Original article

Blood oxygen binding in hypoxaemic calves

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Abstract – Blood oxygen transport and tissue oxygenation were studied in 28 calves from the Belgian White and Blue breed (20 healthy and 8 hypoxaemic ones). Hypoxaemic calves were selected according to their high respiratory frequency and to their low partial oxygen pressure (PaO_2) in the arterial blood. Venous and arterial blood samples were collected, and 2,3-diphosphoglycerate, adenosine triphosphate, chloride, inorganic phosphate and hemoglobin concentrations, and pH, PCO_2 and PO_2 were determined. An oxygen equilibrium curve (OEC) was measured in standard conditions, for each animal. The arterial and venous OEC were calculated, taking body temperature, pH and PCO_2 values in arterial and venous blood into account. The oxygen exchange fraction (OEF%), corresponding to the degree of blood desaturation between the arterial and the venous compartments, and the amount of oxygen released at the tissue level by 100 mL of blood (OEF Vol%) were calculated from the arterial and venous OEC combined with the PO_2 and hemoglobin concentration. In hypoxaemic calves investigated in this study, the hemoglobin oxygen affinity, measured under standard conditions, was not modified. On the contrary, in vivo acidosis and hypercapnia induced a decrease in the hemoglobin oxygen affinity in arterial blood, which combined to the decrease in PaO_2 led to a reduced hemoglobin saturation degree in the arterial compartment. However, this did not impair the oxygen exchange fraction (OEF%), since the hemoglobin saturation degree in venous blood was also diminished.

blood oxygen binding / oxygen equilibrium curve / tissue oxygenation / hypoxaemia / calf

Résumé – Transport de l'oxygène chez les veaux hypoxémiques. Le transport de l'oxygène par le sang et l'oxygénation tissulaire ont été étudiés chez 28 veaux de race Blanc Bleu Belge (20 veaux sains et 8 veaux hypoxémiques). Les veaux hypoxémiques ont été sélectionnés selon les critères suivants : une fréquence respiratoire élevée et une faible pression partielle en oxygène (PaO_2) dans le

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sang artériel. Des échantillons sanguins ont été prélevés au niveau artériel et veineux, les concentrations en 2,3-diphosphoglycérate, adénosine triphosphate, chlore, phosphate inorganiques et hémoglobine ont été déterminées, ainsi que les valeurs de pH, PCO_2 et PO_2. La courbe de dissociation de l'oxyhémoglobine (OEC) a été tracée en conditions standards chez chaque animal. Les courbes de dissociation de l'oxyhémoglobine correspondant aux compartiments artériel et veineux ont ensuite été calculées, en tenant compte de la température corporelle ainsi que des valeurs de pH et de PCO_2 dans le sang artériel et veineux. Le degré de désaturation du sang entre le compartiment artériel et le compartiment veineux (OEF %) a été calculé, ainsi que la quantité d'oxygène libérée au niveau tissulaire, par 100 mL de sang (OEF Vol %), considérant l'OEC artérielle et l'OEC veineuse ainsi que les valeurs de PO_2 et de la concentration en hémoglobine. Chez les veaux hypoxémiques étudiés au cours de cette étude, l'affinité de l'hémoglobine pour l'oxygène, mesurée en conditions standards, n'était pas modifiée. En revanche, in vivo, l'acidose et l'hypercapnie ont induit une diminution de l'affinité de l'hémoglobine pour l'oxygène au niveau artériel qui, combinée à la diminution de la PaO_2, s'accompagnait d'une baisse du degré de saturation de l'hémoglobine au niveau artériel. Cependant, ceci ne perturbait pas l'extraction de l'oxygène au niveau tissulaire, le degré de saturation de l'hémoglobine étant également diminué dans le compartiment veineux.

transport de l'oxygène / courbe de dissociation de l'oxyhémoglobine / oxygénation tissulaire / hypoxémie / veau

1. INTRODUCTION

The influence of naturally occurring diarrhea on blood oxygen binding has been recently reviewed in neonate calves. It has been demonstrated that a left shift in the oxygen equilibrium curve (OEC) occurs in calves with diarrhea, as a result of hypothermia, hypocapnia and a decrease in blood concentration of 2,3-diphosphoglycerate (2,3-DPG). This left shift is partially or totally counterbalanced by the development of acidosis, depending on its intensity. In addition, an increase in partial oxygen pressure in venous blood can contribute to limiting hemoglobin desaturation and oxygen extraction at the tissue level in diarrheic calves [7]. In this context, the aim of the present study was to investigate the modifications of tissue oxygenation occurring during hypoxaemia, i.e. another pathophysiological trouble frequently observed in bovine neonatology. The aetiology of hypoxaemia is various, the latter being particularly observed in double-muscled calves from the Belgian White and Blue breed. Clinically, the affected animals are highly depressed and often show tachypnea.

In man, the modifications of the oxygen equilibrium curve (OEC) have been investigated in patients suffering from hypoxaemia due to the respiratory distress syndrome [11] and sleep apnea syndrome [21]. These works have shown that hypoxaemia induces an increase in the 2,3-diphosphoglycerate concentration, and consequently, a right shift of the oxygen equilibrium curve. In patients suffering from the chronic obstructive pulmonary disease, the hemoglobin oxygen affinity is either decreased [25] or normal [22], whereas the 2,3-DPG level is usually increased [22, 25]. However, it is important to remember that, in all species, a decrease in the hemoglobin oxygen affinity can only induce an improvement in tissue oxygenation during normoxaemia or moderate hypoxaemia [27]. For example, in man, when the partial oxygen pressure in the arterial compartment goes below 50 mm Hg, the right shift of the oxygen equilibrium curve on tissue oxygenation may have deleterious consequences.

Taking all these observations into account, the purpose of the present study was first to assess the modifications of the oxyphoric function of hemoglobin in hypoxaemic calves, in order to compare our results to those obtained in man. In a second step, this paper focussed on the various pathophysiological and biochemical

modifications occurring during hypoxaemia in calves, as well on their consequences on tissue oxygenation. Different parameters were thus taken into account, i.e., body temperature, acid-base balance and partial oxygen and carbon dioxide pressures in arterial and venous blood. The influence of OEC regulating factors such as 2,3-diphosphoglycerate (2,3-DPG), adenosine triphosphate (ATP), inorganic phosphate (Pi) and chloride (Cl^-) was also investigated.

2. MATERIALS AND METHODS

2.1. Animals

Eight diseased 1- to 4-day-old double-muscled calves from the Belgian White and Blue breed were studied. The affected animals suffered from tachypnea, i.e. a respiratory frequency (RF) up to 100 breaths per minute, and hypoxaemia, i.e. an arterial partial oxygen pressure (PaO_2) less than 60 mm Hg.

A control group of healthy double-muscled calves ($n = 20$), in the same range of age as the affected animals, was also investigated.

Calves were considered to be diseased if RF was higher than the mean RF of healthy calves + 2 standard deviations and if PaO_2 was lower than the mean PaO_2 of healthy calves – 2 standard deviations.

2.2. Blood samples

Heparinised syringes were used to collect 1 mL of venous (jugular vein) and 1 mL of arterial (brachial artery) blood under anaerobic conditions. Syringes were placed on ice, and pH, PO_2, and PCO_2 were measured immediately (AVL, Biomedical Instruments, Graz, Austria) and corrected according to rectal temperature. Measuring rectal temperature is indeed a rapid, painless and reproducible method reflecting ac-

curately core temperature, even during hypothermia [29] or hyperthermia [28]. Venous blood samples were also collected into 20-mL syringes containing heparin for the determination of OEC, as well as for biochemical and hematologic analyses. Venous blood was immediately stored at 4 °C, and OEC were recorded within 1 day. Previous studies conducted on bovine blood had shown that OEC was not modified if the analysis was realised within 24 hours after blood sampling. Indeed, the P50 standard values measured on 22 samples immediately after blood sampling and 24 hours later only differed from one another in 0.4%. To determine chloride and inorganic phosphate concentration, 1 mL of plasma was stored at 4 °C after centrifuging venous blood for 15 min at 2 600 g.

2.3. Curve plotting

The OEC was measured by using a dynamic method under standard conditions (pH, 7.4; PCO_2, 40 mm Hg; temperature, 37 °C) [8]. A 15-mL venous blood sample was deoxygenated in a rotary tonometer, according to Farhi [15], with a gas mixture composed of 5.6% CO_2 and nitrogen. The venous sample was deoxygenated during 45 min. The blood was introduced in a balloon, in order to offer a maximal gas exchange surface. The delivery of gas was equal to 150 mL per minute. Thereafter, blood was placed in a home-made analyser and equilibrated with this first gas mixture. For 15 min, oxygen tension in blood samples was slowly increased from 0 to 320 mm Hg by introducing a second gas mixture composed of 5.6% CO_2 and oxygen. Oxygen saturation (SO_2) was measured by photometry (LED 660 nm, Monsanto, St Louis, Mo, USA), as a function of PO_2; PO_2 was measured polarographically (PO_2 electrode, Eschweiler, Kiel, Germany). Changes in plasma pH were corrected automatically to 7.4 ± 0.005 by adding 1 N NaOH or 1 N HCl [8]. A temperature of

37 °C and a PCO_2 of 40 mm Hg were maintained throughout determination of the OEC. Practically, the PCO_2 was determined by the 5.6% CO_2 contained in the gas bottle used for oxygenation and deoxygenation. The accuracy of the mixture is $5.6 \pm 0.12\%$ CO_2, corresponding to 40 ± 0.9 mm Hg. This value was comparable to the accuracy of PCO_2 measurement by a blood gas analyzer and is without measurable effect on the oxygen equilibrium curve. For each curve, 100 points were automatically measured. Values for PO_2 and oxygen saturation were stored on a computer for data processing and curve generation using a home-made software. The accuracy of our method for measuring OEC, expressed by the S.D. of P50, was 0.1 mm Hg for 6 curves determined for the same blood sample (i.e., analytical error of the analyser) and 0.3 mm Hg for 11 curves determined for 11 blood samples collected from the same control subject over a 30-day period (i.e., analytical error associated with intra-individual variations). Oxygen affinity changes were evaluated by measuring PO_2 at 50% hemoglobin saturation under standard conditions (i.e., standard P50).

2.4. Blood analysis

Hemoglobin concentration, expressed in grams per 100 mL of blood, was determined with a hemoximeter (OSM3 Hemoximeter, Radiometer, Copenhagen, Denmark). Hematocrit was measured after microcentrifugation (Universal 30 RF, Hettich, Tuttlingen, Germany). Concentrations of 2,3-DPG and ATP in the blood were determined by enzymatic methods (DPG kit No. 35A, ATP kit No. 366, Sigma Chemical Co, St. Louis, Mo, USA). The concentration of inorganic phosphate in plasma was determined by the commercially available kit (Phosphorus, inorganic kit No. 670, Sigma Chemical Co, St. Louis, Mo). The plasma chloride concentration was determined by the titrimetric method

(Merckotest, Merck, Dormstad, Germany). The measurements of electrolytes were made within 24 h after blood sampling, plasma being stored at 4 °C. The Pi and Cl tests were calibrated with Sigma Standards, with references 955-11 and 661-9, respectively.

2.5. OEF calculation

The oxygen exchange fraction (OEF%) represents the difference between the saturations corresponding to the PO_2 values measured simultaneously in arterial (SO_2 a) and venous (SO_2 v) blood, taking into account the position and shape of the OEC in both compartments. In practice, the arterial and venous OECs were calculated in both breeds from the standard OEC corrected for the effects of pH, temperature [10, 17, 18] and PCO_2.

The amount of oxygen released in vivo by 100 mL of bovine blood at the tissue level (OEF Vol %) was calculated as follows:

$$OEF\ (Vol\ \%) = Hb \times BO_2 \times [OEF\%/100] + \alpha\,(PaO_2 - PvO_2)$$

where BO_2 is the hemoglobin oxygen capacity (1.39 mL O_2/g Hb) [17], Hb the hemoglobin concentration (g/100 mL), α the oxygen solubility coefficient for blood at the temperature of the experiment (0.003 mL·100 mL^{-1}·mm Hg^{-1}), and PaO_2 and PvO_2 the partial oxygen pressures in arterial and venous blood respectively (mm Hg).

2.6. Statistical analyses

Data were expressed as means $\pm$ S.D. Values were tested for normal distribution with the Omnibus test. All data were normally distributed. They were compared using a Student t-test or an Aspin-Welch test, depending on whether the variances were equal or not. Practically all data were compared using a Student T-test, except the hemoglobin saturation in arterial blood. For

all analyses, values of $P < 0.05$ were considered significant.

3. RESULTS

All the results are summarised in Table I. This table shows that age distribution, standard P50, 2,3-DPG, ATP and inorganic phosphate concentrations, rectal temperature, $PvCO_2$, venous P50, as well as the values of OEF%, hemoglobin concentration and OEF Vol% did not differ significantly between healthy and hypoxaemic calves.

Other parameters were significantly modified in diseased animals. The chloride concentration was significantly lower in hypoxaemic than in healthy calves. Diseased animals exhibited a significant acidosis in arterial and venous blood. Furthermore, in hypoxaemic calves, $PaCO_2$ and arterial P50 were significantly higher than in healthy animals. At the same time, diseased animals exhibited lower PaO_2, PvO_2, SO_2 a and SO_2 v values than healthy ones.

4. DISCUSSION

From a physiological point of view, hypoxaemia is correlated to a decrease in the partial oxygen pressure in the arterial blood or to a decrease in the saturation degree of hemoglobin at the arterial level or again to a decrease in the hemoglobin concentration. In hypoxaemia, the "amount" of oxygen held in the arterial blood (CaO_2) is the main cause of clinical seriousness; the lower the oxygen content is, the more hypoxaemic the animal or the patient is. On the contrary, hypoxia is a more general term that describes a deterioration of oxygen layout at the tissue level. Hypoxia takes cardiac output and oxygen collection at the tissue level into account. Therefore, hypoxaemia is one of the causes of hypoxia. Furthermore, it is possible to be hypoxaemic while having a normal oxygen supply at the tissue level thanks to adjustment processes increasing either the cardiac output or oxygen extraction at the tissue level [23].

From a functional point of view, hypoxaemia, which increases peripheral chemoreceptors activity, is often chemically associated with tachypnea [20]. This clinical symptom has therefore been chosen as the first criterion of inclusion in the present study. The decrease in partial oxygen pressure in arterial blood, defined as the hypoxaemia marker, has to be further confirmed by arterial blood gas measurements.

From a pathological point of view in neonatal calves, hypoxaemia may be associated with different pathological processes, i.e. endotoxemia [12], pneumonia [1] and respiratory distress syndrome, being itself associated with a deficiency in the pulmonary surfactant [14]. The purpose of the present paper was to describe the modifications of tissue oxygenation occurring during hypoxaemia, whatever the origin of the latter was. However, it must be mentioned that double-muscled calves, like those from the Belgian White and Blue breed, seem to be more predisposed than standard conformation calves to develop hypoxaemia during respiratory diseases [2, 17]. Furthermore, neonatal calves born by caesarean section, like calves from the Belgian White and Blue breed, seem to be predisposed to develop a respiratory distress syndrome during the first hours of their life [30].

As shown in Table I, the 2,3-DPG values were not significantly modified in calves suffering from hypoxaemia. This may seem amazing since it is extensively described that, in man, hypoxaemia is associated with an increase in 2,3-DPG [11, 21, 22, 25]. However, all these observations were carried out on hypoxaemic but not acidosic patients. In contrast, the calves investigated in the present study suffered additionally from acidosis. The correlation between acidosis and 2,3-DPG values, as reported in men and dogs [9, 24], has been confirmed in calves

Table I. Oxygen equilibrium curve, associated parameters, pH and blood gases in healthy and hypoxaemic double-muscled calves.

Parameters	Control group (n = 20)	Hypoxaemic group (n = 8)
Age (days)	1.8 ± 0.9	2.0 ± 1.2
Respiratory Frequency (breaths/min)	56.6 ± 18.7	140.5 ± 29.5***
P50 std (mm Hg)	19.9 ± 2.1	19.4 ± 3.0
2,3-DPG (μmol/g Hb)	10.0 ± 4.8	9.0 ± 4.6
ATP (μmol/dL)	26.6 ± 5.7	25.2 ± 7.2
Cl^- (mmol/L)	104.5 ± 5.7	98.8 ± 3.6**
Pi (mmol/dL)	2.2 ± 0.3	2.4 ± 0.3
Temperature (ºC)	38.9 ± 0.5	39.3 ± 0.8
pHa (−)	7.38 ± 0.05	7.33 ± 0.06**
pHv (−)	7.35 ± 0.04	7.30 ± 0.04*
$PaCO_2$ (mm Hg)	45.9 ± 3.3	53.0 ± 11.0**
$PvCO_2$ (mm Hg)	53.5 ± 6.3	58.5 ± 9.7
P50 a (mm Hg)	23.1 ± 3.2	25.2 ± 2.0*
P50 v (mm Hg)	25.0 ± 3.4	26.6 ± 2.6
PaO_2 (mm Hg)	77.2 ± 9.9	47.9 ± 12.9***
PvO_2 (mm Hg)	37.8 ± 7.2	31.8 ± 7.7*
SO_2 a (%)	95.0 ± 1.5	81.9 ± 12.7*
SO_2 v (%)	74.2 ± 9.5	59.9 ± 17.5**
OEF (%)	20.8 ± 9.1	22.1 ± 11.0
Hb (g/dL)	10.8 ± 1.5	10.6 ± 2.8
OEF (Vol%)	3.2 ± 1.5	3.2 ± 1.5

Values are expressed as means ±SD. RF: respiratory frequency; P50 std: PO_2 at 50% hemoglobin saturation, measured under standard conditions (pH 7.4, PCO_2 40 mm Hg, temperature 37 ºC); 2,3-DPG: 2,3-diphosphoglycerate concentration; ATP: adenosine triphosphate concentration; Cl^-, Pi: plasmatic concentrations of chloride and inorganic phosphate; pHa, $PaCO_2$, PaO_2: pH and blood gases in arterial blood; pHv, $PvCO_2$, PvO_2: pH and blood gases in venous blood; P50a: PO_2 at 50% hemoglobin saturation in the arterial compartment; P50v: PO_2 at 50% hemoglobin saturation in the venous compartment; SO_2 a: hemoglobin saturation in the arterial compartment; SO_2 v: hemoglobin saturation in the venous compartment; OEF%: oxygen exchange fraction; Hb: hemoglobin concentration; OEF Vol %: amount of oxygen released at the tissue level by 100 mL of bovine blood. * value significantly different between healthy and diseased animals (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

[7]. Acidosis leading into phosphoglycerate mutase inhibition and phosphoglycerate phosphatase activation goes with a 2,3-DPG concentration decrease [3]. In hypoxaemic calves, acidosis may thus have counterbalanced the effects of hypoxaemia on 2,3-DPG synthesis. This lack of alteration of 2,3-DPG concentration might explain that the standard P50 remains unchanged in hypoxaemic calves, 2,3-DPG acting as a regulator of hemoglobin oxygen affinity in calves [6, 19]. Indeed, in healthy double-muscled calves aged from 1 to 26 days, the relationship between P50 std (in mm Hg) and the 2,3-DPG concentration (in μmol/g Hb) was equal to: P50 std = 17.5 + 0.33 × 2,3-DPG [6].

Chloride has been shown to be a regulator of the oxyphoric function of hemoglobin [5, 16, 18, 26]. The hypochloremia noted in hypoxaemic calves might therefore have contributed to limit the rightshift of the oxygen equilibrium curve. This hypochloremia could be due to pseudohypochloremia in hypoproteinemeic calves or to respiratory acidosis [13]. However, in practice, the changes in chloride concentrations

observed in hypoxaemic calves are too low to have an impact on oxygen release at the tissue level [18].

As mentioned previously, the assessment of P50 standard modifications is insufficient to estimate the influence of a pathological process on oxygen transport by the blood. Other regulating factors, such as pH, body temperature and partial carbon dioxide pressure, modulate, in vivo, oxygen equilibrium curves corresponding to arterial and venous compartments and may alter the related parameters such as OEF% and OEF Vol%. In this study, in comparison to healthy calves, hypoxaemic calves showed an arterial hypercapnia and a relative acidosis. The increased $PaCO_2$ values noted in diseased animals appear to be linked to alveolar ventilation disorders [23].

Acidosis and hypercapnia induce a right shift of the oxygen equilibrium curve in several species, including bovines [4]. Accordingly, the arterial P50 values, assessed in the present study, are higher in hypoxaemic calves than in healthy ones. The same trend is present in venous blood, however, the differences between both groups are not significant. Yet the most important thing is to estimate the impact of a right shift of oxygen equilibrium curves on oxygen extraction at the tissue level, in hypoxaemic calves. In these latter animals, PaO_2 is situated between 22.4 and 59.8 mm Hg. These low levels of partial oxygen pressure do not correspond to the plateau of the oxygen equilibrium curve, leading to a decrease in the hemoglobin saturation in arterial blood (SO_2 a being equal to 82% versus 95% in healthy calves), reinforced by the rightshift of the oxygen equilibrium curve. This phenomenon may have a negative effect on tissue oxygenation, as suspected in men suffering from the acute respiratory distress syndrome [11]. In hypoxaemic calves, the significant decrease in PvO_2, which likely reflects the better oxygen extraction at the tissue level,

also leads to a decrease in the hemoglobin saturation degree in venous blood (74% in healthy calves versus 60% in diseased calves), explaining the lack of OEF % modification in hypoxaemic calves. Since hemoglobin values were identical in both groups, OEF Vol % values were not significantly modified in hypoxaemic calves.

In conclusion, this paper shows that in hypoxaemic and acidosic calves, the hemoglobin oxygen affinity, measured under standard conditions, is not modified. However, in vivo acidosis and hypercapnia, accompanying hypoxaemia, induce a decrease in hemoglobin oxygen affinity in arterial blood, which combined to the decrease in PaO_2 leads to a reduced hemoglobin saturation degree in the arterial compartment. However, this does not impair the oxygen exchange fraction (OEF%), since the hemoglobin saturation degree in venous blood is also diminished.

ACKNOWLEDGMENTS

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