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Research in Veterinary Science 80 (2006) 103–108
RVS
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The caprine oxyhemoglobin dissociation curve

Steve C. Haskins [a,*], Marlis L. Rezende [b]

[a] Department of Surgical and Radiological Science, School of Veterinary Medicine, University of California, Davis, CA 95616-8745, United States
[b] Universidade Estadual Paulista, Faculdade de Ciencieas Agrarias e Veterinarias, Jaboticabal, Sao Paulo, Brazil

Accepted 1 April 2005

Abstract

The caprine oxyhemoglobin dissociation curve has not been previously defined. Blood from 10 healthy goats was equilibrated in a tonometer with calibrated gas mixtures of oxygen at concentrations of 95%, 21%, 13%, 12%, 10%, 9%, 8%, 5%, 4%, and 2.5% , 5% carbon dioxide, balance nitrogen. The pH, partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), total hemoglobin, oxyhemoglobin saturation, carboxyhemoglobin, methemoglobin, and oxygen content were measured.

The PO_2/oxyhemoglobin and the PO_2/oxygen content relationships were graphed with curve-fitting software and a formula for calculating oxyhemoglobin from PO_2 was generated. The maximum oxygen content per gram of hemoglobin was 1.29 ml of oxygen per gram of hemoglobin. The PO_2 at which hemoglobin was 50% saturated (P_{50}) from the PO_2/oxyhemoglobin relationship was 28.6 ± 1.5 mmHg and that from the PO_2/oxygen content relationships was 29.1 ± 1.6 mmHg. The Hill coefficient for the PO_2/oxyhemoglobin data was 3.0 ± 0.4.

Keywords: Goat; P_{50}; Oxygen content; Hemoglobin saturation; Blood gases; Methemoglobin; Carboxyhemoglobin

1. Introduction

The oxyhemoglobin dissociation curve is a graphic representation of the relationship between the partial pressure of oxygen and the hemoglobin saturation or hemoglobin oxygen content. The P_{50} is the PO_2 at which the hemoglobin is 50% saturated and is a commonly used value to define the hemoglobin affinity for oxygen. The caprine oxyhemoglobin dissociation curve has not been previously characterized, but the P_{50} has been reported to be between 28 and 33 mmHg (Hilpert et al., 1963; Metcalfe et al., 1967; Parer et al., 1967; Scott et al., 1977). This information can be important for some clinical and research purposes. In cattle, the oxyhemoblogin dissociation curve has been characterized and the P_{50} has been reported to be between 25 and 31 mmHg (Clerbaux et al., 1993; Nakashima et al., 1985; Smith et al., 1979). In sheep, the P_{50} has been reported to be between 30 and 40 mmHg (Meshia, 1961; Nakashima et al., 1985; Scott et al., 1977). The purpose of this study was to characterize the caprine oxyhemoglobin dissociation curve.

2. Materials and methods

Investigational approval by the institutional use and care committee was not required because the goat blood was donated by the goat's owner (SCH) and involved a single jugular venipuncture. Thirty milliliters (ml) of blood was collected into 30 ml plastic syringes from 10 healthy male castrated goats into pre-heparinized (dead space volume of the syringe; liquid sodium heparin [1000 units/ml]), immediately immersed in ice water and transported to the laboratory. The blood from one goat was processed each experimental day.

* Corresponding author. Tel./fax: +1 530 756 3163.
E-mail address: schaskins@ucdavis.edu (S.C. Haskins).

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doi:10.1016/j.rvsc.2005.04.003

Five ml of heparinized blood was placed into each of three separate flasks of a tonometer.[1] The rest of the blood sample was stored in a sealed syringe, immersed in ice water. The volume of blood removed from each flask for each measurement was replaced with blood from the source syringe. The blood samples in the tonometer were equilibrated with gas mixtures containing calibrated oxygen concentrations of 95%, 21%, 13%, 12%, 10%, 9%, 8%, 5%, 4%, and 2.5%, and 5% of carbon dioxide, balance nitrogen. The blood sample in the first flask was sequentially equilibrated with oxygen concentrations of 95%, 21% and 13%, the sample in the second flask was equilibrated with oxygen concentrations of 12%, 10% and 9% and the sample in the third flask was equilibrated with oxygen concentrations of 8%, 5%, 4% and 2.5%. The tonometer water bath temperature was maintained at 37.0 °C. For each gas mixture, the blood sample was equilibrated in the tonometer for one hour in order to achieve full equilibration. The pH was maintained between 7.38 and 7.42 by addition of either sodium bicarbonate or 0.9% saline. The sample was equilibrated for 30 min after any such treatment before analysis.

After equilibration, 1 ml of blood was removed from each flask and the following measurements made: partial pressure of oxygen (PO_2; mmHg), partial pressure of carbon dioxide (PCO_2; mmHg), pH,[2] hemoglobin concentration (Hb; g/l), oxyhemoglobin (HbO_2; %), methemoglobin (MetHb; %), carboxyhemoglobin (COHb; %),[3] and oxygen content ($ContO_2$; ml/dl).[4] The analyzer has a repeatability of 0.006 units for pH, of 0.6 mmHg for PCO_2 in the range of the values of the present experiment, and of 0.8 mmHg at a PO_2 of 14 mmHg, 1.0 mmHg at a PO_2 of 78 mmHg, and 7.4 mmHg at a PO_2 of 562 mmHg. Accuracy was verified with quality control solutions[2] prior to each experiment. Bicarbonate (HCO_3^-; mM/l) and standard base excess (SBE; mM/l) were calculated.[2] The co-oximeter displays functional HbO_2; fractional HbO_2 was calculated: ((100–COHb–metHb) × functional HbO_2)/100. The co-oximeter also calculates and displays a value for oxygen content. No temperature adjustments were made; the blood was equilibrated and measured at 37.0 °C.

The solubility of oxygen in water was determined by equilibrating distilled water with the 95% oxygen gas mixture for 1 h. The PO_2 and $ContO_2$ were measured. The solubility of oxygen was calculated as $ContO_2$ (ml/dl)/PO_2 (mmHg).

[1] Modified Instrumentation Laboratories 137, rotating, 3-flask tonometer.

[2] ABL5 pH and blood gas analyzer, Radiometer, Copenhagen, Denmark.

[3] OSM3 co-oximeter, Radiometer, Copenhagen, Denmark.

[4] LexO_2con oxygen content analyzer, Hospex, Chestnut Hill, MA.

3. Data analysis

Means and standard deviations were calculated for each parameter. Changes in each parameter over time were evaluated by repeated measures analysis of variance applying the Geisser-Greenhouse-Epsilon and Box-Epsilon probability adjustment with time as the within-factor variable.[5] When a significant effect was identified, the Bonferroni all-pairwise multiple comparison test was used to identify the significant differences. Oxygen content per gram of hemoglobin was calculated as ($ContO_2$ – dissolved O_2)/tHb. Dissolved O_2 was calculated as $PO_2 \times 0.00273$. The PO_2:FunctHbO_2 and PO_2:ContO_2 relationships were graphed. A formula was developed which defines the relationship between PO_2 and HbO_2 in this study using Kelman's modification of the Adair formula (Kelman, 1966). The Adair constants (a_1–a_7) were determined by iterative processing using nonlinear regression curve fitting analysis.[5] The general form of the formula for hemoglobin saturation (SO_2) is: $SO_2 = 100 \times (a_1PO_2 + a_2PO_2^2 + a_3PO_2^3 + PO_2^4)/(a_4 + a_5 PO_2 + a_6PO_2^2 + a_7PO_2^3 + PO_2^4)$. Linear regression equations were established for PO_2 (20, 30, and 40 mmHg levels) vs. FunctHbO_2 and PO_2 vs. ContO_2, and P_{50} was determined. A paired t test was used to compare the P_{50} values from the two methods of determination.[5] Wilcoxon signed-rank test was used to compare Functional vs. fractional HbO_2 and the measured ContO_2 vs. that calculated by the co-oximeter. Hill's solubility coefficient was calculated by the formula: log (HbO_2/1–HbO_2)/log (PO_2/P_{50}) for the HbO_2 range 30–60%.

4. Results

The mean and standard deviations for all data are listed in Table 1. The acid–base values were not statistically different throughout the trial with a range of pH values of 7.394–7.411 units; PCO_2 35.7–36.9 mmHg; HCO_3 21.4–22.6 mM/l; and SBE −1.6 to −2.6 mM/l.

Fractional HbO_2 was consistently and significantly lower than functional HbO_2 (Table 1), as expected. The differences between calculated and measured $ContO_2$ were significant and variable. The calculated values were higher than measured at low PO_2; similar between PO_2 values of 100 and 150 mmHg; and lower at very high PO_2 (Table 1). Solubility of oxygen in water in the present experiment was measured to be 0.00273 ml of oxygen per dl of water per mmHg. The maximum hemoglobin oxygen content per gram of hemoglobin was 1.29 ml/g.

The formula, derived from the data, to calculate HbO_2 from measured PO_2 was: $SO_2 = 100 \times$

[5] Number Cruncher Statistical Software, Kaysville, UT.