Effect of two anaesthetic regimens on airway nitric oxide production in horses

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There is evidence that halothane inhibits nitric oxide synthase in vitro, but the effect of intravenous anaesthetic agents is less clear. This study was undertaken to compare the rate of exhaled nitric oxide production (VN O) in spontaneously breathing horses anaesthetized with halothane or an intravenous regimen. Seven adult horses were studied twice in random order. After premedication with romifidine 100 μg kg⁻¹, anaesthesia was induced with ketamine 2.2 mg kg⁻¹ and maintained with halothane in oxygen (HA) or by an intravenous infusion of ketamine, guaiphenesin and romifidine (IV). Inhaled and exhaled nitric oxide (NO) concentrations, respiratory minute ventilation (V̇E), pulmonary artery pressure (PAP), fractional inspired oxygen concentration (FIO₂), end-tidal carbon dioxide concentration (ETCO₂), cardiac output (Q) and partial pressures of oxygen and carbon dioxide in arterial blood (Pao₂, Paco₂) were measured.

Exhaled nitric oxide production rate was significantly lower (40 min, P<0.01; 60 min, P<0.02) during HA [40 min, 1.4 (SD 1.4) pmol l⁻¹ kg⁻¹ min⁻¹; 60 min, 0.7 (0.7) pmol l⁻¹ kg⁻¹ min⁻¹] than during IV [40 min, 9.3 (9.9) pmol l⁻¹ kg⁻¹ min⁻¹; 60 min, 12.5 (13.3) pmol l⁻¹ kg⁻¹ min⁻¹]. Mean pulmonary artery pressure was significantly higher (40 min, P<0.01; 60 min, P<0.001) during HA [40 min, 5.9 (1.1) kPa; 60 min, 5.9 (0.9) kPa] compared with IV [40 min, 4.4 (0.4) kPa; 60 min, 4.4 (0.5) kPa]. NO is reduced in the exhalate of horses anaesthetized with halothane compared with an intravenous regimen. It is suggested that increased mean pulmonary artery pressure during halothane anaesthesia may be linked to the differences in NO production.

Keywords: horse; anaesthesia; pharmacology, nitric oxide; anaesthetics volatile, halothane

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Nitric oxide (NO) has been shown to regulate vascular tone in both the systemic and the pulmonary circulation and has been detected in the exhalate of a number of species, including the horse. In the standing conscious horse, infusion of the NO donor nitroglycerine has been shown to reduce pulmonary vascular pressures. Similarly, infusion of the nitric oxide synthase (NOS) inhibitor L-NAME has been shown to increase resting pulmonary arterial pressures. A number of studies in other species have shown that halothane attenuates endothelium-dependent pulmonary vasodilatation, which may or may not involve NO, both in vitro and in vivo. The interaction of NOS with intravenous anaesthetics is less clear. For example, propofol has been shown to increase NO release from cultured porcine endothelial cells whilst a number of other intravenous anaesthetic agents have been reported to decrease brain NOS activity in vitro. The hypothesis was that horses anaesthetized with halothane would exhibit lower exhaled NO concentrations than horses anaesthetized with a total intravenous regimen.

Methods and results

The study was carried out under Home Office licence PPL.80/1017 and was approved by the Ethics Committee of the Animal Health Trust. Seven healthy adult horses (one female, six castrated males; weight 508 (SD 33) kg; median age 4 yr, range 3–18 yr) were studied on two occasions. Four horses underwent procedure HA first and three underwent procedure IV (see below). Food, but not water, was withheld from 20.00 h on the day preceding each study. In both procedures, the horses were premedicated with romifidine 100 μg kg⁻¹ and anaesthesia was induced with ketamine 2.2 mg kg⁻¹. During procedure HA, anaesthesia was maintained by halothane at an end-tidal concentration of 0.9%. In procedure IV, guaiphenesin 50 mg kg⁻¹ was...
administered as a bolus immediately after induction and anaesthesia was maintained by an infusion of romifidine 82 μg kg⁻¹ h⁻¹ and ketamine 6.6 mg kg⁻¹ h⁻¹. Guaiaphenesin was also infused at the rate of 100 μg kg⁻¹ h⁻¹ for the first 30 min of anaesthesia and the rate was then reduced to 60 μg kg⁻¹ h⁻¹. The trachea was intubated with a 30 mm cuffed tracheal tube and the animals breathed spontaneously from a large-animal circle absorber with a leak (a partial rebreathing circuit) supplied with oxygen at a fresh gas flow rate of 10 litre min⁻¹. Carbon dioxide was absorbed from the expired gas using soda lime. The distal end of the tracheal tube and the circle Y-piece were fitted with adaptors to allow insertion of a pneumotachometer and the collection of expired gases. Measurements of pulmonary artery pressure (P_Pa) were made using a strain-gauge transducer mounted on the tip of a 150 cm, 8F, woven Dacron catheter (Gaeltec, Dunvegan, Isle of Skye, UK) interfaced to a pressure amplifier (Model 13–4615–52; Gould, Essex, UK). The catheter was introduced via an 8F arterial sheath introducer (Haemaguet-Steri Lock; Bard, Crawley, UK) into the right jugular vein. Cardiac output (Q) was measured using transoesophageal Doppler echocardiography. Arterial blood oxygen (P_O2) and carbon dioxide (P_CO2) tensions were measured at 37°C with a blood gas analyser (model 248; Chiron Diagnostics, Essex, UK).

Respiratory airflow was measured with a Fleisch No. 3 heated pneumotachometer connected to a differential pressure transducer (DP41–14; Validyne, Northridge, CA, USA). The pneumotachometer was calibrated with a known volume (7 litres) from a calibrated volume syringe (Series 4900; Hans Rudolph, Kansas City, KA, USA). Respired gases (oxygen, nitrogen, carbon dioxide and halothane) were measured continuously with a respiratory mass spectrometer (MGA200; Morgan Medical Ltd, Rainham, UK). The mass spectrometer was calibrated with two certified gas mixtures representing the range to be encountered during the studies (BOC Speciality Gases, Guildford, UK). The sampling capillary was inserted into the circuit distal to the pneumotachometer to sample both inspired and expired gases.

Concentrations of NO (p.p.b.) in the inspired and expired limbs of the circuit approximately 15 cm on each side of the Y-piece were measured with a dedicated chemiluminescence analyser (270B; Sievers Instruments, Boulder, CO, USA). Measurements of both inhaled and exhaled NO were made over 30 s during periods of regular breathing to reduce the effect of variations throughout each breath in NO concentration and the inherent variation of the analyser, which in the HA procedure was operating near the manufacturer’s reported lower limit of sensitivity (5 p.p.b.). This approach was considered preferable to making single point measurements of NO concentration. As the gas in the expired limb continues to be sampled by the NO analyser during the subsequent period of inspiration, this approach could potentially bias the average exhaled NO concentration according to the NO concentration in the last portion of gas exhaled. In order to reduce this error, measurements were made only during periods of regular breathing, without periods of apnoea between breaths, which would have prolonged the period in which the end-tidal NO continued to be sampled. In addition, the exhaled NO concentration varied minimally throughout exhalation. Net NO production was estimated by subtracting inspired NO concentration from expired NO concentration. Before each measurement of inspired and expired NO, a two-point calibration was performed with zero (100% nitrogen) and mixtures made by volume dilution of a certified NO standard mixture (400 p.p.b.; BOC Speciality Gases).

To determine that halothane did not affect the NO analyser, the anaesthetic system was set up without being connected to a horse and the connection to the tracheal tube was capped. The system was filled and flushed with oxygen and, once the oxygen concentration was above 98%, the flow rate was adjusted to 10 litre min⁻¹. Nitric oxide from a cylinder at 1000 p.p.m. (BOC Speciality Gases) was metered into the circuit to achieve a concentration at the Y-piece of -5 p.p.m., as determined by the NO analyser. Halothane was then added to the circuit to a maximum concentration of 3%, as determined by a calibrated piezoelectric agent monitor (Lamtec 605; Pneupac, Luton, UK), with the sample line positioned at the Y-piece of the breathing circuit.

In order to express respired gas volumes according to BTPS (body temperature and pressure, saturated) convention, gas was drawn continuously from the inspiratory limb of the circuit by a vacuum pump (flow rate <3 litre min⁻¹) across a combined temperature and humidity sensor (HMP35B; Vaisala, Cambridge, UK). A second probe in the operating theatre recorded ambient temperature and humidity.

The voltage signals from the pressure amplifier, pneumotachometer and NO analyser were digitized at 500 Hz and analysed with a commercial data acquisition and analysis system (Po-Ne-Mah; Linton Instrumentation, Diss, UK). Measurements of diastolic (P_PA_DIA), systolic (P_PA_SYS) and mean pulmonary artery pressure (P_PA_MEAN), respiratory minute ventilation (V_E) and net NO production were made at 40 and 60 min of anaesthesia in both groups. Effects of time and treatment were investigated using analysis of variance and Tukey’s test. Where data were not normally distributed (net exhaled NO concentration, V_NO, P_CO2 and Q), they were transformed by conversion to the natural logarithm before statistical analysis.

Net exhaled NO concentration, tidal volume, respiratory minute ventilation, NO production rate, fractional inspired oxygen, end-tidal carbon dioxide, partial pressures of oxygen and carbon dioxide in arterial blood, cardiac output and pulmonary artery pressures after 40 and 60 min of HA and IV anaesthesia are shown in Table 1. There was no significant within-group difference between the 40 and
60 min time points for any of the measurements with either technique.

Net exhaled NO, \( \dot{V}_E \) and \( \dot{V}_{NO} \) were significantly higher and systolic, diastolic and mean pulmonary artery pressures were significantly lower in the IV than in the HA group. During in vitro testing, the NO concentration in the circle, when not connected to a horse, showed no change during or after addition of halothane. Thus, it was concluded that the reduced exhaled NO is unlikely to be an artefact due to an effect of halothane on the NO analyser.

**Comment**

Previously we have reported mean exhaled NO concentrations of 1–3 p.p.b. in conscious, unsedated, nasal-breathing healthy adult horses at rest.\(^1\) In the present study, net exhaled NO concentrations in the IV procedure were similar to those in resting horses, although the rate of NO production was reduced because of lower minute ventilation. Net exhaled NO concentration and the rate of NO production during halothane anaesthesia were lower than when the same horses where anaesthetized with an intravenous regimen. The most simple explanation for a reduction in exhaled NO concentration would be an inhibition of NOS. However, there is currently little evidence to support the contention that halothane inhibits NOS directly.

Reduced airway NO production has also been reported in association with increased inspired carbon dioxide, but in the present study there was no significant difference in arterial carbon dioxide between procedures. It has also been suggested that the degree of lung distension may effect NO production in anaesthetized rabbits\(^8\) and exercising horses.\(^1\) Tidal volume was not different between anaesthetic regimens, although minute ventilation was significantly higher with the IV procedure. A change in functional residual capacity could have occurred, but there is no reason to expect that this would have been different between procedures. Measured exhaled NO could have been reduced if removal of NO by the lower airways was enhanced, although how this might be mediated is unclear.

NO has been shown to be involved in the regulation of pulmonary vascular tone in both systemic and pulmonary circulations in many species, including the horse.\(^2\) A consequence of reduced NO production or increased removal during anaesthesia might therefore be expected to be manifested as an increase in pulmonary arterial pressure. In the present study, mean pulmonary arterial pressure was greater in the HA group than in the IV group. Other mechanisms that could have resulted in a higher mean pulmonary artery pressure include increased cardiac output and arterial carbon dioxide tension, although in the present study these were not different between procedures. Alternatively, the lower pulmonary pressures in the IV procedure could have been due to the intravenous drug mixture per se, but romifidine, a \( \alpha_2 \)-adrenoreceptor agonist, usually increases systemic vascular resistance and pulmonary artery pressures in conscious horses. In addition, with the IV procedure mean pulmonary artery pressures were similar to those in conscious, unsedated horses at rest.

In the present study we have shown that NO production is reduced in horses anaesthetized with halothane compared with an intravenous regimen, and that this is associated with an increase in mean pulmonary artery pressure. We have also shown in a separate study that administration of exogenous inhaled NO (10 p.p.m.) did not reduce pulmonary artery pressure during halothane anaesthesia.\(^9\) Therefore, the present study supports the hypothesis that halothane causes in vivo suppression of NOS. However, failure of exogenous inhaled NO to reduce pulmonary artery pressure during halothane anaesthesia implies either that NO is not important for the regulation of pulmonary

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**Table 1** Net exhaled (exhaled–inhaled) nitric oxide concentration (NO), tidal volume (\( \dot{V}_E \)), mass specific respiratory minute ventilation (\( \dot{V}_{E}/T \)), NO production rate (\( \dot{V}_{NO} \)), fractional inspired oxygen (\( F_{O_2} \)), end-tidal carbon dioxide (\( E_{CO_2} \)), arterial oxygen and carbon dioxide tensions (\( P_{O_2}, P_{CO_2} \)), cardiac output (\( Q \)) and systolic (\( P_{PA\ SYST} \)), diastolic (\( P_{PA\ DIA} \)) and mean (\( P_{PA\ MEAN} \)) pulmonary artery pressures at 40 and 60 min of anaesthesia with halothane (HA) or an intravenous regimen (IV). Data are mean (SD), n=7. IV was significantly different from HA at the corresponding time: \(*P<0.05\); \( \dagger P<0.02\); \( \ddagger P<0.01\); \( \ddagger\ddagger P<0.001\).

<table>
<thead>
<tr>
<th>Duration of anaesthesia</th>
<th>40 min</th>
<th>60 min</th>
<th>40 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \dot{V}_E ) (litre)</td>
<td>5.0 (1.5)</td>
<td>4.7 (0.8)</td>
<td>5.1 (1.7)</td>
<td>4.9 (0.7)</td>
</tr>
<tr>
<td>( E_{CO_2} ) (ml kg(^{-1}) min(^{-1}))</td>
<td>86 (35)</td>
<td>108 (45)*</td>
<td>82 (26)</td>
<td>109 (51)*</td>
</tr>
<tr>
<td>( P_{NO} ) (pmol l(^{-1}) kg(^{-1}) min(^{-1}))</td>
<td>1.4 (1.4)</td>
<td>9.3 (9.9)*</td>
<td>0.7 (0.7)</td>
<td>12.5 (13.3)*</td>
</tr>
<tr>
<td>( P_{O_2} ) (%)</td>
<td>94.4 (2.5)</td>
<td>96.6 (1.3)</td>
<td>96.7 (1.4)</td>
<td>97.6 (2.9)</td>
</tr>
<tr>
<td>( E_{O_2} ) (%)</td>
<td>6.4 (1.1)</td>
<td>6.2 (0.9)</td>
<td>6.2 (0.6)</td>
<td>6.2 (0.6)</td>
</tr>
<tr>
<td>( P_{PA\ SYST} ) (kPa)</td>
<td>46.7 (10.8)</td>
<td>48.9 (8.4)</td>
<td>47.9 (11.2)</td>
<td>50.1 (8.5)</td>
</tr>
<tr>
<td>( P_{PA\ DIA} ) (kPa)</td>
<td>8.1 (0.9)</td>
<td>7.7 (0.7)</td>
<td>8.8 (1.3)</td>
<td>7.7 (0.5)</td>
</tr>
<tr>
<td>( Q ) (litre min(^{-1}))</td>
<td>30 (10)</td>
<td>32 (6)</td>
<td>35 (13)</td>
<td>33 (7)</td>
</tr>
<tr>
<td>( P_{PA\ MEAN} ) (kPa)</td>
<td>5.0 (1.1)</td>
<td>3.6 (0.4)*</td>
<td>5.0 (0.9)</td>
<td>3.5 (0.3)*</td>
</tr>
</tbody>
</table>

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**Exhaled NO in horses during anaesthesia**
vascular tone under these conditions or that there is inhibition of the action of NO, possibly through interference with cGMP-mediated relaxation. In conclusion, it is possible that the increased mean pulmonary artery pressure in these horses during halothane anaesthesia may be linked to the observed differences in NO production.

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Use of cisatracurium during fast-track cardiac surgery

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We prospectively studied spontaneous recovery from cisatracurium-induced neuromuscular block in 18 patients scheduled for cardiac surgery, and its suitability for fast-track cardiac surgery. Neuromuscular block was induced by an i.v. bolus (range 0.15–0.3 mg kg⁻¹) and maintained by a continuous infusion (range 1.1–3.2 μg kg⁻¹ min⁻¹) of cisatracurium until sternal closure. In the intensive care unit (ICU), spontaneous recovery was evaluated by the train-of-four (TOF) ratio measured at the adductor pollicis muscle. The ICU medical staff were unaware of the TOF ratios until sedation was stopped. At that time, if the TOF ratio was less than 0.9, sedation was recommenced. On arrival in ICU, all patients had residual paralysis. The mean time to reaching a TOF ratio of at least 0.9 was 102 min (range 74–144 min) after discontinuation of the cisatracurium infusion. Fifteen patients (83%) were successfully extubated during the first 8 h after stopping the cisatracurium infusion. Only one patient showed residual paralysis when sedation was discontinued. These results support the use of cisatracurium as a suitable neuromuscular blocking agent for fast-track cardiac surgery.

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Keywords: neuromuscular block, cisatracurium; surgery, cardiovascular

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