Effects of anesthetics on systemic hemodynamics in mice

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Running head: Anesthetic effects on cardiac output in mice

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Abstract

The aim of this study was to compare the systemic hemodynamic effects of four commonly used anesthetic regimens in mice chronically instrumented for direct and continuous measurements of cardiac output (CO). Mice (CD-1, Swiss and C57BL6) were instrumented with a transit-time flow probe placed around the ascending aorta for measurement of CO. An arterial catheter was inserted four to five days later into the aorta for blood pressure measurements. After full recovery hemodynamic parameters including, stroke volume (SV), heart rate (HR), CO, mean arterial pressure (MAP) and total peripheral resistance (TPR) were measured in the conscious state. General anesthesia was then induced in these mice using isoflurane (ISO), urethane (UR), pentobarbital (PB), or ketamine/xylazine (K/X). The dose and route of administration of these agents was given as is required for general surgical procedures in these animals. Compared to the values obtained in the conscious resting state, MAP and CO fell during all anesthetic interventions with hemodynamic effects being smallest for ISO (MAP = -24±3%, CO = -5±7%, n=15) and greatest for K/X (MAP = -51±6%, CO = -37±9%, n=8), respectively. The hemodynamic effects of K/X were fully antagonized by administration of the α₂-antagonist atipamezole (n=8). These results indicate that the anesthetic isoflurane has fewer systemic hemodynamic effects in the mouse than the non-volatile anesthetics.

Key Words: cardiac output, systemic hemodynamics, anesthesia, analgesia, buprenorphine.
Introduction

Anesthetic and analgesic regimens are often required for many experimental interventions and phenotypic evaluations in mice. However, the type of anesthetic that is used may have a significant impact on cardiovascular measurements. A wide range of anesthetic regimens has been applied to the mouse and dosing regimens vary between laboratories (23) because of strain differences (33), personal experience (18), or due to institutional regulations. For example, in the Netherlands, animal ethics committees strongly disapprove the use of ether, chloral hydrate, and tri-bromo-ethanol (Avertin). The type of anesthetic also may vary, depending upon the type of experimental intervention required, or the study design. Some anesthetics have cardio- or reno-protective effects, which may be relevant to designing ischemia/reperfusion protocols (17,21,29). Several studies have examined the influence of commonly used anesthetics for short-term non-invasive assessment of cardiac function by echocardiography (4,8,16,32). Anesthetic regimens that can by applied over prolonged periods of time to maintain stable blood pressures recently were described by Zuurbier et al. (33). At present, however, there are no data on the effects of different anesthetic regimens on cardiac output as assessed by direct continuous measurements. Data on systemic blood flows during anesthesia may be of importance for deciding on surgical procedures or designing experimental interventions in mice. Therefore, using transit-time flow probes chronically placed around the ascending aorta in mice, we compared the direct systemic hemodynamic effects of four different anesthetic agents used for surgical or experimental interventions in this species. The studies were conducted at two different institutions (Wake Forest University, USA and the Universiteit Maastricht, NL). In the first set of studies (Wake Forest University) the effects of three anesthetics on CO, HR and SV were examined in CD-
1 mice. In the second study (Universiteit Maastricht), these findings were extended to additional hemodynamic parameters, anesthetic regimens, and mouse strains (Swiss and C57BL6). First, after chronic instrumentation and recovery from surgery of the mouse, arterial blood pressure and cardiac output were recorded under conscious conditions during periods in which the mouse was actively moving in its home cage as well as in non-moving, resting conditions. These conditions were monitored to achieve an indication of the upper and lower limits of these parameters during the conscious state. These hemodynamic values then were compared to those obtained following induction of anesthesia by isoflurane, ketamine/xylazine, pentobarbital Na, or urethane. In addition, the hemodynamic effects of the $\alpha_2$-antagonist atipamezole, an antidote for K/X, were studied.
Materials and Methods

General

Study protocols were performed according to institutional guidelines and were approved by the Animal Care and Use Committee of the Wake Forest University and Universiteit Maastricht. Before and after instrumentation the animals were housed under standard laboratory conditions with water and food ad libitum and environmental temperature set at 22±2 °C.

Study 1

Surgical Protocol: Sixteen CD-1 male mice (20-28g at the time of surgery) were used for these studies. Mice were anesthetized with ketamine/xylazine (50/10 mg/kg i.p.), placed on a warmed surgical platform and intubated transpharyngeally. Anesthesia was maintained with isoflurane (1-1.25%, 96 breaths/m, 12 cm H2O peak inspiratory pressure) using a RSP 1002 respiratory pump (Kent Scientific Corp., Torrington, CT, USA). A 1.5 SL transit time flow probe (Transonic Systems Inc., Ithaca, NY, USA) was implanted on the ascending aorta for determination of CO and HR. Briefly, a thoracotomy was made in the right third intercostal space. The pericardial sack was opened and aorta was isolated. A flow probe was placed onto the aorta and the probe cable was tunneled to the mid-scapulae region. The muscle layers were approximated and a chest tube was inserted to evacuate the thoracic cavity and reinflate the lungs. Skin incisions were closed and the chest tube removed. The flow probe connector was fixed to the back with subcutaneous Dacron mesh and a delrin skin button. Animals were weaned from the respirator and kept in a warm cage for approximately one hour after beginning spontaneous breathing. Animals were allowed to recover at least 10 days before testing response to anesthesia.
Study 2

Surgical Protocol: Ten Swiss mice and ten C57Bl6 of either sex were used. The Swiss mice were purchased from Charles River, the C57Bl6 were derived from an internal breeding line originally derived from Jackson Laboratory mice. The study was intentionally conducted in a heterozygous group of mice to examine whether potential species and gender differences are of relevance. Body weight ranged from 22-44 grams, with Swiss mice being heavier (30±7 grams±SD) than the C57BL6 mice (26±4 grams). Mean body weight was 28±6 grams for both groups. The procedure for the implantation of the transit time flow probe (Transonic, model 1.5 SL, Ithaca, USA) around the ascending aorta has been described in detail previously (12) and is quite similar to the procedure used in study 1. The animals were allowed to recover for 4 days. Then an arterial catheter was implanted using isoflurane anesthesia (1.5-2%). The femoral artery was exposed and a heat-stretched polyethylene cannula (PE-25) was placed with its tip in the abdominal aorta as described in greater detail previously (12). The animals were allowed to recover for an additional 2 days before the following experimental protocols were started. In 16 out of 20 mice the flow probe and catheter were successfully implanted in 16 of 20 mice. The survival rate was slightly higher in Swiss mice (9 out of 10) than in C57BL6 mice (7 out of 10), most likely because the feasibility of this type of surgery is greater when the animal is larger.

Experimental protocols

Study 1

Mice were attached to the flow meter with special lightweight cables and returned to the home cage for at least 45 minutes before measurements were taken. Recordings
were taken when the animals were resting (non-moving) for at least 1 minute for baseline determination. After recording during resting conditions, animals were administered one of four anesthetic regimens. Pedal withdrawal tests were performed when the first signs of deep anesthesia level were observed. The effect of the anesthetic on CO, heart rate (HR) and stroke volume (SV) was determined when the animal first showed a lack of pedal withdrawal to a pinch. At least 7 days were allowed between testing different anesthetics.

1. **Isoflurane**: Mice (n=10, BW=35±1 grams) were placed into a Plexiglas induction chamber (24x11x9cm) which had been filled with either 1% (n=10) or 2% (n=5) isoflurane in O$_2$. Pedal reflex was tested when the mice first showed no eyeblink response to tapping the chamber (approximately 2 minutes).

2. **Ketamine/Xylazine**: Mice (n=9, BW=35±1 grams) were injected i.p. with a mixture of ketamine and xylazine at 50 and 10 mg/kg respectively. Two of seven mice required an additional anesthetic (15 and 3 mg/kg) before showing loss of pedal withdrawal (approximately 6 minutes after the second injection). The mice were placed onto a warmed surgical station and body temperature was monitored by rectal temperature probe (CyQ 111, Cybersense Inc., Lexington, KY, USA) and maintained 36-38° C. In seven mice (BW=35±1 grams) we tested if additional administration of isoflurane (1% in O$_2$) on top of K/X would further reduce CI. After 30 minutes of the combined anesthetic regimen hemodynamic parameters were taken again.

3. **Pentobarbital**: Mice (n=8, BW=35±3 grams) were injected with sodium pentobarbital (50 mg/kg, i.p.). One of the eight mice required an additional injection (15 mg/kg) before showing loss of reflex withdrawal (approximately 6 minutes after the second injection).
Data-acquisition and statistics

Ascending aortic blood flow was sampled at 1 kHz using a Windaq Acquisition system (version 2.18, DATAQ Instruments, Akron, OH, USA). CO was determined by averaging ascending aorta flow signal and the values were normalized as cardiac index (CI) by dividing by body weight. HR was calculated by counting beats during the sampling period. Stroke index (SI) was calculated dividing CI by HR for the sampling period. Differences between pre- and post-anesthetic values were compared using a paired t test. Differences among drug treatments were compared with one way ANOVA with Bonferroni post hoc comparison with a significance level of \( P < 0.05 \) used for all comparisons.

Study 2

The physiological range of the systemic hemodynamics in the mouse was determined by recording under aroused (moving) as well as resting (non-moving) conditions. Maximal values were recorded immediately after the mouse was connected to the measuring equipment when the animal usually is intensely exploring the cage or actively grooming. The levels of cardiac output that were achieved in this way are comparable, or even higher, to those obtained with dobutamine infusion or volume loading (13). The data were recorded for 2-5 minutes and maximal values were extracted from these recordings. The mouse then was allowed to settle down, generally after about 30 minutes, the animal assumes a resting, non-moving position in the corner of its cage, where it usually shelters under nest material. Cardiac output usually is lowest during these resting conditions. Data were recorded over a 10 – 15 minute resting period and both average and minimal values were determined for comparison with the data obtained during the anesthetic regimens.
Measurements in anesthetized conditions.

Recordings were made during 4 different anesthetic regimens. Because cardiovascular parameters are vulnerable to temperature (31), the anesthetized mice were placed on a warmed table and body temperature was maintained at 37 °C using a rectal thermistor probe (Hugo Sachs Temp-Regler, March-Hugstetten, Germany) coupled to an infrared lamp. During all studies (unless specified otherwise) the anesthesia plane was kept on a surgical level by repeatedly (every 15 minutes) testing whether the mouse demonstrated a pedal withdrawal reflex. Data acquisition was initiated 20 minutes after the induction of anesthesia, during a period when hemodynamics were stabilized. Details for each anesthetic regimen are given below.

1. Isoflurane

Anesthesia was induced by placing the mice in a small cylinder filled with air saturated with isoflurane. Anesthesia was maintained through voluntary breathing of a mixture of 1.5-2% isoflurane in normal air using an Univentor type 994650-AU-400 vaporizer (Technical Scientific Equipment, Bad Homburg, Germany). After stabilization, hemodynamic data were acquired for a period of 15 minutes. This protocol was completed in 8 Swiss and 7 C57Bl6 mice (7 males, 8 females, average BW=28±2 grams). Ketamine / Xylazine

Anesthesia was induced by injection of ketamine (100 mg/kg) i.m and of xylazine (5 mg/kg) s.c. When anesthetized the hemodynamics were recorded for a period of 10-15 minutes after stabilization of blood pressure. Then, atipamezole (2.5 mg/kg) was given i.p. to antagonize the alpha-adrenergic blocking effects of xylazine and
recordings were made for an additional 15 minutes. This protocol was completed in 5 Swiss and 3 C57Bl6 mice (5 males, 3 females, average BW=30±3 grams).

2. Pentobarbital Na

Pentobarbital Na was given initially at a dose of 60 mg/kg i.p. to induce anesthesia. This amount is not always enough to suppress the pedal withdrawal reflex and, if necessary, additional amounts of pentobarbital Na, up to 90 mg/kg total dose, were administered. Data were acquired for a 10-15 minute period after stabilization of hemodynamics. This protocol was completed in 5 Swiss and 2 C57Bl6 mice (4 males, 3 females, average BW=29±2 grams).

3. Urethane

Urethane is not a popular anesthetic for chronic mouse preparations because it is carcinogenic (28) and not suitable for survival surgery. The recovery time is very long making it a useful drug for conducting terminal experiments of long duration. Urethane is usually administered i.p in a dose range between 0.8-1.3 g/kg, either alone or combined with alpha-chloralose. With the recommended dose range we found that mice were not anesthetized at all. Following an initial dose of 1.25 g/kg, supplemental injections (2 times 0.5 mg/kg) were needed to achieve surgical depth of anesthesia. Thus the total dosage we applied was 2.5 g/kg. This protocol was completed in 4 Swiss and 3 C57Bl6 mice (4 males, 3 females, average BW=30±2 grams).

The order of the first 3 anesthetic regimens was randomized, allowing 48 h between recordings. Because of its carcinogenic properties and hepatotoxic effects, urethane was always applied in the terminal study. If the arterial catheter had become non-
functional, a new arterial line was inserted into the other femoral artery (in 3 out of 7 animals).

Data-acquisition and calculations

During the experiments the flow probe was connected to the flowmeter (Transonic Systems, type T206) and the arterial line was connected a low-volume pressure transducer. The pressure and flow signals were sampled at 2 kHz (12 bits) using hemodynamic data acquisition software (HDAS) developed by the instrument services of the Universiteit Maastricht. Using special modules included in this software package, mean arterial pressure (MAP), stroke volume (SV), heart rate (HR), cardiac output (CO) and total peripheral resistance (TPR) were calculated as explained in detail before (12). Data were stored on hard disk every 2-5 seconds for later analysis.

Calculations and statistics

Because of the wide range in body size SV, CO and TPR were normalized to body size (and expressed per gram bodyweight) and defined as its index (i.e. SI, CI, and TPRI, respectively). For comparison of the anesthetic effects, both absolute and relative differences were calculated between the average values obtained during the conscious resting state and the average values during the different anesthetized states. For each anesthetic agent, these differences between the conscious and anesthetized state were compared using paired t-tests. Because it was impossible to complete all four protocols in the same mouse (due to catheter failure), the differences between drugs mutually were compared using analysis of variance, followed by a Bonferroni post-hoc t-test. Significance was accepted at $P<0.05$. 
Results

Study 1
Baseline and post-anesthetic hemodynamic values of this study are presented in Table 1. All of the anesthetic treatments produced a significant decreased in both CI and HR. The suppression of CI by anesthetic administration ranged from 24±8% with 1% isoflurane to 68±2% with combined ketamine/xylazine along with 1% isoflurane. The decrease in CI produced by 1% isoflurane was significant less than that produced by all of the other anesthetic regimens. There were no significant differences among the other treatments in the suppression of CI. In all cases, the decrease in CI appeared to be primarily due to a depression in HR. Only with pentobarbital was the depression of CI accompanied by a decrease in SI (t=-2.7, p<0.03).

Study 2
Figure 1 summarizes the average hemodynamic values as recorded in conscious conditions in this study. Data were obtained in 16 mice. Three C57Bl6 and 1 Swiss mouse died during/after the implantation of the flowprobe. There were no significant strain- or sex-dependent differences and therefore all data were pooled. The maximal and minimal values in Figure 1 indicate the physiological range of the various parameters as compared to the average value obtained during a 10-15 minute resting, non-moving period. During the aroused state, CI is increased (when compared to the resting state) primarily via stroke volume and to lesser extent HR. The increase in CI was accompanied by an increase in blood pressure although peripheral resistance fell, most likely because of decreases in muscle vascular resistance.
An example of the hemodynamic effects obtained in one mouse during the conscious state and also after administration of isoflurane, ketamine/xylazine followed by atipamezole is given is Figure 2. Note that the changes in blood pressure were primarily due to changes in CO, which, in turn, was predominantly effected by changes in HR and not stroke volume. A more detailed description of the effect of atipamezole is given in the legend to the figure. The average effects of the various anesthetic regimens on central hemodynamics are presented in Figure 3. The data are given in percentages to allow a comparison in changes between the various parameters. Compared to the average resting values of MAP (105±3 mmHg) and CI (0.46±0.02 ml/min.g) obtained in the conscious resting state, anesthesia with isoflurane (1-2 %) reduced MAP by about 20% to 79±3 mmHg and CI by about 5% to 0.43±0.03 ml/min.g. In contrast, the mixture of ketamine/xylazine induced a more pronounced fall in blood pressure (down to 46±5 mmHg) and CI (to 0.25±0.04 ml/min.g) which was in large part due to a decrease in HR. When the antidote to the $\alpha_2$ agonist xylazine (atipamezole) was administered, all parameters returned to near control values within minutes (see Fig 1). Following pentobarbital Na anesthesia, the reduction in blood pressure (down to 60±8 mmHg) and CI (to 0.39±0.03 ml/min.g) was less severe than observed after ketamine/xylazine. Also, unlike the latter regimen, the reduction in CI was not so much due to a fall of HR but rather due to a decrease of SI. Lastly, following the administration of urethane, the decrease in blood pressure (to 82±8 mmHg) and decrease in CI (to 0.43±0.04 ml/min.g) was relatively modest and not different from values obtained during isoflurane. TPRI was unchanged from control values with all anesthetics.
Discussion

This study demonstrates that different anesthetics have significantly different effects on CI as measured by transit-time blood flowmetry of the ascending aorta in mice. Using this technique the present data extend previous reports regarding the differential hemodynamic effects of anesthetics on blood pressure and HR (14,33) or on various echocardiographic measures (4,8,16,32). The advantage of the present animal preparation is that the systemic hemodynamic effects of anesthetics were obtained in the absence of acute surgery. In addition, the present design allowed a comparison of anesthetic effects not only between different agents, but also to minimal and maximal values obtained under conscious conditions.

We chose to combine the data of two different laboratories to be able to compare the anesthetic effects in a heterozygous group of mice (CD-1, Swiss and C57BL6). In general, as is shown in detail below, the effects of the different anesthetic drugs were qualitatively very comparable between the two laboratories. However, in absolute terms the reduction in CI and HR was generally larger under the experimental conditions of study 1 than of study 2. We therefore decided to present data of both studies separately. Obviously, the most straightforward explanation for the difference in magnitude of effect between labs is the strain difference. CD-1 mice were used in study 1 versus a mixture of Swiss and C67BL6 in study 2. However, this does not corroborate with the findings of Zuurbier et al (33). They reported that at dosages producing a surgical depth of anesthesia, the hemodynamic effects of different anesthetics did not differ between four mouse strains (Swiss, CD-1, BalbC, and C57BL6) of either sex. Moreover, in study 2, we also found no differences between Swiss and C57Bl6 mice. The second explanation is that baseline values of CI were different between the studies. In study 1 CI under non-moving conditions was about
0.55 ml/g.min whereas in study 2 baseline CI was about 0.46 ml/g.min. This may explain part of the difference between studies and illustrates the difficulty in defining baseline conditions of hemodynamic parameters which are very dependent on the animals behavior. As illustrated in Figure 1 we determined the physiological range of the systemic hemodynamics and found that CI may vary up to a factor 2 in conscious mice. In fact the variation in CI may even be greater when continuous measurements over 24-h would have been performed and heart rates reach nadirs of about 400 beats/min (data not shown). However, in the present study, resting baseline heart rates were relatively high (range 600-750) when compared to telemetric studies in mice (30,31). This may be explained by the light arousal due to the tethering of the animals. In addition, separate housing of mice at environmental temperatures below thermoneutrality (28-30 °C) is associated with increased heart rates (31). We have not observed that the flow-probe itself compromises aortic blood flow.

From Figure 1 it follows that, in contrast to other species, that the increase in CI is mainly due to an increase in SI and not HR. This is not surprising, since in these particular conditions with relatively high HR, a further increase in CI can be gained only by increasing SV. We have discussed recently that cardiac reserve is probably limited in the mouse and that therefore small perturbations in venous return directly influence stroke volume and CI (13). This seems to occur in a frequency-dependent manner. We found, especially for frequencies > 0.1 Hz, that the coherence between SV and CI was higher than the coherence between HR and CI (12). Therefore, changes in CI that occur slowly over time (<0.1 Hz), such as those caused by the anesthetic regimens were mainly determined by HR, as will be discussed below.
Compared to the resting conscious state, isoflurane-associated reductions in CI were relatively minor. In the two studies, a light dose of isoflurane reduced CI between 5 and 25% below conscious values. In both studies, the reduction in CI with a low dose of isoflurane was the lowest of all the anesthetics. The reductions in CI following urethane were also modest (-12%). In contrast, CI fell considerably following both ketamine/xylazine (-37 to -63 %) and pentobarbital Na anesthesia (-24 to -40 %). For all anesthetic agents it was found that the decrease in CI was mainly due to a decrease in HR. Stroke volume did not change or even increased a little, probably due to prolonged filling times. Similar observations, i.e. a decrease in HR but no change or increase in SI, have been made when anesthetic effects on left ventricular function were studied by echocardiography (4,16). Only during pentobarbital Na was this pattern different and both HR and SI were decreased. This latter effect has also been observed in rats (27) and may be related to a direct negative inotropic action on myocardial tissue (21). Alternatively, it may also result from an inhibition of cardiac sympathetic tone. The study was not designed to elucidate these mechanisms, but the well-known parasympatholytic effect as it occurs in other species (19) is unlikely to be of importance in mice.

The present data confirm that in mice, isoflurane anesthesia preserves cardiac function better than other anesthetic regimens (18,24,25). Comparable observations have been obtained in rats (5). This may also explain why the outcome of various surgical interventions is generally more successful under isoflurane anesthesia than when non-volatile anesthetics are used. Obviously control of depth of anesthesia is much easier with inhalation anesthetics than with injection anesthetics. However, the relatively high systemic blood flow during isoflurane anesthesia preserves the peripheral organ perfusion during surgical interventions. In addition isoflurane induces an increase in
regional cerebral cortical blood flow in mice (15), which may add to the fast recovery.

Lastly, isoflurane and related volatile anesthetics are known to exert a protective preconditioning-like effect on myocardial tissue (10,20).

These actions make isoflurane the anesthetic of preference among those tested because 1) it easy to administer and to titrate, 2) it has a rapid onset and recovery, 3) it produces reproducibly adequate anesthetic depth, and 4) it causes minimal cardiac depression and maintains blood pressure very well (25). In some circumstances, a reduced cardiac function may be beneficial for the outcome of surgery. In our experience the success rate of an ischemia/reperfusion protocol, in which the coronary artery of a mouse is temporarily ligated for a 30 minutes period, is greater during pentobarbital (7) than during isoflurane anesthesia. The reduced workload of the heart during this type of anesthesia may contribute to recovery from surgery and prevent potential lethal arrhythmias (3,6). Furthermore it has been reported that pentobarbital may facilitate spontaneous recovery from hypoxic apnoea (11).

During anesthesia with isoflurane, mean arterial pressure was reduced to about 80 mmHg and HR to 600, values corresponding to the steady state values observed in Swiss and C57BL6 mice when kept anesthetized for 3 hours (33). We took great care to keep the animal at a surgical plane of anesthesia and regularly checked the pedal withdrawal reflex, which is still one of the most reliable parameters for testing analgesia during standard laboratory conditions in mice (2). For this purpose we needed isoflurane concentrations of about 2%, a concentration that is regularly reported in the literature. In contrast, in study 2 doses of pentobarbital (up to 90 mg/kg) and urethane (2.5 g/kg) were needed that are higher than generally advised in experimental animal text books or websites. Obviously, circumstances vary between
labs and even within one laboratory doses for injection anesthetics should be adapted to the particulars of each experimental design (2). However, the reasons for the incongruity in anesthetic doses between labs remain unclear. Paradoxically, the simplest explanation for the incongruity in anesthetic dosing could be that although many papers mention that supplemental (to the standard dose) amounts of anesthetics were needed, these latter amounts are rarely specified.

The ketamine/xylazine mix had potent cardiodepressive effects. The present data show that these can be quickly reversed by i.p. injection of the alpha-2 adrenoceptor antagonist atipamezole. The peripheral alpha-2 adrenoceptor blockade may explain why blood pressure declined for a very short period even to lower values (see Fig 2), but then the central effect clearly overruled the peripheral action. The data suggest that the NMDA-antagonism due to ketamine is not responsible for the cardiodepressive effects, rather centrally-mediated inhibition of sympathetic tone by the alpha-2 agonist xylazine accounts for the severe cardiodepressive effect observed. The parasympathetic nervous system is probably not involved in this effect. Zuurbier et al. (33) found that the addition of atropine to anesthesia induced by ketamine/medetomidine, another alpha-adrenergic agonist, did not prevent the pronounced fall in HR. The rapid (within minutes) effect of atipamezole is helpful in rescuing mice in critical stages during surgery, especially when heart rates fall under 300 beats/min and cardiac contractility is additionally threatened (8).

During urethane anesthesia arterial blood pressure and CO were reasonably well maintained and not much lower than during isoflurane. In contrast, Jong et al. (14) observed that hemodynamic conditions were relatively unstable during the
combination of urethane/alpha-chloralose in mice. It may be that alpha-chloralose, often added to urethane to preserve autonomic reflexes, is not a stabilizing agent in mice as it is in rats. One of the side-effects of intraperitoneally administered urethane is that plasma osmolality increases and induces vasopressin release (26). The extent to which this contributes to the maintenance of cardiovascular homeostasis during urethane anesthesia in the mouse is not known.

The type of analgesic agent that should be combined with an anesthetic as well as the timing, route of administration, and dosage that should be given in a mouse after surgery is a question that is seldom addressed in the literature (22). The long-acting morphine mimetic buprenorphine is often advocated for this purpose. However, the dose that is recommended for alleviating pain in this species varies considerably in textbooks (0.01-2.5 mg/kg, (1,9)) and institutional websites. In our hands co-administration of buprenorphine to a light (0.5-1%) isoflurane anesthesia dose-dependently depressed blood pressure and cardiac index further in mice. Following a relatively high dose (2 mg/kg) of buprenorphine, MAP fell within 15 minutes by 40 % and CI by 27% (data not shown). The co-administration of 2 mg/kg buprenorphine was associated with increased mortality rates (4 out of 8 mice) despite the fact that animals were withdrawn from the isoflurane exposure. We therefore suggest that high doses of buprenorphine should not be combined with anesthetics in mice. This finding seems not to stand alone. In his practical guide on evaluating physiological functions in mice Lorenz (18) wrote that they tried to combine isoflurane with buprenorphine to help stabilize the anesthetic plane, “but many of the benefits were diminished by this approach”.

19
In summary, compared to resting conscious conditions in mice, CI was only slightly depressed during anesthesia with the volatile anesthetic isoflurane. Severe reductions of CI were observed during anesthesia with the ketamine/xylazine mixture, but these could be reversed by the alpha-2 adrenergic blocker atipamezole.

Acknowledgements:

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Table 1. Effects of anesthesia on Cardiac Index (CI, ml/m/g body wt.) and Heart Rate (HR, bpm) in male CD-1 mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>1% Isoflurane (n=10)</th>
<th>2% Isoflurane (n=5)</th>
<th>Ketamine/Xylazine (n=9)</th>
<th>Combined (n=7)</th>
<th>Pentobarbital (n=8)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>CI</td>
<td>HR</td>
<td>CI</td>
<td>HR</td>
<td>CI</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.53±0.02</td>
<td>726±21</td>
<td>0.58±0.02</td>
<td>711±44</td>
<td>0.51±0.04</td>
</tr>
<tr>
<td>Anesthesia</td>
<td>0.40±0.04*</td>
<td>544±31*</td>
<td>0.33±0.04*</td>
<td>438±45*</td>
<td>0.20±0.04*</td>
</tr>
<tr>
<td>% change</td>
<td>-24±8</td>
<td>-24±5</td>
<td>-42±10</td>
<td>-38±6</td>
<td>-63±6</td>
</tr>
</tbody>
</table>

Hemodynamic values were obtained under baseline (non-moving, resting conditions) and after induction of anesthesia (showing no response to paw pinch). All values are expressed as mean ± standard error of the mean and differences were analyzed as a paired test. * indicates a significant difference between baseline and anesthesia. Combined anesthesia = Ketamine/Xylazine in combination with isoflurane.
**Figure 1:**

Average systemic hemodynamics in the conscious state in mice (n=15). Maximal ■ values were obtained during a period of high locomotor activity, minimal □ values during a period in which the mouse was in a resting (non-moving) position and mean values ■ in this last condition are presented. The 95% confidence intervals are indicated by the shaded areas. Values for cardiac output, stroke volume and total peripheral resistance are expressed relative to the body weight. Abbreviations: MAP: mean arterial pressure, HR: heart rate, CI: cardiac index, SI: stroke index, TPRI: total peripheral resistance index.
**Figure 2:**

Representative tracing of hemodynamics in a Swiss mouse during conscious conditions (CON), during isoflurane (ISO) and during ketamine/xylazine (K/X) followed by i.p. injection of atipamezole (ATI, indicated by arrow). Values are plotted on a beat-to-beat basis in this graph. The different experiments were performed on different days, but for comparative reasons selected parts of each tracing were compiled on a continuous time axis. The time-dependent variation in the data in each condition is due to spontaneously occurring fluctuations (mainly of respiratory origin). Abbreviations are: CO; cardiac output, HR: heart rate, SV: stroke volume, MAP: mean arterial pressure, TPR: total peripheral resistance. Note that following the injection of the alpha-2 adrenoceptor antagonist atipamezole, there is a transient increase in TPR and MAP followed by a quick recovery of the hemodynamic parameters.
Figure 3:

Average systemic hemodynamic changes induced by anesthetic regimens in the mice in the absence of surgery. Data are means ± SE and expressed relative to the average resting values obtained in conscious conditions. Anesthetic regimens:

- black: isoflurane 2% (n=15),
- purple: ketamine/xylazine (n=8),
- grey: K/X + atipamezole (n=8),
- coarse black: pentobarbital (n=7),
- white: urethane (n=7).

* P<0.05 different between from isoflurane. $ P<0.05$ different between K/X and K/X+ATI. For abbreviations see the legend of figure 1.