Blood Gases Tensions and Acid CID Base Imbalance of Cayman Latirostris Submitted to APNEA and Pure Oxygen Breathing

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KEY WORDS: Cayman latirostris, forced apnea, acid base imbalance, blood gases tensions

ABSTRACT

This work intended to test the broad nosed caiman (Cayman latirostris, Daudin, 1802) pattern of acid base imbalance during aerobic apnea from six broad nosed caiman with different sizes. Animals were kept under forced apnea and blood was collected before and during the apnea. Thus, animals were ventilated with pure oxygen. Blood collects were done also during the onset of oxygenation. We evaluated pH, pCO2, pO2, temperature, and heart rate. As expected, in our study, pO2 fell and pCO2 levels rose during forced apnea. Apnea's bicarbonate and pH mean values started at 15.7 mmol.l-1 and 7.09, respectively, until an end-apnea value of 14.3 mmol.l-1 and 6.99, respectively. However, between 15 and 20 minutes and at 15 minutes of apnea, there was a small rise in bicarbonate and pH levels, respectively. Crocodilians in our study had bradycardia that could be associated to the smaller fall of pO2 levels between 15 and 20 minutes of apnea. A significant dependence on body weight just in end-apnea pCO2 and pO2 levels was seen. After lactic acidosis, bicarbonate decreased significantly according to body mass. This could corroborate that CO2 body storage is effective in blood pCO2 control during prolonged aerobic apnea, helping to control pH and lung oxygen uptake.

Table 1: Biological data of experimental animals.

Animal number	Sex	Weight (Kg)
1	F	2,0
2	F	2,7
3	F	3,5
4	M	8,0
5	F	17,0
6	F	25,0

INTRODUCTION

Crocodilian blood has adaptations to their life's style, and these adaptations, although conservative between the two major families, Alligatoridae and Crocodilidae, can present some small variation between them. Their blood pH has great oscillation compared to mammal's blood pH. In alligators, postprandial alkalosis could reach pH 8.0 due to massive HCL secretion in the stomach.[1] On the other hand, when they do great physical exercise (when struggling to food or fighting to escape threat), their major source of energy is obtained by anaerobic metabolism. They can reach high acidosis, with minimums of 6.5to 6.6 pH.[2]

Hemoglobin is an allosteric protein,[3] with its oxygen affinity influenced by pH and CO^2 (Bohr Effect). To support high acid-base variation, crocodilian's hemoglobin has its oxygen affinity driven much more by CO^2 blood concentration (CO^2 Bohr effect) than by the fixed acid influence (fixed acid Bohr effect). The low fixed acid Bohr effect permits an uptake of oxygen even during acidosis events, and the high CO^2 Bohr effect isn't disadvantageous in recovering due to quick CO^2 cleaning trough ventilation.[1,4]

In voluntary dives, crocodiles use their oxygen stock in their lungs to maintain an aerobic metabolism during submergence. Since reptiles have low metabolic rates, their dives can be prolonged. Lactate concentration doesn't arise to significant levels in dives up to 1 hour of duration.[5]

Size has a marked influence on metabolism.[6] The bigger the animal, the lower its specific metabolic rate. The influence of size on anaerobic metabolism is shown by demonstrating that big animals have higher anaerobic capacity then do small ones, and so they have an inverse correlation between mass and pH after exhaustive exercise.[2] In order to test the broad nosed caiman (Cayman latirostris, Daudin, 1802) pattern of acid base imbalance during aerobic apnea. We analyzed animal's blood gas, pH, heart rate, and temperature during a forced apnea and subsequent recovery, using caimans of different sizes.

MATERIAL AND METHODS

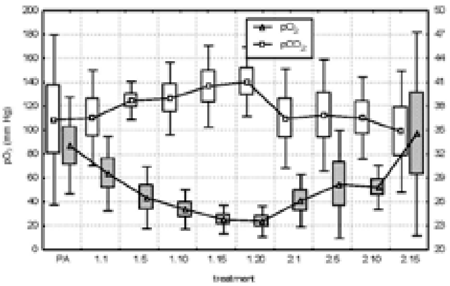
Animals

The study consisted of six broad nosed caiman, five females and one male, from Fundação RIOZOO. Animals were clinically healthy. They were fasted a week previous to the experiment, but had free access to water. Analysis was conducted at ambient temperature, and the caimans' temperature was monitored during the experiments. Their basic biological data are in Table 1.

EXPERIMENTAL DESIGN

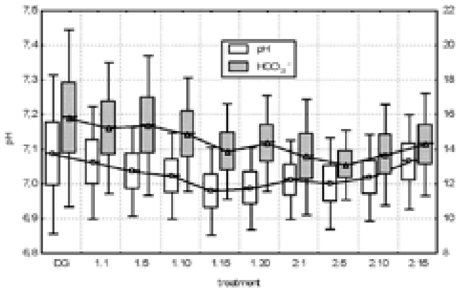
All experiments were conducted during the day, following the same protocol. Each animal was initially restrained with galamin (Flaxedil, 0.4 mg/Kg) by intramuscular injection.7 The restraint was done in a way to cause minimal disturbance to the animal, as physical activity could raise lactate levels and acidify blood. Notwithstanding, some movement was unavoidable. After immobilization, the animal was weighed and placed on a surgical table. A cuffed endotracheal tube in their trachea permitted oxygenation and ventilation control. A blood collect

Figure 1: *pCO2 (mmHg) and pO2 (mmHg) variation during each time in treatments. Horizontal lines links animal parameters means, box are mean ± standard error and whiskers are mean ± standard deviation.*



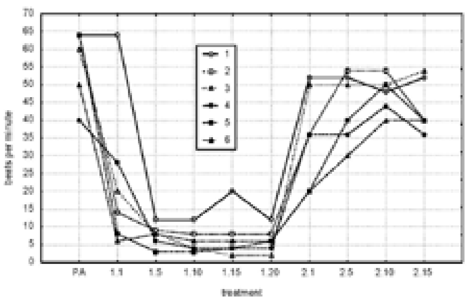
X-axis corresponds to the times in different treatments. Thus, PA concerns to pre apnea collect, 1.1 concerns to 1 minute of the first treatment, and successively.

Figure 2*: pH and HCO3- (mmol.l-1) variation during each time in treatments. Horizontal lines links animal parameters means, box are mean ± standard error and whiskers are mean ± standard deviation.*



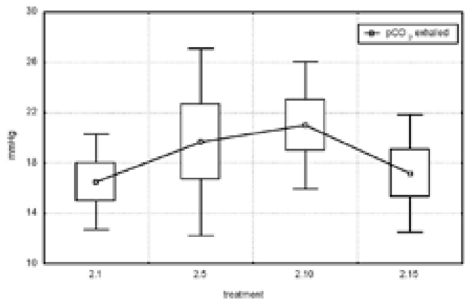
X-axis corresponds to the times in different treatments. Thus, PA concerns to pre apnea collect, 1.1 concerns to 1 minute of the first treatment, and successively.

Figure 3: *Heart rate (beats per minute) in six broad nosed caiman during apnea and subsequent recover.*



X-axis corresponds to the times in different treatments. Thus, PA concerns to pre apnea collect, 1.1 concerns to 1 minute of the first treatment, and successively.

Figure 4: *pCO2 (mmHg) lung exhalation during pure oxygen breathing. Horizontal lines links animal parameters means, box are mean ± standard error and whiskers are mean ± standard deviation.*



X-axis corresponds to the times in last treatment. Thus, 2.1 concerns to 1 minute of the second treatment, and successively.

needle was positioned in their caudal ventral vena cava, and its patency was achieved by perfusing with a saline heparinized solution (100 UI heparin/ml). The animals were artificially ventilated at a rate of 4 movements per minute. A telethermometer (TTE II, +-0,2oC precision) was introduced through its esophagus with an esophagi stethoscope to monitor internal body temperature and heart rate (bpm).

Animals were submitted to two treatments. First, they were kept under forced apnea, done by total occlusion of the endotracheal tube. Blood collects were done just before the apnea started and then at 1, 5, 10, 15 and 20 minutes after it. After last collect, endotracheal tube was opened and animals were ventilated with pure oxygen. Blood collections were performed at 1, 5, 10, and 15 minutes after oxygenation beginning. We evaluated pH, pCO^2, pO^2, temperature and heart rate at each one of these times during both treatments. A circuit multiparameter gas analyzer (Multigas Monitor 9100) was attached as soon as oxygenation started, to evaluate the exhaled CO^2 at each time.

BLOOD ANALYSIS

All analyzed parameters were transformed to 30o C temperature to allow the comparison of values avoiding body temperature influence (latter value was the mean caiman's temperature). When necessary, transforming factors came from Alligator mississipensis, due to their phylogenetic proximity. Analysis was done in a blood gas analyzer (Drake, AGS-2) using a reference electrode Ag/AgCl with a salt bridge KCl 4M as reference membrane associated to a pH glass electrode, for pH measurements. Samples and electrodes were kept equilibrated at 37+-0,2°C. Results were submitted to correction to 30o C, using the relative alkalinity concept; with the coefficient = -0,013.8

The pCO^2 electrode consisted of a pH glass electrode immersed in an electrolytic solution separated from sample by a nylon spacer permeable CO^2 disc. Electrode was calibrated by gases with two different pCO^2. To transform the informed 37o C pCO^2 value, assuming HCO3- concentration was invariable with temperature.[9] HCO^3 was calculated with a Henderson-Hasselbalch equation, pK' values1 and CO^2 solubility coefficient[10] were calculated.

The pO^2 electrode had a platinum cathode and an Ag/AgCl anode, with an electrolytic solution and an oxygen permeable polypropylene membrane. The informed pO^2 was also corrected to 30° C by taking CO^2 Bohr effect, with fixed acid Bohr effect and temperature shift in account.1 The work formula was adapted.[11]

In order to evaluate a possible animal size influence on blood gases parameters, simple regression of each result (pCO2, pO^2, pH and HCO^3-) was done on body weight at the end of the two treatments.[12]

RESULTS AND DISCUSSION

Crocodiles can dive voluntarily for extended time without great disturbance to their acid base imbalance.[5] In our work, we could confirm this situation. As expected, pO2 fell and pCO^2 levels rose during forced apnea (Figure 1). Mean pO2 started at 86 mmHg, falling constantly during apnea, until reaching the lowest mean value of 23 mmHg after 20 minutes. In the other way, mean pCO2 values started at 36 mmHg, rising during apnea to a maximum mean value of 41 mmHg at the end of apnea.

Figure 2 shows a continuous fall in bicarbonate levels during apnea because its change to carbonic acid in presence of CO2 that was continuously produced. Apnea's bicarbonate mean values started at 15.7 mmol.l-1 until an end-apnea value of 14.3 mmol.l-1. However, between 15 and 20 minutes of apnea, we could notice a small rise in bicarbonate levels. The pH levels followed the same pattern; an apnea's starting value of 7.09 reaching end-apnea's value of 6.99, but with an small mean value at 15 minutes of apnea (6.98).

Dive bradycardia is a described event in crocodilians,[13] occurring when water temperature falls or in prolonged dives, associated with a right to left cardiac shunt. Crocodilians in our study, submitted to simulate dive, also demonstrated bradycardia (Figure 3). This bradycardia and the shunt (sometimes associated) diminish metabolic use of oxygen during dive, permitted the animal a lesser acid base disturbance and a better use of oxygen stocks. The smaller fall of pO2 levels between 15 and 20 minutes of apnea could be associated to this event, although an experimental artifact cannot be discarded because the experimental protocol could not ascertain the cardiac shunt.

In our study, rapid respiratory compensation corrected a major disturbance on pH through CO^2 blood removal.[4] This can be seen in Figure 4. CO^2 was continuously exhaled, with its level arising until 10 minutes of pure oxygen breathing and then falling to normal levels. In Figure 1, we can see the sharp fall in pCO^2 blood levels after 1minute of pure oxygen breathing, reflecting CO^2 removal through respiration. It's interesting to note that blood bicarbonate just started to rise after the fifth minute of oxygenation. Thus, even with CO^2 cleaning through respiration, there was bicarbonate utilization

Table 2: *Regression resume of pCO2 (mmHg), pO2 (mmHg), pH and HCO3- (mmol.l-1) on weight, grouped on last time of each treatment.*

Parameter	Treatment	n (number of animals)	a (intercept)	b (angular coefficient)	r (correlation coefficient)	Fisher's F calculated
pCO2	1.20	6	44,88	-0,395	-0,86	11,20*
	2.15	6	30,41	0,45	0,55	1,77
pO2	1.20	6	35,21	-1,18	-0,86	11,37*
	2.15	5	98,76	-3,07	-0,80	5,23
pH	1.20	6	6,93	0,01	0,45	1,01
	2.15	6	7,11	-0,01	-0,36	0,59
HCO3-	1.20	6	13,83	0,05	0,18	0,13
	2.15	5	12,66	0,06	0,34	0,40

Observation: In some parameters in regression, values were not considered when they were considered to be out of normal distribution; in these cases n=5. Fisher's F value for H0: b=0: F 0,05(1)1,4=7,71; F0,05(1)1,3=10,1. Asterisks indicates b coefficient significantly different from zero for $p<0,05$.

during this time. Other authors described the same situation.[4] There is some level of tissue CO^2 sequestration in body fluids in crocodilians, and this CO^2 could be released, driving down bicarbonate levels until excess CO^2 could be corrected.

Regression analysis showed a significant dependence on body weight and end-apnea pCO^2 and pO^2 levels. The r coefficient indicated this relationship was negative, ie, the bigger the animal, the lower the blood gas levels in end-apnea. The lower levels of pO^2 in big animal's end-apnea means that those individuals used more (per unit of time) their oxygen stocks in relation to smaller ones. This occurs because those large size animals have proportionally smaller stocks (ie, smaller lungs) or they use their stocks more quickly (higher specific metabolic rate). The latter proposition is denied by literature.[6] The former must be proved.

In counter part, small blood pCO^2 levels in end-apnea of big animals could be attributed to the CO^2 sequestration in body fluids, that could be size-related.[4,13] Another interesting point in our study was that after lactic acidosis, bicarbonate decreased significantly according to body mass,4 but after respiratory acidosis, did not (Table 2). This could corroborate that CO^2 body storage is effective in blood pCO^2 control during prolonged aerobic apnea, helping to control pH and lung oxygen uptake.

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