EBM

Experimental Biology and Medicine

Blood gases and energy metabolites in mouse blood before and after cerebral ischemia: the effects of anesthetics

Tina M Schwarzkopf, Tobias Horn, Dorothee Lang and Jochen Klein

Updated information and services can be found at:
http://ebm.rsmjournals.com/content/238/1/84

This article cites 18 articles, 5 of which can be accessed free at:
http://ebm.rsmjournals.com/content/238/1/84#BIBL

Original Research

Blood gases and energy metabolites in mouse blood before and after cerebral ischemia: the effects of anesthetics

Tina M Schwarzkopf, Tobias Horn, Dorothee Lang and Jochen Klein

Department of Pharmacology, University of Frankfurt College of Pharmacy, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany
Corresponding author: Jochen Klein. Email: klein@em.uni-frankfurt.de

Abstract

The levels of blood gases and energy metabolites strongly influence the outcome of animal experiments, for example in experimental stroke research. While mice have become prominent animal models for cerebral ischemia, little information is available on the effects of anesthetic drugs on blood parameters such as blood gases, glucose and lactate in this species. In this work, we collected arterial and venous blood samples from female CD-1 mice before and after cerebral ischemia induced by middle cerebral artery occlusion (MCAO), and we tested the influence of different anesthetic drugs. We found that all of the injectable anesthetics tested (ketamine/xylazine, chloral hydrate, propofol and pentobarbital) caused a decrease in blood pH and partial pressure of oxygen (pO_2) and an increase of partial pressure of carbon dioxide (pCO_2), indicating respiratory depression. This was not observed with inhalable anesthetics such as isoflurane, sevoflurane and halothane. Significant and up to two-fold increases of blood glucose concentration were observed under isoflurane, halothane, ketamine/xylazine, chloral hydrate, and propofol anesthesia. Lactate concentration rose significantly by 2–3-fold during inhalation of isoflurane and halothane treatment, but decreased by more than 50% after administration of pentobarbital. Permanent cerebral ischemia induced respiratory acidosis (low pH and pO_2, high pCO_2) which was most prominent after 24 h. Postsurgical treatment with Ringer-lactate solution (1 mL, intraperitoneal) caused a recovery of blood gases to basal levels after 24 h. Use of isoflurane for surgery caused a minor increase of blood glucose concentrations after one hour, but a strong increase of blood lactate. In contrast, anesthesia with pentobarbital did not affect glucose concentration but strongly reduced blood lactate concentrations one hour after surgery. All values recovered at three hours after MCAO. In conclusion, anesthetic drugs have a strong influence on murine blood parameters, which should be taken into account in experiments in mice.

Keywords: anesthetic drugs, blood gases, glucose, isoflurane, lactate, mouse/mice, pentobarbital, respiratory acidosis

Introduction

Experimental procedures in laboratory animals, such as middle cerebral artery occlusion (MCAO), require anesthesia, but anesthetic procedures may strongly influence the outcome of the experiment. While the mechanism of action of anesthesia is beginning to be understood,[1] their effect on respiration and blood parameters in experimental animals have received less attention Data are particularly scarce in mice, a species whose use has dramatically increased in recent years. According to the literature, volatile anesthetics such as isoflurane induce minimal cardiovascular depression in rodents and have the fewest hemodynamic effects.[2,3] A rise in blood glucose concentration during anesthesia was described for the fixed combination ketamine/xylazine and for the volatile anesthetics isoflurane and sevoflurane.[4,5] However, the effects of anesthetic drugs on blood lactate concentration are, to our knowledge, still unknown.

Mice in particular have gained prominence as experimental animals in many fields, including experimental stroke research. However, during our recent work, we noticed that anesthetic drugs strongly affect brain glucose and lactate concentration[6] and that the choice of anesthetic drug can strongly affect the outcome of cerebral ischemia (Horn and Klein, unpublished). Therefore, here we present the results of a study on the effects of several anesthetic drugs on blood parameters in mice. We report that several injectable anesthetics, but not inhalable anesthetics, induce respiratory acidosis in mice. Furthermore, we show the effects of cerebral ischemia on blood parameters that were measured after anesthesia with isoflurane, an inhalable
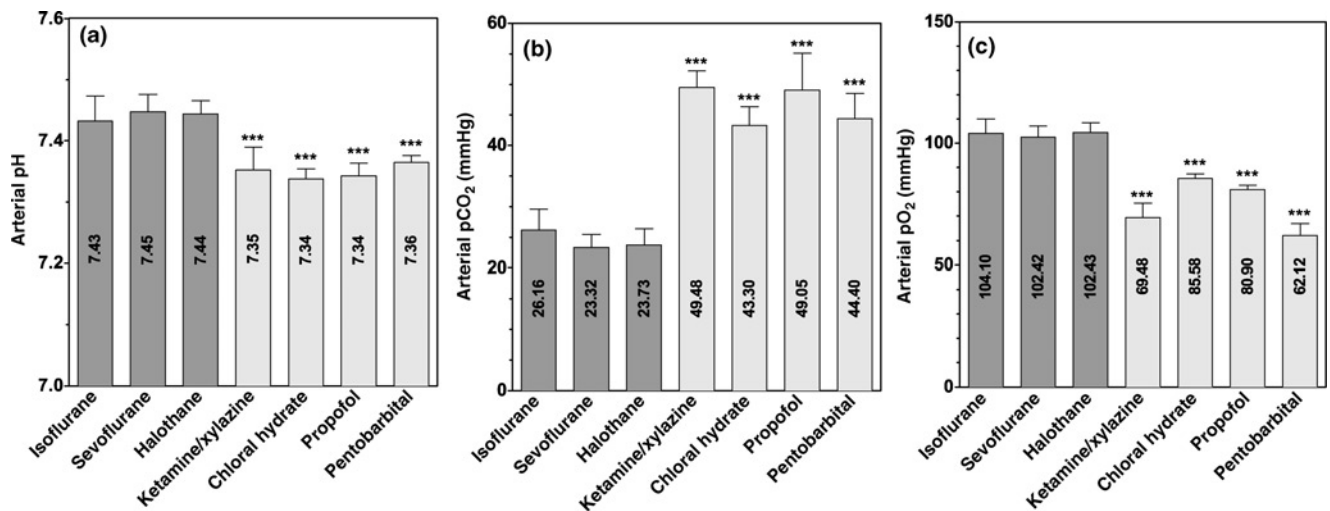
Figure 1 Arterial blood gases of mice anesthetized by different anesthetic procedures. (a) Arterial pH. (b) Partial pressure of carbon dioxide (pCO_2). (c) Partial pressure of oxygen (pO_2). Values were measured in arterial blood taken from the common carotid artery. Data are expressed as means $\pm$ SD (N = 5–11); means are shown within columns. Statistical significance was determined by one-way analysis of variance (Graph Pad Prism®) with Bonferroni post-tests. ***$P < 0.001$ compared with the isoflurane group

anesthetic, or pentobarbital, an injectable anesthetic. The results should help researchers to select the appropriate anesthetic drugs for experimental procedures in mice.

Materials and methods

Animals

Female CD-1 mice (28–32 g, Charles River) were kept under standardized conditions: 12 h-light/dark circle, 22°C and 70% humidity. Food and water were available *ad libitum*. All animal procedures were carried out to minimize animal suffering in accordance with German and European law. The study was registered with the local authorities (Regierungspräsidium, Darmstadt, Germany).

Effects of different anesthetic drugs on blood parameters

Mice were randomly selected for the different procedures. For anesthesia, the following anesthetic drugs were used (doses in brackets): isoflurane (2%), sevoflurane (2%), halothane (2%), ketamine/xylazine (100/10 mg/kg), chloral hydrate (400 mg/kg), propofol (400 mg/kg) and pentobarbital (60 mg/kg). Volatile anesthetics were vaporized in synthetic air. Injectable anesthetics were diluted with physiological saline for intraperitoneal administration. For the withdrawal of arterial blood, mice were anesthetized with various anesthetic drugs and arterial blood was taken as described below from the left common carotid artery (Figures 1–3). For measurements of venous blood parameters, blood was drawn by puncturing the facial vein (Figures 2 and 4). After 24 h, mice were decapitated in deep anesthesia, and rump blood was collected (Figure 4).

Changes in blood parameters after cerebral ischemia

Ischemia in mouse brains was induced by occlusion of the middle cerebral artery as described.[7] For the results shown

in Figures 3 and 4, isoflurane (2% in synthetic air) or pentobarbital (60 mg/kg in physiological saline, intraperitoneal) were used as anesthetics. During surgery, mice were kept at 37°C using a thermostatic blanket coupled to a rectal thermometer (Harvard/Hugo Sachs, March-Hugstetten, Germany). Through a cervical incision, the left bifurcation of the common carotid artery (CCA) was dissected and all three branches (CCA, external carotid artery [ECA] and internal carotid artery [ICA]) were ligated. A 20 mm monofilament (Doccol corporation, Redlands, California, USA size 6–0) was inserted into the ECA and gently advanced through the ICA into the brain until its tip occluded the origin of the middle cerebral artery (MCA). Local cerebral blood flow was measured by laser Doppler flowmetry (Moor Instruments, Devon, UK; AP −0.5, L +3.5 from the bregma) and dropped to 10–15% of basal flow during occlusion. In the permanent stroke model (Figures 3 and 4) the filament was fixed in position and remained for 24 h. In the transient procedure (data not illustrated), the filament was removed after 60 min of occlusion to allow reperfusion (>50% of basal flow). The skin incision was closed with surgical clips and mice were allowed to recover in their home cages. Blood samples were collected before and after (1 and 3 h) MCAO either from the CCA (arterial blood, Figure 3) or from the facial vein (venous blood, Figure 4). In selected mice, the effect of a 1 mL bolus of Ringer-lactate solution given intraperitoneally directly after the end of MCAO surgery was tested (Figure 3). Mice were decapitated 24 h after MCAO and rump blood was collected.

Chemical analysis of blood samples

Blood samples from the CCA, facial vein and trunk were collected in 0.5 mL heparinized Eppendorf cups. The retroorbital venous plexus was punctured with a heparinized capillary (100 × 0.9 mm). The whole blood samples were
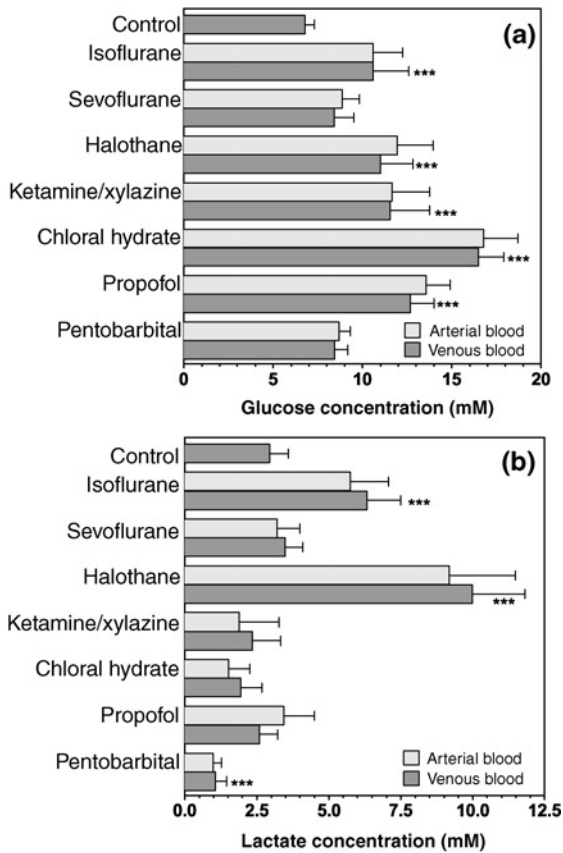
Figure 2 Plasma (a) glucose and (b) lactate concentrations in arterial and venous blood samples taken from mice anesthetized by different procedures. Arterial blood was taken from the common carotid artery, and venous blood samples from the facial vein. Controls were untreated mice; no arterial blood could be withdrawn from these animals. Data are expressed as means $\pm$ SD ($N = 6$–9). Statistical analysis was done using one-way analysis of variance (Graph Pad Prism®) with Bonferroni post-tests. ***$P < 0.001$ compared with venous controls

injected either from the Eppendorf cup or directly from the capillary into a pHOX analyzer (Nova Biomedical, Waltham, Massachusetts, USA) for the determination of blood gases. The pH value and partial pressure values for carbon dioxide (pCO_2) and oxygen (pO_2) were then determined electrochemically. For the determination of blood glucose and lactate concentrations, heparinized blood samples were centrifuged for 30 s at 1000 rpm. Photometric measurement of blood plasma was performed by a CMA-600 analyzer (CMA Microdialysis, Stockholm, Sweden); the lower limits of detection for glucose and lactate were 0.02 mmol/L.

Chemicals

Most anesthetic drugs were obtained from the local hospital pharmacy: Sevoflurane (Sevorane®, Abbott, Wiesbaden, Germany), isoflurane (Forene®, Abbott, Wiesbaden, Germany), halothane (Sigma-Aldrich), ketamine/xylazine (Ketavet®, Pfizer/Rompun®, Berlin, Germany, Bayer, Leverkusen, Germany), pentobarbital (Narcoren®, Merial, Halbergmoos, Germany), chloral hydrate (Fagron®, Barsbüttel, Germany) and propofol
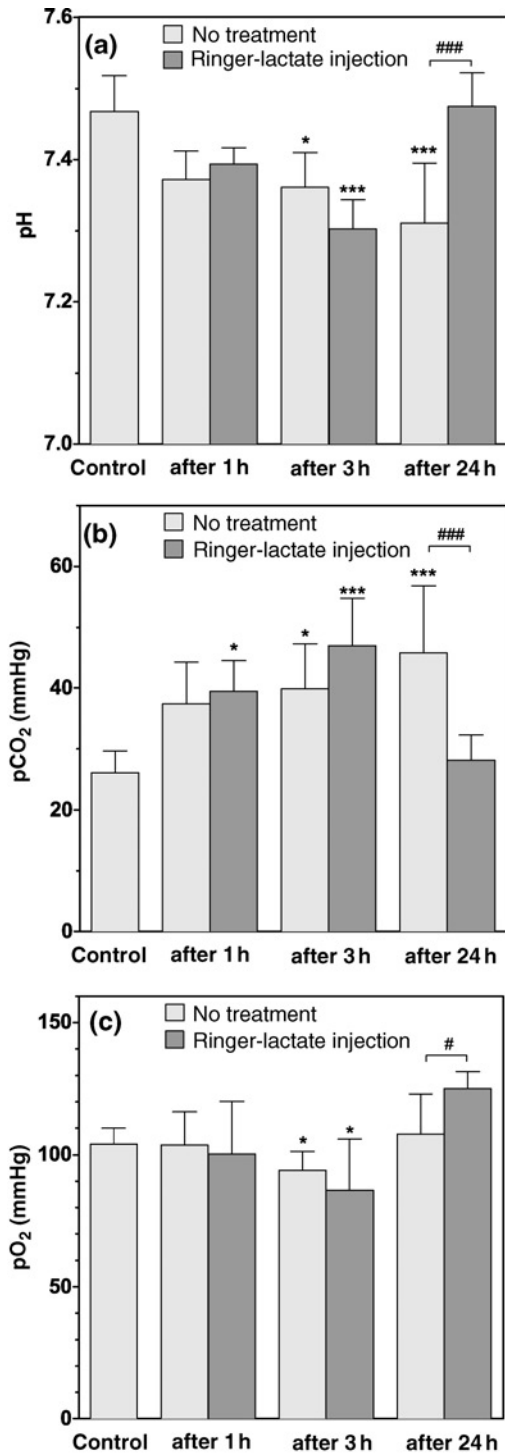

Figure 3 Arterial values of (a) pH, (b) Partial pressure of carbon dioxide and (c) Partial pressure of oxygen measured in blood taken from the common carotid artery before and at different time points after an experimental stroke. Cerebral ischemia was induced by middle cerebral artery occlusion (isoflurane anesthesia) and was sustained for 24 h. Selected mice were treated with an intraperitoneal injection of Ringer-lactate solution (1 mL) which was given immediately after the end of surgery which lasted for 20 min. Blood samples were withdrawn after 1, 3 and 24 h. Data are expressed as means $\pm$ S.D. ($N = 5$–11). Statistical significance was calculated by one-way analysis of variance (ANOVA) and by t-test (Graph Pad Prism®). *$P < 0.05$; ***$P < 0.001$ compared with a control group (ANOVA with Bonferroni post-test). #$P < 0.05$, ###$P < 0.001$ comparing mice with and without Ringer-lactate treatment (t-test)
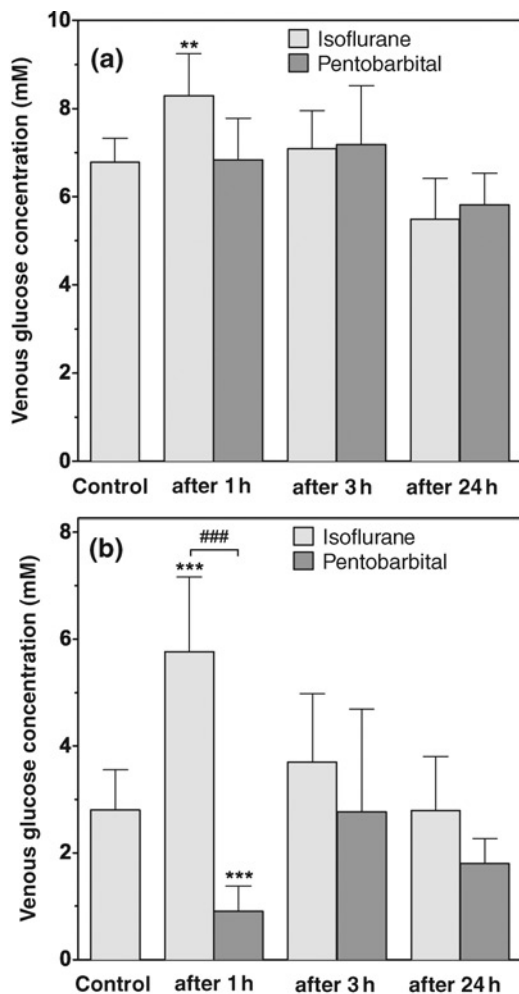
Figure 4 Venous concentrations of (a) glucose and (b) lactate measured in blood taken from the facial vein (24 h: rump blood) before and at different time points after an experimental stroke. Cerebral ischemia was induced by middle cerebral artery occlusion and was sustained for 24 h. Mice were anesthetized either with isoflurane or with pentobarbital. Data are expressed as means ± S.D. ($N = 5$–11). Statistical significance was determined by analysis of variance (ANOVA) with Bonferroni post-test or by t-test (Graph Pad Prism®). **$P < 0.01$; ***$P < 0.001$ compared with a control group (ANOVA). ###$P < 0.001$, isoflurane versus pentobarbital

(Propofol Lipuro®, B. Braun, Melsungen, Germany). Ringer-lactate solution (Delta Select®, Pfullingen, Germany) and heparin (Heparin-Natrium-25000-ratiopharm) were purchased in a local pharmacy.

Results

Effect of different anesthetic drugs on blood parameters

Using a variety of anesthetic drugs, we measured blood parameters (pH, pCO_2, pO_2, glucose and lactate) in mice. As shown in Figure 1, volatile anesthetics (isoflurane, sevoflurane and halothane, all used at 2%) did not change blood gases from physiological levels: pH was retained close to 7.4, and partial pressure of oxygen pO_2 (100–105 mmHg) and partial pressure of carbon dioxide (pCO_2) values

(20–25 mmHg) were normal. All of the injectable anesthetics used in the present study (ketamine/xylazine 100/10 mg/kg; chloral hydrate 400 mg/kg; propofol 400 mg/kg and pentobarbital 60 mg/kg), however, caused acidosis with pH levels around 7.35. As indicated by low oxygen concentration and significantly increased pCO_2, this acidosis was due to respiratory depression.

When glucose and lactate concentrations were measured in blood under anesthesia, no simple correlation was found between inhalable and injectable anesthetic drugs. Figure 2a demonstrates that glucose concentrations were increased significantly by isoflurane and halothane, but not by sevoflurane. The strongest increase, however, was observed with the injectable anesthetic chloral hydrate, followed by propofol and ketamine/xylazine. Pentobarbital, in contrast, was metabolically inactive and did not affect glucose concentration.

Surprisingly, lactate concentration responded very differently to individual anesthetic drugs. Lactate concentration were increased several-fold under isoflurane and halothane anesthesia, while injectable drugs did not affect lactate concentration. The exception was pentobarbital which caused a strong (50%) decrease in blood lactate concentration. It should be noted that according to animal regulations, no control experiment for arterial blood parameters without anesthesia could be done; therefore, statistics in Figure 2 only refer to venous blood samples taken from untreated mice. Of note, arterial and venous blood levels of glucose and lactate did not differ significantly when taken from arterial or venous blood.

Changes in blood parameters after cerebral ischemia

Cerebral ischemia was induced by MCAO performed under isoflurane or pentobarbital anesthesia. Arterial blood samples were collected 1, 3 and 24 h after surgery. As displayed in Figure 3, MCAO caused long-lasting respiratory depression. While pO_2 values were relatively stable, pH values were significantly decreased after ischemia and pCO_2 values significantly increased for up to 24 h. Interestingly, the administration of a bolus of Ringer-actate solution (1 mL intraperitoneally) immediately after MCAO surgery increased pO_2, decreased pCO_2 and restored pH to control levels after 24 h. This clearly indicates the usefulness of Ringer-lactate application after MCAO surgery in mice.

In separate experiments, transient occlusion of the middle cerebral artery was performed, in which the filament was withdrawn after 1 h of occlusion, allowing reperfusion. In these experiments, only a minor tendency towards respiratory acidosis was observed, but the changes of the blood pH values did not reach significance (data not shown). After 24 h, blood gases recovered to basal levels. In this transient model, administration of Ringer-lactate solution did not have significant effects (data not shown).

For a comparison of anesthetic effects on glucose and lactate concentration, we used the permanent ischemia model and compared two different anesthetic drugs: isoflurane and pentobarbital. Both anesthetic drugs displayed significant effects after 1 h of occlusion. As in naïve mice (Figure 2), isoflurane produced an increase in glucose

concentration and a strong (2-fold) increase of lactate concentration (Figure 4) while pentobarbital narcosis did not affect glucose and reduced lactate concentration by half. All values recovered 3 h after induction of cerebral ischemia (Figure 4).

Discussion

Understanding the physiological effects of anesthesia is a prerequisite for the correct interpretation of experimental values. We recently became aware that the effects of anesthetic drugs on blood gases in mice are poorly characterized, while effects on energy metabolism were basically unknown. In the present study, our measurements of blood pH, pCO_2 and pO_2 in mouse arterial blood demonstrated that basal values in the mouse correspond to previous measurements in the rat.[8–10] Importantly, several volatile anesthetics had little effect on respiration and blood gases. In contrast, under anesthesia induced by several different injectable anesthetics (ketamine/xylazine, chloral hydrate, propofol and pentobarbital), blood gases were changed significantly. The combination of lowered pH values, lowered pO_2 values and increased pCO_2 levels indicated respiratory acidosis at drug concentrations that are routinely applied in experimental research. For comparison, in humans, respiratory acidosis is diagnosed at pH < 7.36 and at $pCO_2 > 45$ mmHg.

Interestingly, even under isoflurane anesthesia, cerebral ischemia caused a respiratory depression which lasted for less than three hours after transient MCAO, but more than 24 h after permanent MCAO. The mechanism of this effect is not known but may involve an influence of the ischemic area in the forebrain on the respiratory control center in the medulla oblongata.[11,12] Importantly, the present data show that the effect of MCAO on blood gases can be alleviated by an administration of Ringer-lactate solution immediately after surgery. As previously shown, Ringer-lactate solution improves surgical outcomes by refilling lost blood volume and electrolytes but also by buffering the change in pH levels due to metabolism of lactate.[13] When given after MCAO, the effect is highly significant and this finding should help to reduce long-term acidosis following experimental stroke.

In addition to blood gases, we monitored blood glucose and lactate concentration as indicators of energy metabolism in the mouse *in vivo*. Basal values for blood glucose in mice (approx. 6 mmol/L) were similar to those in rats[10,14,15] and humans, but lactate concentration were found to be higher in mice (2.5–3 mmol/L). Interestingly, the tested anesthetic drugs had completely different effects on glucose and lactate concentration. Inhalable anesthetics such as isoflurane and halothane increased both glucose and lactate concentrations. A rise of glucose concentration during anesthesia can be explained by a rise of stress hormones such as epinephrine and cortisol during anesthesia, which mobilizes glucose from glycogen and amino acids.[16] A decrease of insulin concentration has also been proposed to explain increases in blood glucose during isoflurane anesthesia.[4,5,17] Sevoflurane did not affect blood glucose concentration in our experiments; it should be noted, however, that such increases have been observed when sevoflurane was applied at a higher dose.[5,17]

The effects of isoflurane and halothane on lactate concentration were surprising. Isoflurane caused a two-fold increase, and halothane caused an almost three-fold increase in blood lactate concentrations. These increases were evidently not due to anaerobic metabolism, because they were accompanied by parallel increases of glucose and occurred in the absence of acidosis. Lactate formation by muscle tissue is also unlikely because all animals were immobile during anesthesia. The increased lactate concentrations are reminiscent, however, of increases of lactate in the brain which we recently observed under isoflurane anesthesia in the mouse brain.[6] The mechanism of this effect is unknown, but we speculate that it may involve mitochondrial functions; for example, increased formation or blocked degradation of lactate.[6]

Glucose concentration also rose significantly with ketamine/xylazine, chloral hydrate and propofol anesthesia. Here, the fixed combination ketamine/xylazine is well known to produce a rise in blood glucose concentrations, which is explained by xylazine's agonistic actions on adrenergic alpha-2 receptors in the pancreas and the consequent reduction in insulin release.[4,18] However, the actions of propofol and chloral hydrate were unexpected and their mechanisms are not known. Trichloroethanol, the active metabolite of chloral hydrate, is actually expected to cause hypoglycemia due to the well-known actions of ethanol on glucose concentration. A possible reason for the increase of glucose may be a reduction of glucose utilization in tissues, but that remains to be investigated.

In agreement with data from the literature,[4,17] pentobarbital did not induce hyperglycemia but, surprisingly, pentobarbital is the only anesthetic drug to lead to a *reduction* of blood lactate concentration In the present study, this reduction was observed both in untreated mice and in mice after transient cerebral ischemia, and it disappeared when mice recovered and started moving. Again, the mechanism behind this effect is not obvious and remains to be investigated.

Summarizing our results, the anesthetic drugs tested each differently influenced blood pH, pCO_2, pO_2, glucose and lactate concentrations. Inhalable anesthetic gases caused little alteration in blood gases and, therefore, should be preferentially used for surgery in mice; however, their distinct metabolic effects, leading to increases in blood glucose and lactate concentrations, should be considered. Injectable anesthetic drugs induce respiratory acidosis and an increase in blood glucose. Cerebral ischemia induced by MCAO also leads to acidosis, a consequence that can be attenuated by the application of Ringer-lactate solution. Metabolically speaking, pentobarbital is the odd drug because it does not change glucose, but decreases blood lactate concentration to a significant extent. The consequences of lactate changes in the blood induced by anesthetics in the absence of acidosis, are not yet known.

REFERENCES

1 Franks NP. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. *Nat Rev Neurosci* 2008;**9**:370–86

2 Janssen BJ, De Celle T, Debets JJ, Brouns AE, Callahan MF, Smith TL. Effects of anesthetics on systemic hemodynamics in mice. *Am J Physiol Heart Circ Physiol* 2004;**287**:1618–24

3 Rao S, Verkman AS. Analysis of organ physiology in transgenic mice. *Am J Physiol Cell Physiol* 2000;**279**:C1–C18

4 Saha JK, Xia J, Grondin JM, Engle SK, Jakubowski JA. Acute hyperglycemia induced by ketamine/xylazine anesthesia in rats: mechanisms and implications for preclinical models. *Exp Biol Med* 2012;**230**:777–84

5 Tanaka T, Nabatame H, Tanifuji Y. Insulin secretion and glucose utilization are impaired under general anesthesia with sevoflurane as well as isoflurane in a concentration-independent manner. *J Anesth* 2005;**19**:277–81

6 Horn T, Klein J. Lactate levels in the brain are elevated upon exposure to volatile anesthetics: a microdialysis study. *Neurochem Int* 2010;**57**:940–7

7 Lang D, Kiewert C, Mdzinarishvili A, Schwarzkopf TM, Sumbria R, Hartmann J, Klein J. Neuroprotective effects of bilobalide are accompanied by a reduction of ischemia-induced glutamate release in vivo. *Brain Res* 2011;**1425**:155–63

8 Milmer KE, Clough DP. Optimum ventilation levels for maintenance of normal arterial blood pO_2, pCO_2, and pH in the pithed rat preparation. *J Pharmacol Methods* 1983;**10**:185–92

9 Hoffman WE, Pelligrino D, Werner C, Kochs E, Albrecht RF, Schulte am Esch J. Ketamine decreases plasma catecholamines and improves outcome from incomplete cerebral ischemia in rats. *Anesthesiology* 1992;**76**:755–62

10 Abel EL. Alcohol-induced changes in blood gases, glucose, and lactate in pregnant and nonpregnant rats. *Alcohol* 1996;**13**:281–5

11 Meyer JS. Studies of cerebral circulation in brain injury. IV. Ischemia and hypoxemia of the brain stem and respiratory center. *Electroencephalogr Clin Neurophysiol* 1957;**9**:83–100

12 Malik AB, Krasney JA, Royce GJ. Respiratory influence on the total and regional cerebral blood flow responses to intracranial hypertension. *Stroke* 1977;**8**:243–9

13 Khajavi MR, Etezadi F, Moharari RS, Imani F, Meysamie AP, Khashayar P, Najafi A. Effects of normal saline vs. lactated ringer's during renal transplantation. *Ren Fail* 2008;**30**:535–9

14 Benthem L, van der Leest J, Steffens AB, Zijlstra WG. Metabolic and hormonal responses to adrenoceptor antagonists in exercising rats. *Metabolism* 1995;**44**:245–53

15 Kofke WA, Hawkins RA, Davis DW, Biebuyck JF. Comparison of the effects of volatile anesthetics on brain glucose metabolism in rats. *Anesthesiology* 1987;**66**:810–3

16 Lattermann R, Schricker T, Wachter U, Georgieff M, Goertz A. Understanding the mechanisms by which isoflurane modifies the hyperglycemic response to surgery. *Anesth Analg* 2001;**93**:121–7

17 Zuurbier CJ, Keijzers PJ, Koeman A, Van Wezel HB, Hollmann MW. Anesthesia's effects on plasma glucose and insulin and cardiac hexokinase at similar hemodynamics and without major surgical stress in fed rats. *Anesth Analg* 2008;**106**:135–42

18 Kawai N, Keep RF, Betz AL. Hyperglycemia and the vascular effects of cerebral ischemia. *Stroke* 1997;**28**:149–54