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The normal acid–base status of mice

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ABSTRACT

Rodent models are commonly used for various physiological studies including acid–base regulation. Despite the widespread use of especially genetic modified mice, little attention have been made to characterise the normal acid–base status in these animals in order to reveal proper control values. Furthermore, several studies report blood gas values obtained in anaesthetised animals. We, therefore, decided to characterise blood CO_2 binding characteristic of mouse blood *in vitro* and to characterise normal acid–base status in conscious BALBc mice. *In vitro* CO_2 dissociation curves, performed on whole blood equilibrated to various P_{CO_2} levels in rotating tonometers, revealed a typical mammalian pK' (pK' = 7.816 – 0.234 × pH (r = 0.34)) and a non-bicarbonate buffer capacity (16.1 ± 2.6 slyke). To measure arterial acid–base status, small blood samples were taken from undisturbed mice with indwelling catheters in the carotid artery. In these animals, pH was 7.391 ± 0.026, plasma [HCO_3^-] 18.4 ± 0.83 mM, P_{CO_2} 30.3 ± 2.1 mmHg and lactate concentration 4.6 ± 0.7 mM. Our study, therefore, shows that mice have an arterial pH that resembles other mammals, although arterial P_{CO_2} tends to be lower than in larger mammals. However, pH from arterial blood sampled from mice anaesthetised with isoflurane was significantly lower (pH 7.239 ± 0.021), while plasma [HCO_3^-] was 18.5 ± 1.4 mM, P_{CO_2} 41.9 ± 2.9 mmHg and lactate concentration 4.48 ± 0.67 mM. Furthermore, we measured metabolism and ventilation ($\dot{V}_E$) in order to determine the ventilation requirements ($\dot{V}_E/\dot{V}_{O_2}$) to answer whether small mammals tend to hyperventilate. We recommend, therefore, that studies on acid–base regulation in mice should be based on samples taken for indwelling catheters rather than cardiac puncture of terminally anaesthetised mice.

1. Introduction

Given their amenability to genetic manipulation, and relative short generation time, mice have become a favoured model-organism for physiological studies. As an example, numerous recent studies seek to investigate the role of various proteins for renal acid–base regulation. While appropriate control values for arterial acid–base status are of obvious importance for such studies, published values for murine arterial pH vary considerably, while arterial carbon dioxide pressure (P_{CO_2}) and plasma bicarbonate concentration ([HCO_3^-]) are rarely reported. Because even small acid–base disturbances may significantly alter receptor affinities, function of ion channels and transporters as well as the conformation of enzymes and structural proteins (e.g. Cuthbert and Alberti, 1978; Hamra et al., 1997; Joo et al., 1998), it is important that acid–base status is established correctly during experimental interventions (Sjöblom and Nylander, 2006).

While arterial acid–base status is remarkably similar amongst mammals, reflecting that effective alveolar ventilation is similarly matched to CO_2 production in most species, there is very little data on arterial blood gases in small mammals obtained from minimally disturbed individuals without the interference of anaesthesia. Because the high tissue specific metabolism of small mammals is attended by lower blood oxygen affinities (Schmidt-Nielsen and Larimer, 1958; Hall, 1965; Gray and Steadman, 1964), it is possible that small mammals exhibit higher rates of effective ventilation, and hence, lower arterial P_{CO_2} (Lahiri, 1975). To investigate this possibility, we have measured normal acid–base status in mice, and include a description of CO_2 binding properties of mouse blood *in vitro*. To assess the low arterial P_{CO_2} measured in our study, we also measured ventilation and gas exchange to evaluate air-convection requirements in relation to arterial acid–base status. Finally, because our initial studies revealed pronounced metabolic acidosis when large blood samples were taken from conscious or anaesthetised mice, we also evaluate the influence of lactate levels on the acid–base status measured in samples taken from conscious mice.

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2. Materials and methods

2.1. Experimental animals

Twenty-nine 12-week-old BLABc mice of both sexes were purchased from Taconic (Denmark) and maintained at the animal facility at Department of Bioscience (Aarhus University). Here they were kept at a standard light (12:12 dark–light cycles) and temperature regime with *ad libitum* access to water and standard mouse chow. Body mass of the mice was 24.9 ± 1.0 g when the experiments were performed. All experiments were approved by the Danish Animal Experiment Inspectorate.

2.2. Surgical procedure

Mice were anaesthetised with an intraperitoneal injection of a combination of ketamine and xylazine (75 and 10 mg kg^{-1}, respectively, in 0.10 ml 10 g^{-1}). A small thermistor was inserted into the rectum for continuous measurements of body temperature. Mice were then placed on a sterile surgical table with heating lamps and body temperature was maintained at 36–37.5 °C throughout surgery. The eyes were protected against dehydration with an eye ointment (Ophtha A/S, Copenhagen, Denmark).

The carotid artery was cannulated under a dissecting microscope (Olympus, SZ-STB1, Japan) through a small ventral incision (1 cm) in the neck, using stretched PE-10 tubing (Portex fine bore polythene tubing, Smiths Medical ASP, Inc., Keene, USA) containing heparinised saline (50 UI ml^{-1}). The catheter was led out though the skin behind the ear and secured by a single suture, before the incision was closed by 6 stitches (sterile 6-0 black silk, AgnThoïs AB, Sweden). A 1 ml subcutaneous injection of saline was given to prevent dehydration, and the mice were allowed to recover for at least 24 h at 32 °C and a 12 h light–dark cycle. The mice were given two subcutaneous injections of Temgesic (Buprenorphinum, 0.05 mg kg^{-1}) for analgesia during recovery.

2.3. Construction of buffers curves on true plasma in vitro

After 24 h recovery, a 1 ml blood sample was withdrawn from the catheter in 5 mice (24.3 ± 2.4 g). These mice were killed by cervical dislocation immediately after blood withdrawal. The blood was transferred to a rotating Eschweiler tonometer (Kiel, Germany), submerged in water at 37 °C and allowed to equilibrate with humidified gas mixtures containing 2, 4 and then 7% CO_2 (P_{CO_2} of 14.5, 29.0, and 50.8 mmHg, respectively), balanced with air (P_{O_2} of 142–150 mmHg). Gas mixtures were delivered from a precision gas-mixing pump (Wösthoff, Bochum, Germany). When the blood sample had equilibrated to either P_{CO_2} level for 30 min, 50–80 μl samples were drawn into heparinised hematocrit tubes for determination of blood pH, hematocrit (Hct), and total CO_2 concentration of plasma ($[CO_2]$pl).

Blood pH was measured with a Radiometer (Copenhagen, Denmark) capillary pH electrode thermostated to 37 °C and connected to a Radiometer PHM 73 acid–base analyzer. Total plasma CO_2 concentration ($[CO_2]$pl) was measured as described by Cameron (1971), and plasma bicarbonate concentration ($[HCO_3^-]$)pl) was calculated as $[CO_2]\text{pl} - (\alpha_{CO_2} \times P_{CO_2})$ using a plasma CO_2 solubility (α_{CO_2}) at 37 °C of 0.0301 mmol l^{-1} $mmHg^{-1}$ (Siggaard-Andersen, 1976).

The apparent pK' for the CO_2/HCO_3^- system in mouse plasma at 37 °C was calculated from measured total plasma CO_2 and pH values, using the rearranged Henderson–Hasselbalch equation:

$$pK' = pH - \log\left[\left(\frac{[CO_2]pl}{\alpha_{CO_2} P_{CO_2}}\right) - 1\right]$$

2.4. Arterial blood gases and lactate concentrations in vivo

Acid–base parameters and lactate concentrations were measured in six mice (23.2 ± 0.7 g) anaesthetised in a chamber with isoflurane where systemic arterial blood was withdrawn by cardiac puncture as quickly as possibly (i.e. within 5 min). In another 18 undisturbed and conscious mice (25.7 ± 1.5 g), 350 μl blood was sampled from the indwelling catheters. Blood pH, hematocrit, total CO_2 and lactate concentration were determined in both groups. Arterial P_{CO_2} was calculated from the re-arranged Henderson–Hasselbalch equation:

$$P_{CO_2} = \frac{[CO_2]\text{tot}}{\alpha_{CO_2}(1 + 10^{(pH - pK')})}$$

In addition, 150 μl arterial blood samples were taken from an additional 5 mice (28.6 ± 0.3 g) with indwelling catheters and analysed for pH, P_{CO_2}, P_{O_2}, [lactate] and Hct using a full automatic blood gas analyser GEM 3500 Premier (Instrumentation Laboratory, MA, USA).

2.5. Measurements of gas exchange and ventilation using barometry

Metabolism was determined in 16 BALB/c mice (8 females and 8 males; 16.9 ± 1.5 g) using an open-flow system. The animals were weighed and placed overnight within thermostated chambers (1.1 l). The chambers were translucent on one side allowing natural light conditions as well as visual inspection of the animal. Room air was pumped (EHEIM 400) through the chamber at approximately 300 ml min^{-1}. The outflow was dried and passed through a mass flow meter (Sierra Instruments, Inc., Monterey, CA, USA) before being monitored by a calibrated gas analysing system (Ametek Applied Electrochemistry) consisting of an O_2 and CO_2 sensor and analysers (P-61B, N-22M, CD-3A, and S-3A/I). Both sensors were calibrated immediately before each experiment with dried outside air (20.95% O_2 and 0.04% CO_2 and with a mixture of 5% CO_2 in dried outside air). The gas mixture was delivered by a precision Wösthoff gas-mixing pump (Bochum, Germany). A humidity sensor (Waisala) was connected to the outflow. All data was collected by a data acquisition system (BIOPAC MP 100). Rates of oxygen consumption ($\dot{V}_{O_2}$ ml STPD min^{-1}) and O_2 excretion ($\dot{V}_{O_2}$) were calculated on basis from three equations:

$$\dot{V}_{O_2} = \dot{V}_I F_{I_{O_2}} - \dot{V}_E F_{E_{O_2}} \quad (1)$$

$$\dot{V}_{CO_2} = \dot{V}_I F_{I_{CO_2}} - \dot{V}_E F_{E_{CO_2}} \quad (2)$$

$$\dot{V}_{N_2} = \dot{V}_I F_{I_{N_2}} - \dot{V}_E F_{E_{N_2}} = 0 \quad (3)$$

where $\dot{V}_I$ and $\dot{V}_E$ are the inspired and expired volume, respectively, and FI and FE are the fractions of O_2 or CO_2 in the inspired or expired gases, respectively. From Eq. (3), $\dot{V}_I$ can be calculated from:

$$\dot{V}_I = \frac{\dot{V}_E F_{E_{N_2}}}{F_{I_{N_2}}}$$

where F_{N_2} is given by $1 - F_{O_2} - F_{CO_2}$.

All mice were placed individually in each chamber and $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were measured over-night. Basal metabolic rate was measured in the early morning when the animal was quiet and resting within the chamber.

Ventilation was measured using the barometric method (Drorbaugh and Fenn, 1955) by closing the in- and out-flow pipes of the respirometers. The volume-related pressure signal created by ventilation was measured immediately after obtaining resting metabolism (described above), and after we disturbed the mice to