), P\text{PA mean} 45 (8) mm Hg; HA1NO: SF 0.21 (0.04), P\text{PA mean} 44 (7) mm Hg) or after 85 min (HA: SF 0.22 (0.07), P\text{PA mean} 45 (8) mm Hg; HA1NO: SF 0.20 (0.03), P\text{PA mean} 43 (7) mm Hg). There were no significant effects of time or nitric oxide inhalation on any other variable. There was a significant correlation (r=0.80, P<0.05) between calculated shunt fraction 65 min after induction of anaesthesia and body weight.

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Endogenous nitric oxide is responsible for maintenance of basal dilator tone in the systemic and pulmonary vasculatures of many species. Inhaled nitric oxide reverses hypoxic pulmonary vasoconstriction (HPV) in humans and it is now used therapeutically to reduce pulmonary hypertension and intrapulmonary shunting during acute respiratory distress syndrome.1 Large discrepancies between alveolar and arterial oxygen tension (P\text{A O}_2−P\text{a O}_2) are common during general anaesthesia in horses, differences which result from mismatching between ventilation and perfusion (V\text{A}/Q\text{O}_2).2 It is generally accepted that halothane directly interferes with nitric oxide activity on vascular endothelium3 and that potent inhalation anaesthetics also inhibit HPV in many species. Therefore, it is possible that disruption of nitric oxide activity and abolition of HPV may contribute to ventilation–perfusion abnormalities in anaesthetized horses. It was our hypothesis that inhaled nitric oxide might selectively induce vasodilatation in ventilated areas of lung, thereby promoting blood flow to these areas and reducing venous admixture. The purpose of this study was to evaluate whether inhaled nitric oxide modulated intrapulmonary shunting in spontaneously breathing adult horses during halothane–oxygen anaesthesia.

Methods and results

The study conformed to the UK Animal (Scientific Procedures) Act 1986 and was approved by the Ethics Committee
of the Animal Health Trust. Seven adult horses were studied on two occasions, in a randomized sequence, with each procedure separated by a period of at least 4 weeks. During procedure HA, anaesthesia was maintained with halothane at an end-tidal concentration of 1.1 MAC in oxygen, for 100 min. In procedure HA+NO, nitric oxide 10 ppm was added to the inspired gases for 20 min after 65 min of anaesthesia.

The group of horses comprised one female and six castrated males of median age 4 yr (range 3–18 yr), weighing 490–570 kg (mean 507 kg). Three months before the studies were performed, a portion of the right carotid artery was raised to a subcutaneous position under general anaesthesia. Food, but not water, was withheld from the horses from 20:00 on the day preceding each study. In a padded anaesthesia induction area, the α₂ adrenoceptor agonist romifidine 100 µg kg⁻¹ was administered i.v. via a jugular catheter. Anaesthesia was induced 5 min later with ketamine 2.2 mg kg⁻¹ i.v. The trachea was intubated and general anaesthesia was subsequently maintained with halothane in oxygen. Each horse breathed spontaneously from a large animal circle system. Halothane concentration in the respired gases was measured using respiratory mass spectroscopy (MGA2000, Case, Biggin Hill, Kent, UK). The instrument was calibrated before each anaesthetic using calibration gas containing 2% halothane, 5% carbon dioxide, 63% oxygen and 30% nitrogen (BOC Speciality Gases, Guildford, Surrey, UK). Two-point calibration of the thermistor was performed against a precision mercury manometer (Poddy meter, Kingston-upon-Thames, Surrey, UK) before catheter insertion, and baseline drift was checked on removal. Similarly, two-point calibration of the thermistor was performed against a precision mercury thermometer using two heated water baths. Thermistor and transducer outputs were linear within the measured range.

Respiratory airflow was measured with a heated Fleisch No. 3 pneumotachometer, connected to a Validyne differential pressure transducer. The device was calibrated by forcing a known volume (7 litre) of air through the pneumotachometer using a calibrated syringe (Series 4900, Hans Rudolph, Kansas City, USA). The pneumotachometer was connected via an adapter to the breathing circuit Y-piece and the tracheal tube. The adapter was equipped with ports to allow sampling of respired gases for measurement of oxygen, carbon dioxide, nitrogen and halothane by respiratory mass spectroscopy (MGA2000, CASE, Biggin Hill, Kent, UK). The instrument was calibrated before each anaesthetic using calibration gas containing 2% halothane, 5% carbon dioxide, 63% oxygen and 30% nitrogen (BOC Speciality Gases, Guildford, Surrey, UK). In order to express respired gas volumes according to BTPS (body temperature and pressure saturated) convention, the catheter contained an internal lumen for collection of mixed venous blood. A 16-gauge over-the-needle Teflon catheter was placed in the raised carotid artery for collection of arterial blood. Carotid arterial pressure was measured using an external strain gauge transducer secured at the vertical height of the horse’s sternum. Bridge excitation voltage to the strain gauge transducers was supplied by two high-gain amplifiers (Pressure Processor, Model 13-4615-52, Gould, Essex, UK). The thermistor was interfaced with a custom-made bridge and commercial amplifier (Universal Preamplifier, Model 13-4615-58, Gould, Essex, UK). Two-point calibration of the pressure transducers was performed using a precision mercury manometer (Poddy meter, Kingston-upon-Thames, Surrey, UK) before catheter insertion, and baseline drift was checked on removal. Similarly, two-point calibration of the thermistor was performed against a precision mercury thermometer using two heated water baths. Thermistor and transducer outputs were linear within the measured range.

Immediately after induction of anaesthesia, a 160-cm long, 3.75-MHz transoesophageal echocardiographic probe (Vingmed Sound, Horton, Norway) was inserted into the oesophagus via the ventral nasal meatus. Cardiac output was calculated from the aortic velocity spectra according to the methods of Young and colleagues. Measurements of pulmonary arterial pressure were made using a strain-gauge transducer, mounted on a 150-cm long 8F woven Dacron catheter. The catheter was also equipped with a thermistor to record pulmonary artery temperature to allow correction of blood-gas tensions for body temperature and to express respired gas volumes according to BTPS (body temperature and pressure saturated) convention. The catheter contained an internal lumen for collection of mixed venous blood. A 16-gauge over-the-needle Teflon catheter was placed in the raised carotid artery for collection of arterial blood. Carotid arterial pressure was measured using an external strain gauge transducer secured at the vertical height of the horse’s sternum. Bridge excitation voltage to the strain gauge transducers was supplied by two high-gain amplifiers (Pressure Processor, Model 13-4615-52, Gould, Essex, UK). The thermistor was interfaced with a custom-made bridge and commercial amplifier (Universal Preamplifier, Model 13-4615-58, Gould, Essex, UK). Two-point calibration of the pressure transducers was performed using a precision mercury manometer (Poddy meter, Kingston-upon-Thames, Surrey, UK) before catheter insertion, and baseline drift was checked on removal. Similarly, two-point calibration of the thermistor was performed against a precision mercury thermometer using two heated water baths. Thermistor and transducer outputs were linear within the measured range.

After 65 min of anaesthesia in procedure HA+NO, gas from a cylinder containing nitric oxide 1000 ppm in nitrogen was metered into the patient breathing circuit to achieve a steady inspired concentration of 10 ppm. Nitric oxide was delivered to the circuit for 20 min, then administration ceased and the fresh gas flow to the breathing circuit was...
Table 1 Heart rate (HR), cardiac output (Q\dot), mean pulmonary artery pressure (P_{PA\,mean}), mean carotid artery pressure (P_{CA\,mean}), pulmonary vascular resistance (PVR), systemic vascular resistance (SVR), partial pressure of oxygen in arterial blood (P_{A\,O2}), partial pressure of carbon dioxide in arterial blood (P_{A\,CO2}) and respiratory minute ventilation (V\dot_E) in seven adult horses (mean (sd)) breathing spontaneously during 100 min of halothane anaesthesia (end-tidal halothane=1.1 MAC). The procedures were identical except that during HA+NO, nitric oxide 10 ppm was administered for 20 min (italicized), 65 min after induction of anaesthesia. There was no significant effect of time or treatment (ANOVA)

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<thead>
<tr>
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<th>40 min</th>
<th>65 min</th>
<th>+10 min</th>
<th>+20 min</th>
<th>+35 min</th>
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</thead>
<tbody>
<tr>
<td>HR (beat min^{-1})</td>
<td>HA (30)</td>
<td>HA+NO (31)</td>
<td>HA (32)</td>
<td>HA+NO (32)</td>
<td>HA (30)</td>
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<tr>
<td>Q (litre min^{-1})</td>
<td>HA (30)</td>
<td>HA+NO (31)</td>
<td>HA (32)</td>
<td>HA+NO (32)</td>
<td>HA (30)</td>
</tr>
<tr>
<td>P_{PA,mean} (mm Hg)</td>
<td>HA (46)</td>
<td>HA+NO (43)</td>
<td>HA (45)</td>
<td>HA+NO (44)</td>
<td>HA (46)</td>
</tr>
<tr>
<td>P_{CA,mean} (mm Hg)</td>
<td>HA (68)</td>
<td>HA+NO (68)</td>
<td>HA (56)</td>
<td>HA+NO (65)</td>
<td>HA (66)</td>
</tr>
<tr>
<td>PVR (dyn s cm^{-5})</td>
<td>HA (128)</td>
<td>HA+NO (112)</td>
<td>HA (112)</td>
<td>HA+NO (104)</td>
<td>HA (112)</td>
</tr>
<tr>
<td>SVR (dyn s cm^{-5})</td>
<td>HA (192)</td>
<td>HA+NO (176)</td>
<td>HA (160)</td>
<td>HA+NO (160)</td>
<td>HA (160)</td>
</tr>
<tr>
<td>P_{A,O2} (kPa)</td>
<td>HA (46.4)</td>
<td>HA+NO (42.8)</td>
<td>HA (47.5)</td>
<td>HA+NO (47.5)</td>
<td>HA (46.7)</td>
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<tr>
<td>P_{A,CO2} (kPa)</td>
<td>HA (8.1)</td>
<td>HA+NO (8.9)</td>
<td>HA (8.8)</td>
<td>HA+NO (8.5)</td>
<td>HA (8.5)</td>
</tr>
<tr>
<td>V\dot_E (litre min^{-1} BTPS)</td>
<td>HA (43)</td>
<td>HA+NO (44)</td>
<td>HA (42)</td>
<td>HA+NO (44)</td>
<td>HA (42)</td>
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increased to encourage a rapid decrease in inspired nitric oxide concentration. The concentration of nitric oxide at the Y-piece was monitored constantly using a dedicated chemiluminescence analyser (270B, Sievers Instruments Inc, CO, USA). The analyser was zeroed with nitrogen and calibrated with a nitric oxide standard containing nitric oxide 40 ppm in nitrogen (BOC Speciality Gases, Guildford, Surrey, UK).

In both procedures, arterial and mixed venous blood was collected 65 min after the start of halothane anaesthesia. Sixty-five minutes was selected as the control data collection point to allow sufficient time for complete instrumentation of all horses and to allow adequate denitrogenation. In procedure HA, further samples were collected after 75 min (+10 min), 85 min (+20 min) and 100 min (+35 min) of anaesthesia. In procedure HA+NO, nitric oxide was added to the circuit immediately after withdrawal of blood at 65 min. Additional blood samples were collected at 10 (+10 min) and 20 (+20 min) min after inhalation of nitric oxide 10 ppm, and 15 min (+35 min) after nitric oxide administration was discontinued.

Mixed venous and arterial blood was withdrawn into heparinized syringes and blood-gas analysis was performed within 10 min of collection. Haemoglobin concentrations in the mixed venous and arterial blood samples were measured using a colorimetric method. Venous admixture was calculated using the shunt equation. Pulmonary end-capillary oxygen tension was assumed to be equal to alveolar oxygen tension.

All measured signals were recorded and analysed using commercial hardware and a software package for acquisition and analysis of physiological data (Po-Ne-Mah, Linton Instrumentation, Diss, Norfolk, UK). The voltage signals corresponding to pulmonary and carotid arterial pressures, pulmonary arterial temperature, ECG, inspired gas temperature and humidity, ambient temperature and humidity, respiratory gas flow, and nitrogen, oxygen, halothane and carbon dioxide fractional concentrations in the respiratory gas mixture and inspired nitric oxide concentration were digitized at 500 Hz.

Data were first checked for normality using normal probability plots. Data were subsequently analysed using repeated measures analysis of variance. A probability of P < 0.05 was considered to reflect statistically significant differences between adjacent times and between the same times in procedures HA and HA+NO. The relationship between shunt fraction and body weight after 65 min of anaesthesia was investigated using correlation and linear regression analyses. The mean of the shunt fraction data for each horse from the HA and HA+NO procedures at 65 min was used.

Inhalation of nitric oxide 10 ppm produced no significant effects on calculated shunt fraction or mean pulmonary artery pressure in spontaneously breathing horses anaesthetized with halothane (Fig. 1). There was no significant difference in any measured or derived cardiopulmonary variable after administration of nitric oxide (Table 1). Data were not significantly different between procedures HA and HA+NO at any time. There was a significant correlation between shunt fraction at 65 min after induction of anaesthesia and body weight (r = 0.799, 5 df, P < 0.05; shunt fraction = 0.0016.weight – 0.6318).

**Comment**

We found that inhalation of nitric oxide 10 ppm for 20 min failed to alter venous admixture in spontaneously breathing horses anaesthetized with halothane, although there was a significant positive correlation between body weight and shunt fraction. Our data suggest that vessels supplying ventilated alveoli may be maximally dilated in anaesthetized horses, as inhaled nitric oxide had no effect on either mean pulmonary artery pressure or pulmonary vascular resistance. The relationship between body weight and shunt fraction supports the hypothesis that compression atelectasis is important in the development of alveolar–arterial oxygen tension differences during anaesthesia in adult horses.2

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References


