Effects of anesthetics on systemic hemodynamics in mice

Ben J. A. Janssen, Tijl De Celle, Jacques J. M. Debets, Agnieszka E. Brouns, Michael F. Callahan, and Thomas L. Smith

1Department of Pharmacology and Toxicology, Cardiovascular Research Institute Maastricht, Universiteit Maastricht, Maastricht 6200 MD, The Netherlands; and 2Department of Orthopaedic Surgery, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157

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Janssen, Ben J. A.; Tijl De Celle, Jacques J. M. Debets, Agnieszka E. Brouns, Michael F. Callahan, and Thomas L. Smith. Effects of anesthetics on systemic hemodynamics in mice. Am J Physiol Heart Circ Physiol 287: H1618–H1624, 2004.—The aim of this study was to compare the systemic hemodynamic effects of four commonly used anesthetic regimens in mice that were chronically instrumented for direct and continuous measurements of cardiac output (CO). Mice (CD-1, Swiss, and C57BL6 strains) were instrumented with a transit-time flow probe placed around the ascending aorta for CO measurement. An arterial catheter was inserted into the aorta 4 or 5 days later for blood pressure measurements. After full recovery, hemodynamic parameters including stroke volume, heart rate, CO, mean arterial pressure (MAP), and total peripheral resistance were measured with animals in the conscious state. General anesthesia was then induced in these mice using isoflurane (Iso), urethane, pentobarbital sodium, or ketamine-xylazine (K-X). The doses and routes of administration of these agents were given as required for general surgical procedures in these animals. Compared with the values obtained for animals in the conscious resting state, MAP and CO decreased during all anesthetic interventions, and hemodynamic effects were smallest for Iso (MAP, −24 ± 3%; CO, −5 ± 7%; n = 15 mice) and greatest for K-X (MAP, −51 ± 6%; CO, −37 ± 9%; n = 8 mice), respectively. The hemodynamic effects of K-X were fully antagonized by administration of the α2-receptor antagonist atipamezole (α2-receptor antagonist atipamezole (n = 8 mice). These results indicate that the anesthetic Iso has fewer systemic hemodynamic effects in mice than the nonvolatile anesthetics.

anesthesia; analgesia; buprenorphine; isoflurane; urethane; ketamine; xylazine; pentobarbital

ANESTHETIC AND ANALGESIC REGIMENS are often required for 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 mice, and dosing regimens vary between laboratories (23) because of strain differences (33), personal experiences (18), and institutional regulations. For example, in the Netherlands, animal ethics committees strongly disapprove of the use of ether, chloralhydrate, and tribromoethanol (Avertin). The type of anesthetic also may vary depending on the type of experimental intervention required or the study design. Some anesthetics have cardio- or renoprotective effects, which may be relevant to designing ischemia-reperfusion protocols (17, 21, 29). Sev-
al studies have examined the influence of commonly used anesthetics for short-term, noninvasive assessment of cardiac function via echocardiography (4, 8, 16, 32). Anesthetic regimens that can be applied over prolonged time periods to maintain stable blood pressure (BP) values 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 (CO) as assessed by direct continuous measurements. Data on systemic blood flow during anesthesia may be important when deciding on surgical procedures or designing experimental interventions in mice. Therefore, by 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 in the USA and Universiteit Maastricht in The Netherlands. In the first set of studies (at Wake Forest University), the effects of three anesthetics on CO, heart rate (HR), and stroke volume (SV) were examined in CD-1 mice. In the second study (at Universiteit Maastricht), these findings were extended to additional hemodynamic parameters, anesthetic regimens, and mouse strains (Swiss and C57BL6). First, after the mice received chronic instrumentation and recovered from surgery, arterial BP and CO values were recorded with the animals under conscious conditions during periods in which they were actively moving in their home cages as well as during nonmoving, resting conditions. These conditions were monitored to achieve an indication of the upper and lower limits of these parameters during the animal’s conscious state. These hemodynamic values were then compared with those obtained after induction of anesthesia by isoflurane (Iso), ketamine-xylazine (K-X), pentobarbital sodium, or urethane. In addition, the hemodynamic effects of the α2-receptor antagonist atipamezole, which is an antidote for K-X, were studied.

MATERIALS AND METHODS

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

Surgical Protocols

Study 1. Sixteen CD-1 male mice (body weight at time of surgery, 20–28 g) were used for these studies. Mice were anesthetized with

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K-X (50 and 10 mg/kg ip, respectively), placed on a warmed surgical platform, and intubated transpharyngeally. Anesthesia was maintained with 1–1.25% Iso (96 breaths/min, 12 cmH2O peak inspiratory pressure) using an RSP 1002 respiratory pump (Kent Scientific; Torrington, CT). A 1.5 SL transit-time flow probe (Transonic Systems; Ithaca, NY) 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 sac was opened, and the aorta was isolated. A flow probe was placed onto the aorta, and the probe cable was tunneled to the midscapulare region. The muscle layers were approximated, and a chest tube was inserted to evacuate the thoracic cavity and reinfurate the lungs. Skin incisions were closed, and the chest tube was removed. The flow-probe connector was fixed to the animal’s back with subcutaneous Dacron mesh and a Delrin skin button. Animals were weaned from the respirator and kept in a warm cage for ~1 h after they began spontaneous breathing. Animals were allowed to recover for at least 10 days before their responses to anesthesia were tested.

**Study 2.** Swiss and C57BL6 mice (n = 10 animals of each) either sex were used. The Swiss mice were purchased from Charles River, and the C57BL6 mice 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 relevant. Body weight ranged from 22 to 44 g with Swiss mice being heavier (30 ± 7 g) than C57BL6 mice (26 ± 4 g). Mean body weight was 28 ± 6 g for both groups. The procedure for implantation of the transit-time flow probe (model 1.5 SL, Transonic Systems) around the ascending aorta has been described in detail previously (12) and was quite similar to the procedure used in **study 1**. The animals were allowed to recover for 4 days. An arterial catheter was then implanted using Iso anesthesia (1.5–2%). The femoral artery was exposed, and a heat-stretched polyethylene (PE)-25 cannula was placed with its tip in the abdominal aorta as described previously in greater detail (12). The animals were allowed to recover for an additional 2 days before the subsequent experimental protocols were started. The flow probe and catheter were successfully implanted in 16 of 20 mice. The survival rate was slightly higher in Swiss mice (9 of 10) than in C57BL6 mice (7 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 flowmeter with special light-weight cables and were returned to their home cages for at least 45 min before measurements were taken. Recordings were made when the animals were resting (nonmoving measurements) for at least 1 min for baseline determination. After recordings were made during resting conditions, animals were administered one of four anesthetic regimens. Pedal-withdrawal tests were performed when the first signs of a deep level of anesthesia were observed. The effects of the anesthetic on CO, HR, and SV were determined when the animal first showed a lack of pedal withdrawal to a pinch. At least 7 days were allowed between testing of different anesthetics.

**ISOFLURANE.** Mice (n = 10; body weight, 35 ± 1 g) were placed in a Plexiglas induction chamber (24 × 11 × 9 cm) that was filled with either 1% (n = 10 mice) or 2% (n = 5 mice) Iso in O2. Pedal reflex was tested when the mice first showed no eye-blink response when we tapped the chamber (~2 min).

**KETAMINE-XYLAZINE.** Mice (n = 9; body weight, 35 ± 1 g) were injected with a K-X mixture at 50 and 10 mg/kg ip, respectively. Two of seven mice required an additional anesthetic (15 and 3 mg/kg) before they showed loss of pedal withdrawal (~6 min after the second injection). The mice were placed into a warmed surgery station. Body temperature was monitored using a rectal temperature probe (CyQ 111, Cybersense; Lexington, KY) and was maintained at 36–38°C. In seven mice (body weight, 35 ± 1 g), we tested whether administration of Iso (1% in O2) in addition to K-X would further reduce the cardiac index (CI). After 30 min of the combined anesthetic regimen, hemodynamic parameters were again recorded.

**PENTOBARBITAL SODIUM.** Mice (n = 8, body weight, 35 ± 3 g) were injected with pentobarbital sodium (50 mg/kg ip). One of the eight mice required an additional injection (15 mg/kg) before it showed loss of reflex withdrawal (~6 min 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). CO was determined by averaging the ascending aorta flow signals, and the values were normalized as the CI by dividing that value by body weight. HR was calculated by counting beats during the sampling period. Stroke index (SI) was calculated by dividing CI by HR for the sampling period. Differences between pre- and postanesthetic values were compared using a paired t-test. Differences among drug treatments were compared using one-way ANOVA with Bonferroni post hoc comparison; a significance level of P < 0.05 was used for all comparisons.

**Study 2.** The physiological range of the systemic hemodynamics in mice was determined by recording measurements while animals were in aroused (moving) as well as resting (nonmoving) conditions. Maximal values were recorded immediately after the mouse was connected to the measuring equipment, which is when the animal usually is intensely exploring the cage or actively grooming. The CO levels that were achieved in this way are comparable to or even higher than those obtained with dobutamine infusion or volume loading (13). The data were recorded for 2–5 min, and maximal values were extracted from these recordings. The mouse then was allowed to settle down. Generally, after ~30 min, the animal assumed a resting, nonmoving position in the corner of its cage, where it usually sheltered under nest material. CO was usually lowest during these resting conditions. Data were recorded over a 10- to 15-min resting period, and both average and minimal values were determined for comparison with the data obtained during the anesthetic regimens.

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

**ISOFLURANE.** Anesthesia was induced by placing the mouse in a small cylinder filled with Iso-saturated air. Anesthesia was maintained through voluntary breathing of a mixture of 1.5–2% Iso in normal air using a Univentor type 994650-AU-400 vaporizer (Technical Scientific Equipment; Bad Homburg, Germany). After stabilization, hemodynamic data were acquired for a period of 15 min. This protocol was completed in 8 Swiss and 7 C57BL6 mice (7 males and 8 females; average body weight, 28 ± 2 g).

**KETAMINE-XYLAZINE.** Anesthesia was induced by injection of ketamine (100 mg/kg im) and xylazine (5 mg/kg sc). Once the mouse was anesthetized, hemodynamics were recorded for a period of 10–15 min after its BP had stabilized. Atipamezole (2.5 mg/kg ip) was then given to antagonize the α-adrenergic receptor-blocking effects of xylazine, and recordings were made for an additional 15 min. This protocol was completed in 5 Swiss and 3 C57BL6 mice (5 males and 3 females; average body weight, 30 ± 3 g).

**PENTOBARBITAL SODIUM.** Pentobarbital sodium was given initially at a dose of 60 mg/kg ip to induce anesthesia. This amount is not always enough to suppress the pedal withdrawal reflex, and, if necessary, additional amounts of pentobarbital sodium up to a total
dose of 90 mg/kg were administered. Data were acquired for a 10- to 15-min period after hemodynamics measurements were stabilized. This protocol was completed in 5 Swiss and 2 C57BL6 mice (4 males and 3 females; average body weight, 29 ± 2 g).

URETHANE. Urethane is not a popular anesthetic for chronic mouse preparations, because it is carcinogenic (28) and therefore unsuitable for survival surgery. The recovery time from urethane is very long, which makes it a useful drug for conducting terminal experiments of long duration. Urethane is usually administered intraperitoneally in a dose range of 0.8–1.3 g/kg either alone or combined with α-chloralose. Within the recommended dose range, we found that mice were not anesthetized at all. After an initial dose of 1.25 g/kg was given, two supplemental injections (0.5 g/kg each) 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 and 3 females; average body weight, 30 ± 2 g).

The order of the first three anesthetic regimens was randomized, and 48 h was allowed between recordings. Because of its carcinogenic properties and hepatotoxic effects, urethane was always applied in the terminal study. If the arterial catheter became nonfunctional, a new arterial line was inserted into the other femoral artery (this occurred in 3 of 7 animals).

DATA ACQUISITION AND CALCULATIONS. During the experiments, the flow probe was connected to the flowmeter (type T206, Transonic Systems), and the arterial line was connected to a low-volume pressure transducer. The pressure and flow signals were sampled at 2 kHz (12 bits) using a hemodynamic data-acquisition software developed by the instrument services of the Universiteit Maastricht. With the use of special modules included in this software package, mean arterial pressure (MAP), SV, HR, CO, and total peripheral resistance (TPR) were calculated as previously explained in detail (12). Data were stored on hard disk every 2–5 s 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 of body weight) and depicted as its index, i.e., SI, CI, and TPR index (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 different anesthetized states. For each anesthetic agent, these differences between the conscious and anesthetized states 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 were mutually compared using ANOVA and a subsequent Bonferroni post hoc t-test. Significance was accepted at P < 0.05.

RESULTS

Study 1

Baseline and postanesthetic hemodynamic values for this study are presented in Table 1. All of the anesthetic treatments produced significant decreases in CI and HR. The suppression of CI by anesthetic administration ranged from 24 ± 8% with 1% Iso to 68 ± 2% with K-X combined with 1% Iso. The decrease in CI produced by 1% Iso was significantly 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 sodium was the depression of CI accompanied by a decrease in SI (t = −2.7; P < 0.03).

Study 2

Table 1. Effects of anesthesia on cardiac index and heart rate in male CD-1 mice

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Cardiac Index, ml/min−1·g body wt</th>
<th>Heart Rate, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Isoflurane</td>
<td>10</td>
<td>0.53±0.02</td>
<td>726±21</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>0.40±0.04*</td>
<td>544±31*</td>
</tr>
<tr>
<td>Percent change</td>
<td></td>
<td>−24±8</td>
<td>−24±5</td>
</tr>
<tr>
<td>2% Isoflurane</td>
<td>5</td>
<td>0.58±0.02</td>
<td>711±44</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>0.33±0.04*</td>
<td>438±45*</td>
</tr>
<tr>
<td>Percent change</td>
<td></td>
<td>−42±10</td>
<td>−38±6</td>
</tr>
<tr>
<td>Ketamine-xylazine</td>
<td>9</td>
<td>0.51±0.04</td>
<td>663±21</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>0.20±0.04*</td>
<td>298±40*</td>
</tr>
<tr>
<td>Percent change</td>
<td></td>
<td>−63±6</td>
<td>−55±6</td>
</tr>
<tr>
<td>Ketamine-xylazine with isoflurane</td>
<td>7</td>
<td>0.49±0.04</td>
<td>645±20</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>0.16±0.02*</td>
<td>255±31*</td>
</tr>
<tr>
<td>Percent change</td>
<td></td>
<td>−68±2</td>
<td>−60±5</td>
</tr>
<tr>
<td>Pentobarbital sodium</td>
<td>8</td>
<td>0.58±0.04</td>
<td>709±31</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>0.34±0.03*</td>
<td>481±47*</td>
</tr>
<tr>
<td>Percent change</td>
<td></td>
<td>−40±7</td>
<td>−31±8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. Hemodynamic values were obtained under baseline (nonmoving, resting) conditions and after induction of anesthesia (showing no response to paw pinch). Differences were analyzed as a paired test. *P < 0.05, significant difference between baseline and anesthesia.

study. Data were obtained from 16 mice. Three C57BL6 and one Swiss mouse died during or after implantation of the flow probe. There were no significant strain- or sex-dependent differences, and therefore all data were pooled. The maximal and minimal values shown in Fig. 1 indicate the physiological range of the various parameters compared with the average value obtained during a 10- to 15-min resting, nonmoving period. During the aroused state, CI was increased (compared with the resting state) primarily via SV and to a lesser extent HR. The increase in CI was accompanied by an increase in BP, although TPR decreased, which was most likely because of decreases in muscle vascular resistance.

An example of the hemodynamic effects identified in one mouse both in the conscious state and after administration of Iso or K-X with subsequent atipamezole is shown in Fig. 2. Note that the changes in BP were primarily due to changes in CO, which in turn were predominantly caused by changes in HR and not SV. Figure 2 provides a more detailed description of the effect of atipamezole. The average effects of the various anesthetic regimens on central hemodynamics are presented in Fig. 3. The data are provided in percentages to allow comparisons of changes in the various parameters. Compared with the average resting values of MAP (105 ± 3 mmHg) and CI (0.46 ± 0.02 ml·min−1·g−1) obtained from animals in the conscious resting state, anesthesia with Iso (1−2%) reduced MAP by ~20% to 79 ± 3 mmHg and reduced CI by ~5% to 0.43 ± 0.03 ml·min−1·g−1. In contrast, the K-X mixture induced a more pronounced decrease in BP (to 46 ± 5 mmHg) and CI (to 0.25 ± 0.04 ml·min−1·g−1), which was in large part due to a decrease in HR. When atipamezole (the antidote to the α2-receptor agonist xylazine) was administered, all parameters returned to near control values within minutes (see Fig. 1). After pentobarbital sodium anesthesia was administered, the reductions in BP (to 60 ± 8 mmHg) and CI (to 0.39 ± 0.03...
ml\cdot min^{-1}\cdot g^{-1}) were less severe than those observed after K-X administration. Also, unlike the latter regimen, the reduction in CI was not so much due to a decrease in HR but rather due to a decrease of SI. Last, after urethane administration, the decreases in BP (to 82 ± 8 mmHg) and CI (to 0.43 ± 0.04 ml\cdot min^{-1}\cdot g^{-1}) were relatively modest and not different from values obtained during Iso administration. TPRIs were 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. With the use of this technique, the present data extend previous reports regarding the differential hemodynamic effects of anesthetics on BP and HR (14, 33) or on various echocardiographic
measures (4, 8, 16, 32). The advantage of the present animal preparation was that the systemic hemodynamic effects of anesthetics were obtained in the absence of acute surgery. In addition, the present design allowed comparison of anesthetic effects not only between different agents but also to minimal and maximal values obtained from animals under conscious conditions.

We chose to combine the data of two different laboratories to enable us to compare the anesthetic effects in a heterozygous group of mice (CD-1, Swiss, and C57BL6 strains). In general, the effects of the different anesthetic drugs were qualitatively very comparable between the two laboratories. However, in absolute terms, the reductions in CI and HR were generally larger under the experimental conditions of study 1 than study 2. We therefore decided to present the data of both studies separately. Obviously, the most straightforward explanation for the difference in the magnitude of effects between laboratories is the strain difference. CD-1 mice were used in study 1 vs. a mixture of Swiss and C67BL6 mice in study 2. However, this does not corroborate with the findings of Zuurbier et al. (33). They reported that at dosages that produced 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 CI values were different between the studies. In study 1, the CI for animals under nonmoving conditions was ~0.55 ml·min⁻¹·g⁻¹, whereas in study 2, baseline CI was ~0.46 ml·min⁻¹·g⁻¹. This may explain part of the difference between studies and illustrates the difficulty in defining baseline conditions of hemodynamic parameters that are dependent on the animals' behavior. As illustrated in Fig. 1, we determined the physiological range of the systemic hemodynamics and found that CI may vary up to a factor of 2 in conscious mice. In fact, the CI variation may even be greater: if continuous measurements over 24 h were performed, and HR would reach nadirs of ~400 beats/min (data not shown). However, in the present study, resting baseline HR values were relatively high (range, 600–750 beats/min) compared with 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 HR (31). We have not observed that the flow probe itself compromises aortic blood flow.

From Fig. 1, it follows that in contrast with other species, the increase in CI is mainly due to an increase in SI and not HR. This is not surprising, because in these particular conditions with relatively high HR, an additional increase in CI can be gained only by increasing SV. We have discussed recently that cardiac reserve is probably limited in mice, and therefore small perturbations in venous return directly influence SV 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 that 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.

Iso-associated CI reductions were relatively minor. In the two studies, a light dose of Iso reduced the CI by between 5 and 25% of values obtained in conscious animals. In both studies, the CI reduction with a low Iso dose was the lowest of all the anesthetics. The CI reductions after urethane administration were also modest (~12%). In contrast, CI decreased considerably after administration of both K-X (~37 to ~63%) and pentobarbital sodium (~24 to ~40%) anesthesia. For all anesthetic agents, it was found that the decrease in CI was mainly due to a decrease in HR. SV did not change or even increased slightly, which was probably due to prolonged filling times. Similar observations, i.e., a decrease in HR but no change or an increase in SI, have been made when anesthetic effects on left ventricular function were studied using echocardiography (4, 16). Only during pentobarbital sodium administration was this pattern different, and both HR and SI were decreased. This latter effect was also observed in rats (27) and may be related to a direct negative inotropic action on myocardial tissue (21). Alternatively, it may also result from 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, Iso 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 with Iso.
anesthesia than when nonvolatile anesthetics are used. Obviously, control of the depth of anesthesia is much easier with inhalation than injection anesthetics. However, the relatively high systemic blood flow during Iso anesthesia preserves peripheral organ perfusion during surgical interventions. In addition, Iso induces an increase in regional cerebral cortical blood flow in mice (15) that may enhance the fast recovery. Last, Iso and related volatile anesthetics are known to exert a protective preconditioning-like effect on myocardial tissue (10, 20).

These actions make Iso the anesthetic of preference among those tested, because 1) it is 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 BP very well (25). In some circumstances, 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-min period, is greater during pentobarbital sodium (7) than Iso anesthesia. The reduced workload of the heart during this type of anesthesia may contribute to recovery from surgery and prevent potentially lethal arrhythmias (3, 6). Furthermore, it has been reported that pentobarbital sodium may facilitate spontaneous recovery from hypoxic anepia (11).

During anesthesia with Iso, MAP was reduced to ~80 mmHg and HR to 600 beats/min. These values correspond to the steady-state values observed in Swiss and C57BL/6 mice that were kept anesthetized for 3 h (33). We took great care to keep each 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 in mice during standard laboratory conditions (2). For this purpose, we needed Iso concentrations of ~2%, a concentration that is regularly reported in the literature. In contrast, in study 2, doses of pentobarbital sodium (≤90 mg/kg) and urethane (2.5 g/kg) were needed that are higher than those generally advised in experimental animal textbooks or on Internet websites. Obviously, circumstances vary between laboratories, 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 laboratories remain unclear. Paradoxically, the simplest explanation for the incongruity in anesthetic dosing could be that although many reports mention that supplemental (to the standard dose) amounts of anesthetics were needed, these latter amounts are rarely specified.

The K-X mixture had potent cardiodepressive effects. The present data show that these can be quickly reversed by intraperitoneal injection of the α2-adrenoceptor antagonist atipamezole. The peripheral α2-adrenergic receptor blockade may explain why BP declined for a short period to even lower values (see Fig. 2) before the central effect clearly overruled the peripheral action. The data suggest that centrally mediated inhibition of sympathetic tone by the α2-receptor agonist xylazine rather than N-methyl-D-aspartate antagonism due to ketamine is responsible for the severe cardiodepressive effects. 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, which is another α2-adrenergic receptor agonist, did not prevent the pronounced decrease in HR. The rapid (within minutes) effect of atipamezole is helpful in rescuing mice in critical stages during surgery, especially when HR decreases to <300 beats/min and cardiac contractility is also threatened (8).

During urethane anesthesia, arterial BP and CO were reasonably well maintained and not much lower than during Iso anesthesia. In contrast, Jong et al. (14) observed that hemodynamic conditions were relatively unstable during the administration of a combination of urethane and α-chloralose in mice. It may be that α-chloralose, which is 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 mice is not known.

The type of analgesic agent that should be combined with anesthetic as well as the timing, route of administration, and dosage that should be given to 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 on institutional websites. In our hands, coadministration of buprenorphine to a light (0.5–1%) Iso anesthesia in mice dose dependently further depressed BP and CI. After a relatively high dose (2 mg/kg) of buprenorphine, MAP decreased within 15 min by 40% and CI decreased by 27% (data not shown). The coadministration of 2 mg/kg buprenorphine was associated with increased mortality rates (4 of 8 mice) despite the fact that animals were withdrawn from the Iso 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 Iso with buprenorphine to help stabilize the anesthetic plane, “but many of the benefits were diminished by this approach.”

In summary, compared with resting conscious conditions in mice, CI was only slightly depressed during anesthesia with the volatile anesthetic Iso. Severe CI reductions were observed during anesthesia with the K-X mixture, but these could be reversed by the α2-adrenergic receptor blocker atipamezole.

GRANTS

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