Original Research

Optimizing anesthetic regimen for surgery in mice through minimization of hemodynamic, metabolic, and inflammatory perturbations

Coert J Zuurbier[1], Anneke Koeman[1], Sander M Houten[2], Markus W Hollmann[1] and Wouter J Florijn[3]

[1]Laboratory of Experimental Intensive Care and Anesthesiology, Department of Anesthesiology, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands; [2]Department of Clinical Chemistry and Department of Pediatrics, Laboratory Genetic Metabolic Diseases Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands; [3]Department of Animal Welfare, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands

Corresponding author: Coert J Zuurbier. Email: c.j.zuurbier@amc.uva.nl

Abstract

The role of anesthetics in animal research models is crucial, yet often ignored, and is almost never the primary focus of examination. Here, we investigated the impact of anesthetic regimens on different parameters of hemodynamics (blood pressure (BP) and heart rate (HR)), metabolism (glucose, insulin, and free fatty acids (FFA)), and inflammation (IL-6 and TNF-α) in two frequently used mouse strains (C57BL/6 and FVB). All animals were at a similar surgical plane of anesthesia, mechanically ventilated, and monitored for 60 min. The following anesthetic regimens were studied: (1) fentanyl–ketamine–midazolam (FKM), (2) fentanyl–midazolam–haldol (FMH), (3) pentobarbital (P), (4) fentanyl–fluanisone–midazolam (FFM), (5) fentanyl–midazolam–acepromazine (FMA), (6) ketamine–medetomidine–atropine (KMA), (7) isoflurane (ISO), and (8) propofol–fentanyl–midazolam (PFM). Metabolic and inflammatory parameters were compared with those obtained from non-anesthetized animals. *Hemodynamics:* BP >80 mm Hg were only obtained with KMA, whereas hypotension (BP <60 mm Hg) was observed with FKM and P. HR >500 beats/min was observed with ISO and PFM, whereas HR <400 beats/min was induced with KMA, FMH (BL/6), P (BL/6), and FKM (FVB). *Metabolism:* Glucose and insulin were most disturbed by KMA and ISO and mildly disturbed by FMA, whereas FFM, PFM, and P did not have any effect. FFA increased largely by FMA, with ISO and FKM having no effects. *Inflammation*: Cytokines were increased least with ISO/FFM/FMA, whereas FKM and KMA induced the largest increases in cytokines. When aiming at achieving surgical anesthesia without large disturbances in hemodynamic, metabolic, and inflammatory profiles, FFM, ISO, or PFM may be the most neutral anesthetic regimens in mice.

Keywords: Anesthesia/anesthetic, mouse/mice, blood pressure, cytokines, metabolites

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Introduction

An ever-increasing number of different transgenic mouse models are being explored by the scientific community, to evaluate the functional and (patho) physiological consequences of gene manipulation. Often, anesthetic and analgesic regimens are needed to perform these phenotypic characterizations. However, such regimens may themselves have a significant impact on the parameters investigated. In addition, the anesthetic regimen may specifically interact with the genetic manipulation. This also applies to many other animal research questions, where the impact of anesthesia on the parameters studied is often ignored. In the early days (mid-1990s) of murine structure–function evaluation of gene manipulation, hemodynamic parameters were often severely depressed due to inadequate anesthetic techniques.[1] Optimizing anesthesia in relation to hemodynamics resulted in the initial advocation of an α-chloralose/urethane combination, presumably due to its minimal depressant effects on cardiovascular reflexes.[1–3] However, we[4] and others[5] found the recommended doses of α-chloralose/urethane to be insufficient to provide a surgical plane of anesthesia, reminiscent of previous literature questioning the anesthetic potency of α-chloralose.[6] This is in accordance

with recent recommendations from cardiovascular scientific journals that prohibit the use of α-chloralose as the sole anesthetic agent.[7] Isoflurane (ISO) or a combination of ketamine with an α_2-agonist (e.g. xylazine or medetomidine) is currently suggested as the anesthetic regimen of choice in mice, providing the most optimal hemodynamics in the presence of a surgical plane of anesthesia.[5,8,9] However, it is unknown how these anesthetic regimens affect other important physiological parameters in mice. Preliminary studies on the rat have already demonstrated that ketamine–xylazine,[10] ketamine–medetomidine[11], or ISO[11,12] may significantly impact glucose homeostasis in the rat. However, anesthetic regimens without large metabolic disturbances are needed in the current area of metabolic disease research. Therefore, this study set out to examine several different anesthetic regimens in relation to metabolic disturbances; since how these and other frequently used, anesthetic regimens affect metabolic parameters in mice is still unknown. Finally, although it has been suggested that local anesthetics may significantly affect the inflammatory response,[13] little data exist describing the possible effects of general anesthetics on inflammation. This is surprising, considering the current awareness of the importance of inflammation and metabolic derangements in human diseases, and consequently the emphasis on these parameters in murine research models.[14–16]

Therefore, we investigated to what extent many commonly used anesthetic regimens affect hemodynamics, inflammatory, and metabolic parameters in two frequently used mice strains, that is, C57BL/6 and FVB mice.

Methods

Mice strains

Two different strains (all male) were investigated (age 10–14 weeks): C57BL/6 J and FVB. All mice were obtained from the same supplier (Charles River, Chatillon-sur-Chalaronne, France). These strains were chosen due to their frequent use in current biomedical research. All animals were kept in cages in groups of 2–6 animals of similar strain subjected to a 12-h dark/12-h light cycle, with water and food *ad libitum*.

Animal preparation and experimental protocol

Animals were treated according to the guidelines of the Declaration of Helsinki and all procedures were in accordance with the requirements of the Animal Welfare and Use Committee of the Academic Medical Center of Amsterdam. Following the induction of anesthesia, the animals were ventilated to prevent impaired blood oxygenation due to the sedative effects of anesthesia.[17] A tracheotomy was therefore performed (trachea tube 1.0 mm OD, 0.6 mm ID) and mechanical ventilation started. The animals were ventilated (Mouse Ventilator Minivent, Hugo Sachs Electronik, March-Hugstetten, Germany) with 50% O_2/50% N_2 at a rate of 120/min, a positive end-expiratory pressure of 4 cm H_2O, and with a tidal volume of 0.21 mL (Schwarte et al, 2000). The carotid artery was cannulated with polyethylene tubing (0.61 mm OD, 0.28 mm ID) and mean arterial pressure (MAP) and heart rate (HR) were recorded using a heparinized saline-filled catheter. The catheter was connected to a blood pressure (BP) transducer (Baxter truwave PX-600 F, Baxter-Edwards, Irvine, CA, USA), which was sampled at 1 k Hz. Heart rate and MAP were subsequently continuously calculated from this 1 h Hz signal, and displayed and stored at 500 ms intervals using LabVIEW^{tm} 5.1 (National Instruments Corporation, Austin, TX, USA) applications. Temperature was controlled at 37°C using rectal temperature monitoring, a temperature-controlled heating pad and an infrared lamp. Mice received an intraperitoneal (i.p.) bolus of 0.8 mL saline and 0.2 mL sodium bicarbonate (200 mM) to compensate for fluid loss and prevent metabolic acidosis.[18] Each experiment lasted for 60 min, after which a 0.2 mL blood sample was drawn from the carotid artery cannula for analysis of blood gasses. Subsequently, an additional 0.2 mL blood was sampled and centrifuged, and plasma stored at −80°C for analysis of IL-6, TNFα, insulin, and free fatty acids (FFA). Blood glucose levels were determined (Freestyle Freedom glucose strips, Abbott Diabetes Care Inc, Alameda, CA, USA) before induction of anesthesia through heel bleeding, and at $t = 0$, 30, and 60 min through tail bleeding. The 60-min experiment was started ($t = 0$) following a short stabilization period (<10 min) after completion of tracheotomy and carotid artery cannulation. A 60-min monitoring period was chosen because our previous work[9] demonstrated that effects of anesthesia on physiological parameters develop during the first 60 min, with minor changes in these effects when extending the monitoring period beyond 60 min. An even shorter period of 60 min would have masked the temporal behavior of blood glucose.

Anesthesia

For the anesthetic regimens that we were inexperienced with (fentanyl–ketamine–midazolam (FKM), fentanyl–midazolam–haldol (FMH), fentanyl–midazolam–acepromazine (FMA), and propofol–fentanyl–midazolam (PFM)), optimal dosage providing surgical anesthesia was initially set at values reported in the literature, and further adopted as necessary in pilot experiments ($n = 5$–15 mice per regimen) until an adequate plane of anesthesia (loss of pedal withdrawal reflex) was obtained. The pedal withdrawal reflex was probed with a customized forceps entailing a metal clip,[19] ensuring a standardized stimulus for inducing pedal reflexes. The pedal withdrawal reflex was used as index of surgical anesthesia in animal research, as recommended and discussed by Flecknell.[20] Every 20 min throughout the experimental protocol, the right or left hind leg (intermittent) was stretched by pulling the fascia in between the toes and subsequently pinching it with the customized forceps. When a sharp withdrawal of the hind leg was observed following the pinching, it was assumed that surgical anesthesia was not present and an additional maintenance bolus of the anesthetic regimen was applied. As a secondary index of depth of anesthesia, when we had an arterial line in place, we also monitored BP and HR responses to pinches to the hind leg. In cases of no presence of pedal reflex, but there was still a considerable change in

hemodynamics, a small additional bolus of anesthesia was also applied.

The following dosages of anesthetic regimens ($n = 6$ animals for each group) were used: (1) FKM, induction with 0.1 mg/kg fentanyl, 100 mg/kg ketamine, and 10 mg/kg midazolam, given as an i.p. bolus of 0.15 mL/10 g; maintenance provided by ¼ induction dose every 15 min; (2) FMH, induction with 0.65 mg/kg fentanyl, 20 mg/kg midazolam, and 12.5 mg/kg haldol, given as an i.p. bolus of 0.2 mL/10 g; maintenance provided by ¼ induction dose every 15 min; (3) P, induction with 80 mg/kg pentobarbital (Euthasol 20%), given as an i.p. bolus of 0.10 mL/10 g; maintenance by 20% induction dose every 20 min; (4) fentanyl–fluanisone–midazolam (FFM), induction with (0.63 mg/kg fentanyl +25 mg/kg fluanison (Hypnorm, VetaPharma Ltd, Leeds, UK) and 12.5 mg/kg midazolam, given i.p. in 0.10 mL/10 g; maintenance through ¼ induction dose every 15 min; (5) FMA, induction with 0.31 mg/kg fentanyl, 6.25 mg/kg midazolam, and 6.25 mg/kg acepromazine, given i.p. as a bolus of 0.15 mL/kg; maintenance provided by ⅓ induction dose every 20 min; (6) ketamine–dexmedetomidine–atropine (KMA), induction with 125 mg/kg ketamine, 0.2 mg/kg dexmedetomidine. and 0.5 mg/kg atropine given i.p. as 0.075 mL/kg; maintenance provided by ⅓ induction dose every 20 min; (7) ISO, induction with 4% ISO, maintenance with continuous inhalation of 1.5–2.0% ISO; and (8) PFM, induction with 35 mg/kg propofol, 1.2 mg/kg fentanyl, and 20 mg/kg midazolam in an i.p. bolus of 0.315 mL/10 g; maintenance by continuous i.p. infusion of 90 mg/kg/h propofol and 1.6 mg/kg/h fentanyl.

To examine effects of anesthesia *per se* on blood values, we also obtained blood by heart puncture from non-anesthetized animals (control; $n = 5$ for each group) that were killed by cervical dislocation just prior to blood sampling.

Blood analysis. Plasma insulin (Ultra Sensitive Mouse Insulin, Bioconnect #900800, Mercodia, Uppsala, Sweden; 10 μL), IL-6 (Duoset Mouse R&D Systems, Abingdon, UK; 25 μL), and TNF-α (Duoset Mouse R&D Systems; 25 μL) were determined by enzyme-linked immunosorbent assay techniques. FFA were measured by a colorimetric determination (WAKO NEFA-C, # 999-75406, Instruchemie, Delfzijl, the Netherlands; 25 μL).

Statistics. Data are presented by mean ± SEM or median ± interquartile range (IQR; 25% IQR, 75% IQR). One-way analysis of variance with repeated measurements (4 time points) for blood glucose levels was performed, followed by Fisher's *post hoc* analysis comparing time points 2, 3, and 4 with time point 1 when an overall significance was observed. Mann–Whitney non-parametric tests were performed to compare each anesthetic regimen with the non-anesthetized, control group for insulin, FFA, Il-6, and TNF-α. Values of $P < 0.05$ were considered to be statistically significant.

Results

MAP and HR for the eight different anesthetic regimens and the two different mouse strains are shown in Figure 1. At similar surgical depths of anesthesia, the highest MAP (~90–100 mm Hg) was observed with KMA anesthesia for both the C57BL/6 and FVB strain (Figure 1(a) and (c)). Conversely, FKM anesthesia resulted in lowest MAP (~40–50 mm Hg) for both strains. ISO, FFM, and FMA had approximately equal effects in relation to MAP (~70–80 mm Hg) for both strains, whereas FMH preserved MAP better in C57BL/6 than in FVB. PFM anesthesia also resulted in MAP of 70 mm Hg in C57BL/6 mice. However, we were unable to obtain a surgical plane of anesthesia with PFM in the FVB mice. Pentobarbital (P) resulted in rather low MAP (~60 mm Hg) for both strains, although MAP recovered partly over time in FVB mice.

The highest HR (~500–550 beats/min) was achieved with ISO anesthesia for both strains, and also with PFM in C57BL/6 and FMA in FVB mice (Figure 1(b) and (d)). In contrast to the highest MAP observed with KMA anesthesia, KMA was also associated with the lowest HR (~350 beats/min) in both strains. FFM and P anesthesia resulted in an in-between HR (~400–450 beats/min). Thus, in general, when achieving a plane of surgical anesthesia, KMA offered superior maintenance of BP at the cost of lowest HR, ISO and PFM maintained both BP and HR at relatively high levels and FFM and FMA anesthesia resulted in acceptable hemodynamic values.

We next examined whether certain anesthetic regimens affected blood glucose levels in C57BL/6 and FVB mice. KMA increased glucose levels immediately following anesthesia compared with pre-anesthesia glucose levels, with hyperglycemia maintained throughout the experiment (Figure 2(a) and (c)). FMA induced a transient increase in glucose levels in both strains (Figure 2(b) and (d)). A transient increase in glucose was also observed with ISO and FKM in the FVB mice. In contrast, a small decrease in blood glucose levels was observed with FFM (Figure 2(b)). Only PFM anesthesia was without significant effects on blood glucose levels (Figure 2(a)). Overall, the results indicate that KMA is associated with persistent, severe hyperglycemia, whereas several other anesthetics (FMA, FKM, and ISO) induce a transient moderate hyperglycemic response. PFM, FFM, P, and FMH only had minor or no effects on blood glucose in the mice.

At the end of the experiment, insulin and FFA plasma levels in blood obtained from carotid artery sampling for the different anesthetic regimens were analyzed (Figure 3). Insulin levels amounted to 1.8 ng/mL for C57BL/6 and 2.4 ng/mL for FVB in the non-anesthetized, control condition. The data indicate that most anesthetic regimens did not have a significant effect on insulin levels, except for FMA, KMA, and ISO. KMA (0.1 ng/mL insulin) and ISO (0.2–0.5 ng/mL insulin) especially resulted in severe hypoinsulinemia in both strains (Figure 3(a)). FMA also reduced insulin in the C57BL/6 animal. FFA levels in the non-anesthetized animals equaled 0.7 mM and 1.1 mM for C57BL/6 and FVB, respectively (Figure 3(b)). Most anesthetic regimens significantly increased these levels to

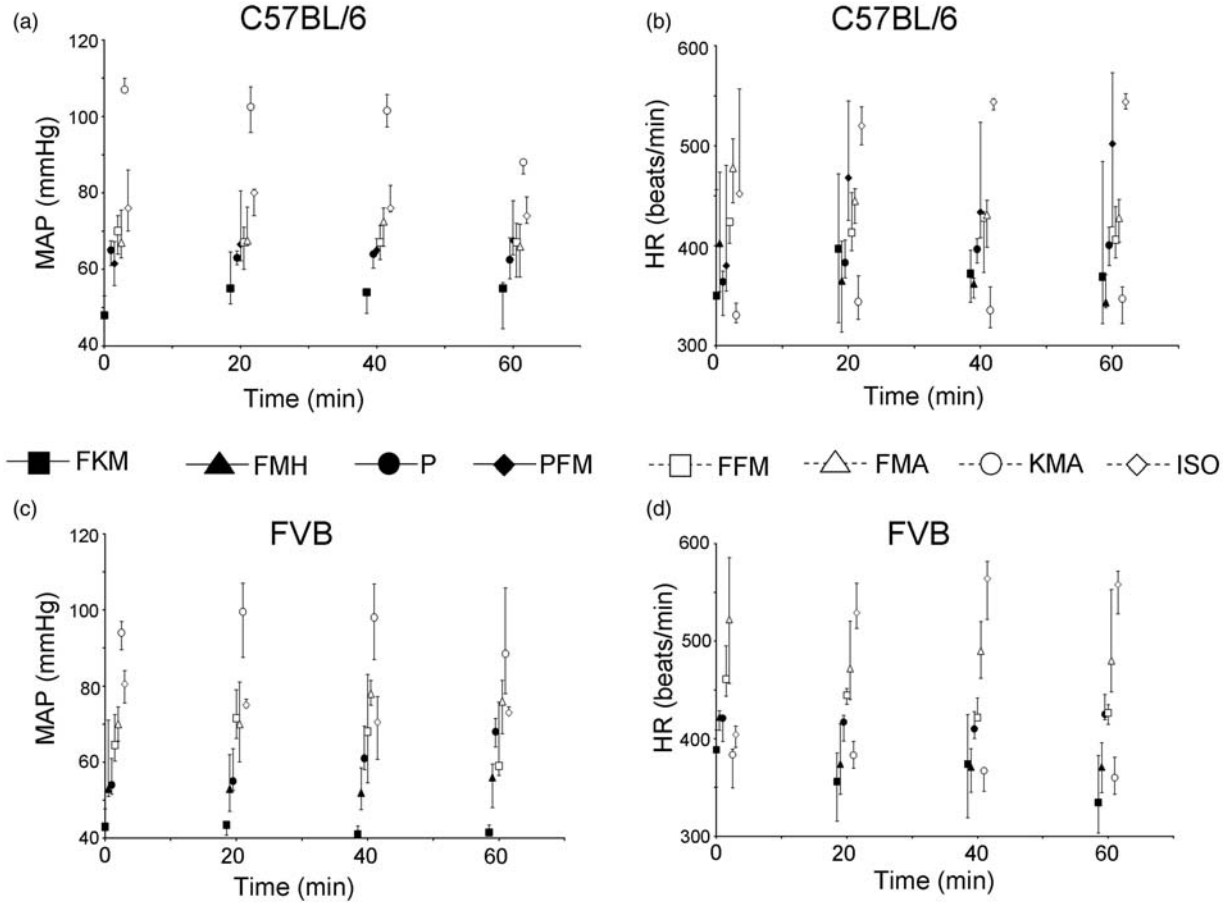

Figure 1 Mean arterial pressure (MAP; a + c) and heart rate (HR, b + d) of ventilated, anesthetized mice for the C57BL/6 and FVB mice and the eight different anesthetic regimens during a 60-min monitoring protocol. The anesthetic regimens were fentanyl–ketamine–midazolam (FKM), fentanyl-midazolam–haldol (FMH), pentobarbital (P), propofol–fentanyl–midazolam (PFM), fentanyl–fluanisone–midazolam (FFM), fentanyl–midazolam–acepromazine (FMA), ketamine–medetomidine–atropine (KMA), and isoflurane (ISO). Data are presented as median with the 25% interquartile range (IQR; downward error bar) and the 75% IQR (upward error bar). In order to improve visualization at each time point, the different groups were artificially spread around the time points

above 2 mM. Surprisingly, in contrast to its effect on insulin and glucose, ISO anesthesia was one of the few anesthetics that was without an effect on FFA in both strains. Besides ISO, only FKM anesthesia was not associated with major alterations of FFA in both strains, as compared with values obtained in the non-anesthetized animals.

Next, we examined to what extent anesthesia may affect inflammatory parameters (Figure 4). Both IL-6 (Figure 4(a)) and TNF-α (Figure 4(b)) were below detection limits in the non-anesthetized, immediately sacrificed, animals. Overall, the data already show an inflammatory response to minimal surgery and anesthesia. Thus, it should be realized that differences between control and anesthetized animals not only pertain to usage of anesthetics but also to the presence of minimal surgical interventions in the anesthetized animals. This inflammatory response appeared attenuated in FVB mice when compared with C57BL/6 mice. All anesthetic regimens significantly increased IL-6 in C57BL/6 mice, with the smallest increase observed for ISO, whereas FKM induced the largest IL-6 increase. FKM was also associated with the highest IL-6 level in the FVB mice. Only KMA induced significant increases in TNF-α in both the C57BL/6 and FVB animals (Figure 3(b)). Overall, the data suggest that ISO anesthesia, closely followed by FFM and FMA anesthesia are the preferred anesthetic regimens when aiming at attenuation of a pro-inflammatory condition within the experimental mice.

Blood gases of all groups at the end of the experiment are given in Table 1. Mechanical ventilation with a set FiO_2 of 0.5 resulted in a relatively similar oxygenation of arterial blood (pO_2 values between 220 and 260 mm Hg) for most anesthetic regimens. We observed very low arterial oxygen tension levels only for FKM anesthesia in C57BL/6 mice. In addition, anesthesia with P was associated with lowest blood oxygenation (190–210 mm Hg) for both mouse strains, whereas ISO anesthesia consistently resulted in the highest arterial blood oxygenation (>300 mm Hg). The high oxygenation with ISO anesthesia was paralleled with the lowest arterial blood carbon dioxide levels, compared with all of the other anesthetics, suggesting that ISO provides most optimal ventilation/perfusion matching in the mechanically ventilated mice. The pH values together with the bicarbonate measurements indicated the presence of moderate alkalosis with all anesthetic regimens, which could probably be ascribed to relatively high bicarbonate levels in the blood.

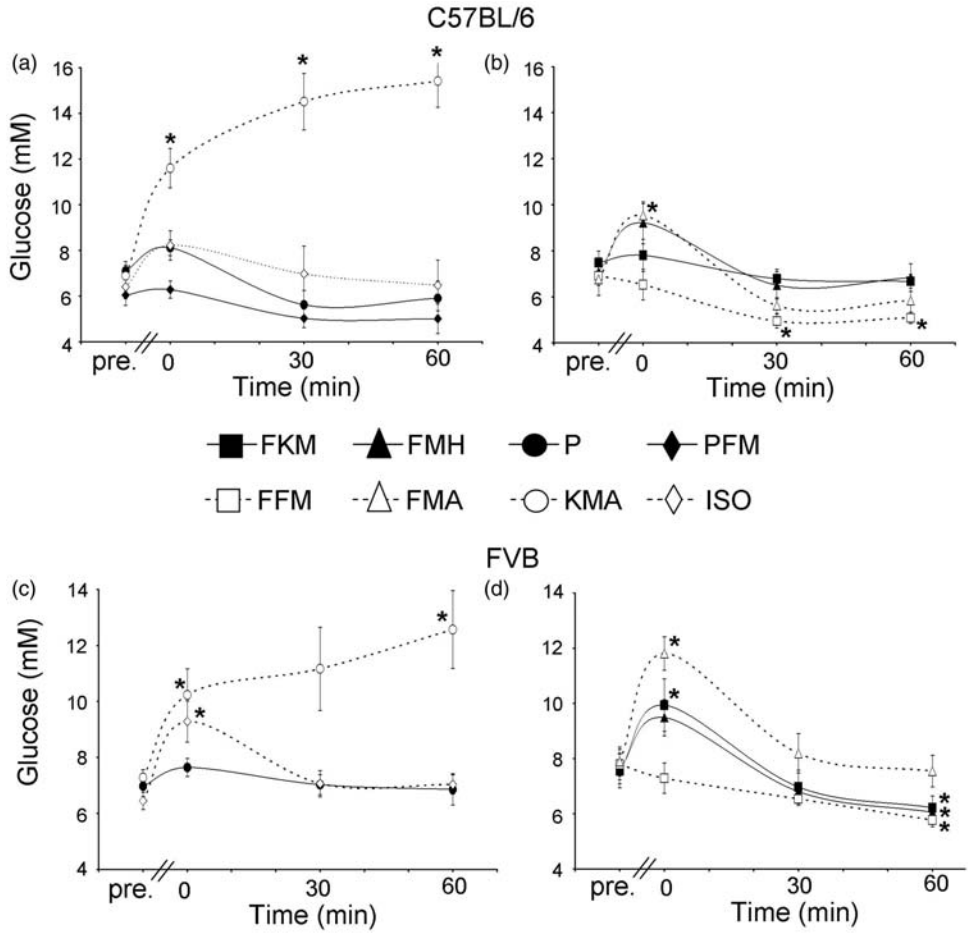

Figure 2 Blood glucose levels determined before anesthesia was applied (pre.) and at $t = 0$, 30, and 60 min in ventilated, anesthetized mice for C57BL/6 and FVB strain and the eight different anesthetic regimens (see Figure 1 for abbreviations). *$P < 0.05$ Fisher's *post hoc* test relative to pre-anesthetic blood glucose value

Discussion

This study demonstrates that every anesthetic affects different physiological systems to varying degrees. There is no ''golden'' anesthetic regimen for all experimental conditions, but our data clearly suggest that the type of anesthetics can be tailored to the physiological system being scrutinized. We suggest that for inflammation research ISO, FFM, or FMA, but not KMA or FKM, are the preferred anesthetic regimens. For hemodynamic studies, ISO, FFM, PFM, or KMA can be used, whereas FKM or P are not recommended. When studying glucose metabolism, FKM, FFM, P, PFM, or FMH can be applied, whereas KMA should be especially avoided. However, although different anesthetics may be preferred depending only on the selected physiological system, it will be equally important to prevent studies being performed in which any physiological system deviates largely from normal, murine physiology. The current study suggests that KMA and FKM should be avoided as anesthetic regimens in mice and offers a warning against the increased use of ketamine in combination with α_2-agonists such as medetomidine and xylazine as anesthetic regimen of choice in the perioperative care of animals.[21]

Hemodynamics and anesthetics in C57BL/6 and FVB mice

KMA anesthesia was superior in terms of the maintenance of arterial BP for both strains. Although the exact mechanism of KMA-induced pressure elevation is unknown, it may be related to ketamine-induced alterations in autonomic nerve activity and baroreflex control[22] and/or ketamine-induced increases in catecholamines[23,24] and cortisol.[25] Additionally, the medetomidine-associated hyperglycemia (see section Metabolic parameters) may also contribute to a higher BP with KMA anesthesia, knowing that acute increases in blood glucose are associated with increased BP.[26] In contrast, Janssen et al.[5] demonstrated strong cardiodepressive effects of ketamine in combination with xylazine, another α_2-agonist. The cardiodepressive effects are likely due to xylazine, inducing stronger central inhibition of the sympathetic tone than medetomidine, thereby overruling ketamine-induced alterations in autonomic activity. It is known that medetomidine is a more specific α_2-agonist than xylazine, probably explaining the higher BP with medetomidine as compared with xylazine, as was also observed in an *in vivo* rabbit study.[27] Therefore, medetomidine is preferred to xylazine as α_2-agonist in small animal studies. However, KMA anesthesia is also associated with bradycardia, probably induced by initial hypertension and

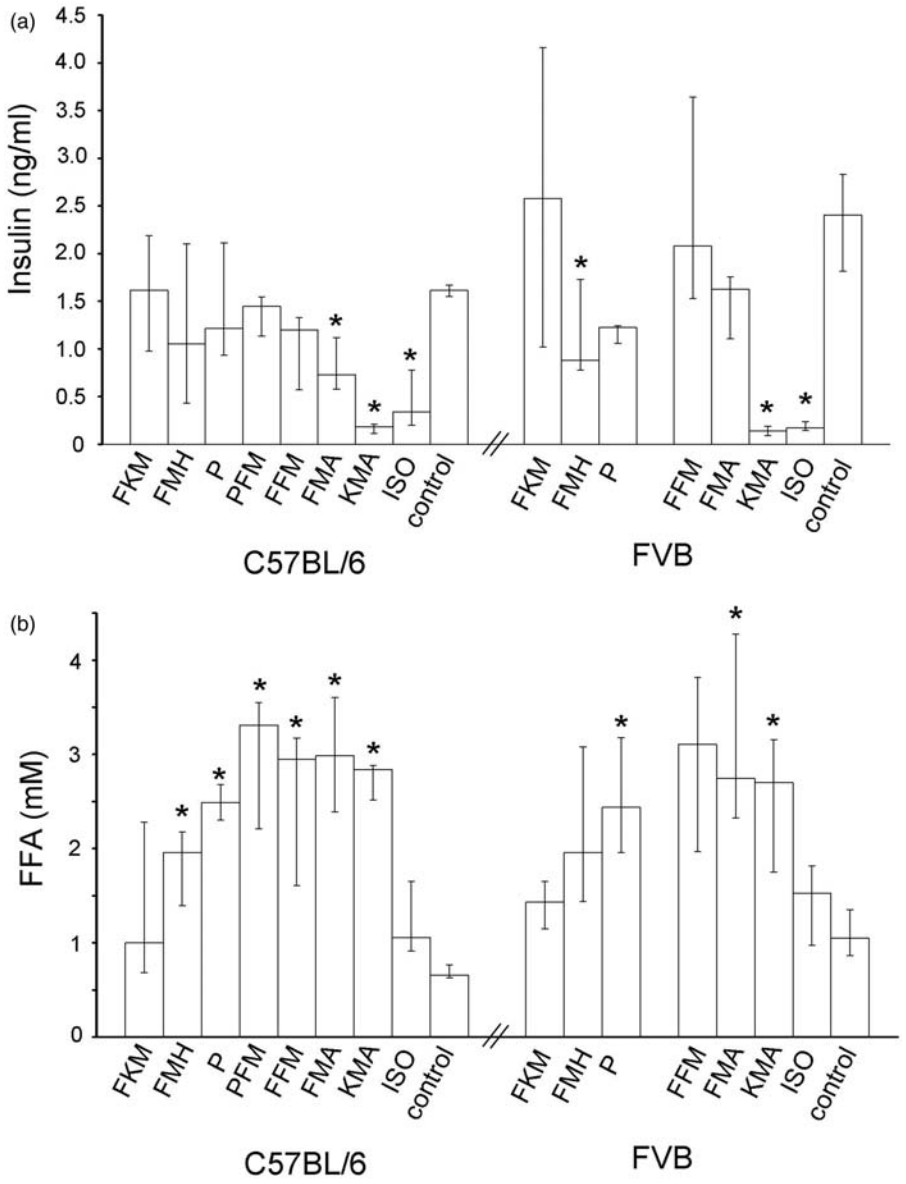

Figure 3 Plasma insulin and free fatty acid (FFA) levels determined at the end of the 60-min monitoring protocol for the two different mice strains, the eight different anesthetic regimens, and the non-anesthetized, control mice. Data are presented as median with the 25% IQR (downward error bar) and the 75% IQR (upward error bar). *$P < 0.05$ vs. control of that specific strain

activation of the baroreceptor-mediated reflex. Atropine can only partly attenuate this bradycardia. Therefore, ISO anesthesia may be preferred above KMA when the focus is on optimal hemodynamics in the mice. Finally, in our hands the combination of fentanyl with ketamine was associated with depressed hemodynamics in mice, excluding the use of this anesthetic regimen for surgical intervention in mice. Fentanyl–ketamine was also associated with low blood oxygenation in the C57BL/6 mice, indicating impairment of gas exchange at the lungs with this type of anesthesia. Future work is necessary to elucidate the mechanism behind this poor gas exchange in the C57BL/6 mice. It should be noted that measurements of arterial BP and HR are insufficient to fully characterize the hemodynamic profile. Measurements of cardiac output[8] and regional (tissue, organ) blood flow and oxygenation will improve the hemodynamic characterization. However, the goal of the present study was first to describe and compare a broad spectrum of many different anesthetic regimens across two different mouse strains for global measurements of hemodynamics, metabolism, and inflammation. Future experiments are now needed that characterize more fully the hemodynamic, metabolic, and inflammatory profiles of only a limited number of the most promising anesthetic regimens that were evaluated in the present work.

Metabolic parameters and anesthetics in C57BL/6 and FVB mice

KMA induced severe persistent hyperglycemia in both mice strains, as was already reported by previous literature.[10,11,28] The hyperglycemia is probably due to

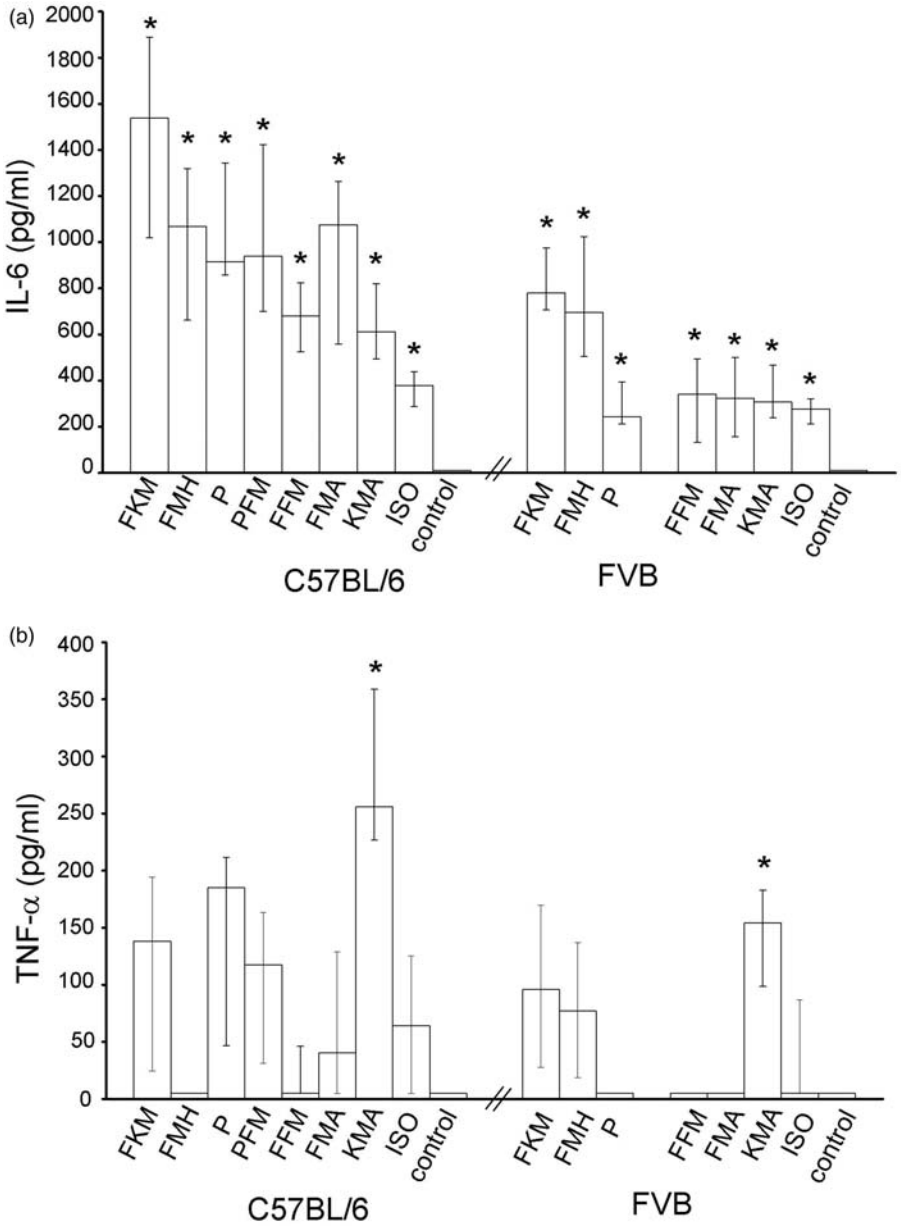

Figure 4 Plasma IL-6 and TNF-α levels determined at the end of the 60-min monitoring protocol for the two different mice strains, the eight different anesthetic regimens, and the non-anesthetized, control mice. Data are presented as median with the 25% IQR (downward error bar) and the 75% IQR (upward error bar). *$P < 0.05$ vs. control of that specific strain

Table 1 Arterial blood parameters for C57BL/6 and FVB mice and the different anesthetic regimens. Values are means (SEM)

	PaO_2 (mmHg)		$PaCO_2$ (mmHg)		pH		HCO_3^- (mmol/L)	
	C57BL/6	**FVB**	**C57BL/6**	**FVB**	**C57BL/6**	**FVB**	**C57BL/6**	**FVB**
FKM	83 (33)	261 (6)	35 (4)	31 (2)	7.47 (0.03)	7.48 (0.03)	24.3 (1.9)	22.7 (0.6)
FMH	222 (25)	240 (11)	34 (3)	28 (3)	7.56 (0.02)	7.54 (0.03)	29.3 (2.2)	23.3 (3.1)
P	193 (15)	210 (19)	34 (2)	34 (3)	7.52 (0.02)	7.52 (0.03)	27.4 (2.3)	26.7 (1.6)
PFM	216 (31)	–	42 (4)	–	7.46 (0.05)	–	29.5 (2.1)	–
FFM	268 (24)	277 (13)	34 (1)	35 (3)	7.60 (0.04)	7.54 (0.02)	33.1 (2.3)	29.9 (3.1)
FMA	264 (14)	262 (10)	34 (2)	37 (2)	7.57 (0.04)	7.49 (0.03)	30.7 (1.3)	28.2 (3.2)
KMA	250 (29)	260 (16)	38 (4)	39 (2)	7.48 (0.03)	7.53 (0.02)	27.7 (1.3)	32.0 (0.4)
ISO	314 (14)	310 (20)	25 (1)	26 (1)	7.59 (0.01)	7.61 (0.03)	23.2 (0.7)	25.3 (1.9)

FKM: fentanyl–ketamine–midazolam; FMH: fentanyl–midazolam–haldol; P: pentobarbital; PFM: propofol–fentanyl–midazolam; FFM: fentanyl–fluanisone–midazolam; FMA: fentanyl–midazolam–acepromazine; KMA: ketamine–medetomidine–atropine; ISO: isoflurane.

medetomidine and not ketamine, since FKM anesthesia did not have an effect on blood glucose. Alpha-2 agonists (medetomidine, xylazine) induce hyperglycemia through the inhibition of insulin release from pancreatic β cells.[10] This is also supported by the present study, demonstrating low insulin levels with KMA anesthesia. An increase in alpha cell glucagon secretion was also reported with alpha-2 agonists.[19,29] The resulting decrease in the insulin/glucagon ratio lowers insulin-dependent glucose uptake and increases hepatic glucose production via gluconeogenesis and glycogenolysis. In addition, hepatic and renal glucose production is regulated directly via alpha-1 and beta-2 adrenergic action.[30–32] The use of alpha-2 agonists when analyzing blood glucose can however lead to artifacts. We observed that ketamine–xylazine did not increase blood glucose in a mouse model for fasting-induced hypoglycemia (i.e. long-chain acyl-CoA dehydrogenase KO),[33] whereas blood glucose levels did increase in fasted WT mice. This interaction between the anesthetic and the genetic perturbation was traced back to profound glycogen depletion in the KO animals. Again, this illustrates that alpha 2-agonists should be avoided in mice.

To our knowledge, the present study is the first to report the direct effects of anesthetics on blood FFA levels. The reported FFA levels are relatively high, which may be caused by ongoing lipolysis within the blood-collecting tube, because lipoprotein lipase (LPL) was not inhibited by an LPL inhibitor. It is likely that LPL was partly released from the inner wall of the vasculature due to the presence of heparin. Small amounts of heparin were needed to allow continuous BP recording without clotting of the sample line. Previous studies demonstrated that omission of an LPL inhibitor can result in 50–130% overestimation of FFA levels.[34,35] Although FMA anesthesia is frequently used for metabolic studies in animals,[36] the current study showed that it is not without significant metabolic perturbations. FMA resulted in an acute, initial increase in blood glucose in both strains that disappeared after 30 min anesthesia. In addition, FMA significantly decreased insulin levels in C57BL/6 mice and was associated with the highest FFA levels measured. It is likely that these metabolic effects are mainly due to acepromazine, since several other combinations using fentanyl and midazolam did not result in similar metabolic alterations. Previous literature has reported acepromazine-induced increases in blood glucose and FFA levels concomitant with low insulin levels in horses,[37] although lower dosages of acepromazine (0.1 mg/kg vs. 6.25 mg/kg in the current study) did not have an effect on blood glucose and insulin sensitivity.[38] Although ISO only transiently increased blood glucose in FVB mice, it inhibited insulin release to a large extent. This is in support of other studies demonstrating an ISO-induced inhibition of pancreatic insulin release[11,12] due to decreased ATP sensitivity of K_{ATP} channels. Although ISO-induced insulin levels were as low as that observed with KMA, blood glucose was much higher with KMA, in support of the literature (see above) that KMA not only inhibited insulin release but also increased hepatic glucose production. ISO also was one of the few anesthetics (together with FKM) that did not significantly increase FFA levels. Why most other anesthetics increased FFA levels is currently unknown, but may be related to the often reported increases in catecholamines with anesthetic agents.[23] This issue will require direct examination in future studies.

Although not monitored in the present study, a recent study demonstrated that blood lactate may also be severely affected by the choice of anesthetics,[28] with halothane and ISO inducing large increases in lactate, whereas pentobarbital resulted in lactate levels below that observed in un-anesthetized animals. It will therefore be of interest to also monitor lactate, besides glucose, insulin, and FFA, and possibly amino acids, in subsequent studies comparing different anesthetics at a similar surgical level of anesthesia without major surgical stress stimuli present.

Inflammatory parameters and anesthetics in C57BL/6 and FVB mice

It is known that anesthetic regimens may differently modulate the inflammatory response to pathologic stressors such as infection, lipopolysaccharides, shock, or ischemia reperfusion. However, in most of these studies the presence of major surgery and/or pathologic stress or varying levels of depth of anesthesia has hindered an unequivocal evaluation of effects of anesthesia on inflammation. The current study now offers a first examination of the effects of various anesthetic regimens on inflammation at a similar (surgical) depth of anesthesia in the absence of any major surgical and/or pathological stressor. We found that inflammation was least activated with ISO, closely followed by the anesthetic regimens FFM and FMA. In contrast, FKM resulted in the largest IL-6 increase in both mice strains, whereas KMA was associated with the largest TNF-α increase in both strains. Attenuation of inflammation by ISO compared with pentobarbital was recently described in a mice model of intestinal ischemia-reperfusion injury, with ISO administered only after ischemia.[39] Interestingly, in that study the attenuation of inflammation was ascribed to an ISO-induced generation of transforming growth factor-β1, a potent anti-inflammatory molecule.[40] It is difficult to distinguish in our *in vivo* study whether the inflammation attenuation by ISO and FFM/FMA anesthesia results from direct cytoprotective effects or modulating effects on inflammatory processes such as neutrophil migration and activation. However, considering the short time course of the experiments (1 h) it seems unlikely that neutrophil processes play an important role in the observed increased cytokine profile, suggesting that it is mostly the cytoprotective action of ISO/FFM/FMA that confer the anti-inflammatory action. This is consistent with the notion that volatile anesthetics and opioids are usually associated with protection against trauma, whereas ketamine and propofol are not.[41]

Limitations

The results obtained in the anesthetized- versus the non-anesthetized groups cannot only be ascribed to anesthesia effects but also to the, albeit relatively minor, surgical stress

of tracheotomy and blood vessel cannulation. Although this surgical stress was similar between all anesthetized groups, and therefore cannot explain differences between anesthetized groups (these are due to the anesthesia, which is the primary goal of this study), it likely contributes to the differences between the anesthetized and non-anesthetized animals (see e.g. Shu et al.[42]).

Previous work in our laboratory demonstrated the presence of mild acidosis (pH 7.2) in anesthetized ventilated mice with fluid support solely provided by saline,[9] which we were able to prevent (pH = 7.4) in a later study through administration of 0.2 mL bicarbonate solution (200 mM).[18] Surprisingly, a similar bicarbonate administration protocol in the present study resulted in mild alkalosis (pH > 7.50) in many of our experimental groups. These values are on averaged 0.1–0.2 pH unit above values obtained in spontaneously breathing, anesthetized mice (pH = 7.42)[18] and non-anesthetized mice with indwelling catheters (pH = 7.39).[43] As blood pH may affect many physiological regulatory systems we cannot exclude the possibility that the results described in the present study are affected by this mild alkalosis. Further studies are therefore warranted to answer these questions. From these results and our previous results,[9] it is at least suggested to use less than a 0.2 mL 200 mM bicarbonate solution or even no bicarbonate solution to obtain physiological blood pH in ventilated, anesthetized mice.

In this study, we specifically choose to keep the ventilation strategy as simple as possible, to facilitate broad application for murine research. It is therefore possible that a more sophisticated ventilation strategy, for example, applying intermittent deep ventilation to prevent the development of atelectasis and using end-tidal continuous CO_2 measurements,[44,45] may further improve the physiology of the ventilated, anesthetized mice. Although the analysis of blood gas at the end of the experiment indicated that for most groups our applied ventilation strategy was indeed sufficient, it is possible that the low oxygenation observed for the FKM anesthesia in the C57BL6 or the low $PaCO_2$ in the ISO-anesthetized mice could have been prevented by applying intermittent deep ventilation and adjusting ventilation according to end-tidal CO_2 measurements and exact quantification of pressure and volume data during ventilation in mice.[45] Finally, a likely contributor of the lower $PaCO_2$ with the dose of ISO used was the persistence of spontaneous breathing in approximately 50% of the animals anesthetized with ISO.

In conclusion, the present work indicates that FFM, ISO, or PFM are the most neutral anesthetic regimens of choice in murine studies necessitating surgical anesthesia. In contrast, KMA or FKM anesthesia is not recommended as anesthetic regimens in the perioperative care of animals.

Author contributions: WJF and CJZ initiated the study, AK performed the experiments, SMH performed the FFA and insulin measurements, CJZ analyzed the data and wrote the manuscript, and SMH, MWH, and WJF edited the manuscript.

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