Severity of Locomotor and Cardiovascular Derangements after Experimental High-Thoracic Spinal Cord Injury Is Anesthesia Dependent in Rats

Yvette S. Nout, Michael S. Beattie, and Jacqueline C. Bresnahan

Abstract

Anesthetics affect outcomes from central nervous system (CNS) injuries differently. This is the first study to show how two commonly used anesthetics affect continuously recorded hemodynamic parameters and locomotor recovery during a 2-week period after two levels of contusion spinal cord injury (SCI) in rats. We hypothesized that the level of cardiovascular depression and recovery of locomotor function would be dependent upon the anesthetic used during SCI. Thirty-two adult female rats were subjected to a sham, 25-mm or 50-mm SCI at T3–4 under pentobarbital or isoflurane anesthesia. Mean arterial pressure (MAP) and heart rate (HR) were telemetrically recorded before, during, and after SCI. Locomotor function recovered best in the 25-mm-injured isoflurane-anesthetized animals. There was no significant difference in locomotor recovery between the 25-mm-injured pentobarbital-anesthetized animals and the 50-mm-injured isoflurane-anesthetized animals. White matter sparing and extent of intermediolateral cell column loss appeared larger in animals anesthetized with pentobarbital, but this was not significant. There were no differential effects of anesthetics on HR and MAP before SCI, but recovery from anesthesia was significantly slower in pentobarbital-anesthetized animals. At the time of SCI, MAP was acutely elevated in the pentobarbital-anesthetized animals, whereas MAP decreased in the isoflurane-anesthetized animals. Hypotension occurred in the pentobarbital-anesthetized groups and in the 50-mm-injured isoflurane-anesthetized group. In pentobarbital-anesthetized animals, SCI resulted in acute elevation of HR, although HR remained low. Return of HR to baseline was much slower in the pentobarbital-anesthetized animals. Severe SCI at T3 produced significant chronic tachycardia that was injury severity dependent. Although some laboratories monitor blood pressure, HR, and other physiological variables during surgery for SCI, inherently few have monitored cardiovascular function during recovery. This study shows that anesthetics affect hemodynamic parameters differently, which in turn can affect functional outcome measures. This supports the need for a careful evaluation of cardiovascular and other physiological measures in experimental models of SCI. Choice of anesthetic should be an important consideration in experimental designs and data analyses.

Key words: animal model; arterial blood pressure; chronic telemetry; critical care; hemodynamics; HR; hypotension; isoflurane; MAP; neurology; pentobarbital; recovery of function

Introduction

CARDIOVASCULAR CONTROL is abnormal and unstable following spinal cord injury (SCI), particularly when the injury has occurred above the mid-thoracic segments (T2–T6); (Baldrige et al., 2002; Bonica, 1968; Furlan and Fehlings, 2008; Maiorov et al., 1998; Mayorov et al., 2001; Wallis et al., 1996). Injuries rostral to this level, such as cervical injuries that make up 55% of human SCI (National Spinal Cord Injury Statistical Center, 2010), may result in interruption of the descending sympathoexcitatory fibers that originate from the

rostroventrolateral medulla and contact preganglionic neurons in the thoracic region. Loss of this descending input leads to unopposed parasympathetic outflow in the acute phase of SCI. This can cause disruption of both tonic and reflex sympathetic control of mean arterial blood pressure (MAP) with resulting hypotension, and cardiac arrhythmias. Systemic vascular alterations such as reduced heart rate (HR), cardiac arrhythmias, hypotension, reduced peripheral vascular resistance, and compromised cardiac output are indeed commonly encountered in humans and experimental animals after SCI (Ball, 2001; Levi et al., 1993; Mathias and Frankel, 1992;

McDonald and Sadowsky, 2002; Vale et al., 1997). Bradyarrhythmias also have been shown to occur in dogs with cervical intervertebral disk herniation (Kube et al., 2003; Stauffer et al., 1988). In addition, experiments have shown that systemic microvascular blood flow was decreased after SCI, in particular in liver, spleen, and muscle (Guizar-Sahagun et al., 2004).

It has been well documented that systemic hypotension occurs for a variable length of time in the acute period following trauma in humans (Casha and Christie, 2009; Levi et al., 1993; Vale et al., 1997) and animal models of SCI (Baldridge et al., 2002; Guha et al., 1989; Leal et al., 2007; Mayorov et al., 2001). Among many other consequences of systemic hypotension, it is considered to play an important role in expansion of the lesion through its negative impact on spinal cord blood flow (Tei et al., 2005). Systemic cardiovascular instability can further compromise blood flow to the injured spinal cord. In addition, ischemia and hypoxia, both of which are detrimental to cell survival, are considered important players in the development of secondary damage following SCI. Spinal cord ischemia is a likely consequence of both local and systemic vascular alterations after severe injury (Tator, 1991, 1998; Young, 1993). Local vascular alterations include immediate mechanical damage of the microvasculature and focal, post-injury vasospasm, both of which lead to loss of autoregulation of spinal cord blood flow (Guha et al., 1989; Kobrine et al., 1976; Senter and Venes, 1979; Young et al., 1982). Systemic hypotension compounds local spinal cord ischemia by further reducing perfusion, ultimately reducing spinal cord tissue delivery of oxygen (DO_2). Inadequate DO_2 to tissues is associated with cell death.

In animal models of SCI, different anesthetics, each with unique properties that affect cardiovascular and respiratory function, are used when inducing SCI. Studies have shown that different anesthetics affect outcomes from both experimental brain and spinal injury differently, when they are used either when the lesion is created or during the postoperative period. Three studies examined a total of 10 anesthetics in experimental brain injury, and showed that isoflurane and urethane had less deleterious consequences on outcomes when compared to morphine, propofol, pentobarbital, and alpha-chloralose (Cochrane et al., 1988; O'Connor et al., 2003; Statler et al., 2006). Three other studies examined a total of 10 anesthetics in experimental SCI, and showed better recovery of function associated with halothane anesthesia and poor recovery associated with ketamine and pentobarbital anesthesia (Grissom et al., 1994; Ho and Waite, 2002; Leal et al., 2007; Salzman et al., 1990). This is an important consideration both for comparing studies across laboratories and when investigating (therapeutic) effects of novel pharmaceuticals. In the present study we sought to compare two anesthetics commonly used in experimental SCI and determine how each of these affected the cardiovascular response to acute SCI, and whether there would be an anesthetic effect on locomotor outcome. We hypothesized that locomotor and cardiovascular outcomes would be affected differently depending upon the anesthetic's effects on lesion size and/or development.

Methods

Study design

Adult, female Long-Evans hooded rats (Simonsen Laboratories, Gilroy, CA; $n=32$) were used in this study. Ani-

mals were subjected to a sham, 25-mm, or 50-mm contusion SCI at the level of thoracic vertebra (T)3–4 either under pentobarbital or isoflurane anesthesia. Groups were: 1: isoflurane + 25-mm SCI, $n=7$ (Iso-25); 2: isoflurane + 50-mm SCI, $n=7$ (Iso-50); 3: isoflurane + sham, $n=3$ (Iso-control); 4: pentobarbital + 25-mm SCI, $n=6$ (Pent-25); 5: pentobarbital + 50-mm SCI, $n=7$ (Pent-50); 6: pentobarbital + sham, $n=2$ (Pent-control).

Rats were housed individually in plastic cages, maintained on a 12-h light/dark cycle, and had free access to food and water. All animal experiments were conducted after approval by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University and University of California, San Francisco and were performed in compliance with National Institutes of Health guidelines and recommendations.

Surgical procedures and postoperative care

Surgical procedures were performed aseptically with the animals under deep anesthesia, determined by testing for withdrawal to foot pinch. Lacrilube ophthalmic ointment (Allergan Pharmaceuticals, Irvine, CA) was applied to the eyes and a pre-operative dose of cefazolin (Cefazolin, West-Ward Pharmaceutical Corp., Eatontown, NJ; 50mg/kg subcutaneously) was administered prior to all surgeries. Body temperature was maintained at $37.5 \pm 0.5^\circ\text{C}$ using a rectal thermal probe and heating pad during all surgeries.

Transducer implantation. A telemetric pressure transducer catheter (TA11PA-C40, Data Sciences International, St. Paul, MN) was implanted into the aortic arch through the left carotid artery 1 week prior to SCI. Anesthesia was induced and maintained by intraperitoneal (i.p.) administration of xylazine (TranquiVedTM, Vedco Inc., St. Joseph, MO; 10mg/kg) and ketamine (ketamine HCl, Abbott Laboratories, N. Chicago, IL; 80mg/kg). Rats were positioned in dorsal recumbency and surgical sites (ventral neck) shaved and cleaned with betadine. A 2-cm midline incision was made in the ventral cervical region. With saline-moistened gauze sponges, the left carotid artery was located and isolated from the surrounding soft tissues. The cranial carotid artery was ligated and a ligature was also placed more proximally but not tightened. Flow in the proximal carotid artery was temporarily obstructed during catheter placement by lifting the artery with the ligature. A small hole was made in the carotid artery between the two ligatures with a 24-gauge needle, through which the catheter was slipped into the artery and advanced ($\sim 2\text{cm}$) until the tip was in the aortic arch. After the puncture site was thoroughly dried, the catheter tip was maintained in place using a veterinary adhesive (Vetbond, 3M Animal Care Products, St. Paul, MN) and tightening the proximal ligature over the catheter. The carotid artery remained permanently ligated¹ (This procedure by itself does not lead to brain lesions: Levine, S. (1960) Anoxic-ischemic encephalopathy in rats. *Am J Pathol* 36, 1-17.). The transducer body was placed subcutaneously behind the shoulder, at the level of the cranioventral thorax. The incisions were closed with staples and telemetric studies commenced 5 days after implantation. Physiological telemetry data was recorded on a PC using DSI equipment.

SCI. Rats were anesthetized with either pentobarbital (Abbott Laboratories, Chicago, IL; 50mg/kg i.p.) or with isoflurane (IsoFlow, Abbott Laboratories, North Chicago, IL; 2–3% maintenance inhalant anesthesia). A 25-mm or 50-mm SCI was delivered with a MASCIS/NYU device as previously described (Gruner, 1992). A dorsal midline incision was made and a laminectomy at T3–4 (spinal level T4–T5; Waibl, 1973) was performed. The spinal cord, with an intact dura mater, was impacted with a 10g rod from a height of 25 mm or 50 mm. The SCI was delivered ~20 min after inducing anesthesia. Animals in the control group underwent the same surgical procedure, were placed in the impactor device, and had the impactor rod leveled to the spinal cord, but no contusion was induced. After closing the muscle layers the skin was apposed with skin staples and the animal was allowed to recover. Cessation of inhalant anesthesia occurred within 5 min after SCI.

Postoperative care for the injured rats included continued administration of cefazolin (50 mg/kg subcutaneously) once daily for 13 days, and a once- or twice-daily animal check and bladder expression throughout the remainder of the study. Furthermore, animals were administered lactated Ringer's solution subcutaneously (Abbott Laboratories, North Chicago, IL; 5–10mL once or twice daily) if there was evidence of dehydration or hematuria, and nutritional supplementation in the form of Nutri-Cal (Evsco Pharmaceuticals, Division of Vétoquinol USA Inc., Buena, NJ) if animals lost >10% of body weight. At 14 days after SCI, animals were anesthetized with xylazine (10mg/kg i.p.) and ketamine (80mg/kg i.p.) and transcardially perfused with 0.9% NaCl followed by 4% paraformaldehyde in phosphate-buffered saline (PBS).

Behavioral outcomes

Locomotor and activity. Open-field walking was evaluated before SCI (baseline [BL]) and at 24 h, 3–5, 7, and 14 days following SCI using the 21-point Basso Beattie Bresnahan (BBB) Locomotor Rating Scale (Basso et al., 1995). Furthermore, level of activity in the cage was determined by telemetry. For this, movement of the transducer over different detection fields of the receiver was recorded and, using Dataquest ART software (Data Sciences International, St. Paul, MN), a moving average in counts per minute was acquired. Data were collected for 2×24 h prior to SCI (BL). Data were then continuously recorded from the start of anesthesia, throughout the SCI surgery, until day 14 after SCI.

Cardiovascular data. Cardiovascular telemetry data, obtained from the left carotid artery, were recorded and analyzed on a PC using Dataquest ART software. For recordings during SCI impact in anesthetized animals, the receiver plate was positioned under the animal on the MASCIS/NYU device. For all other recordings, animals were awake and housed in their regular cages. Data were collected for 2×24 h prior to SCI (BL). Data were then continuously recorded from the start of anesthesia, throughout the SCI surgery, until day 14 after SCI. Cardiovascular parameters determined from the left carotid artery were HR, pulse pressure, and diastolic, systolic, and mean arterial pressure. Quality of recordings was assessed by examining the pulse pressure waveform obtained during a 10-sec period once daily at 2 a.m. Quality of the recording was considered good if hemodynamic parameters

were within normal limits at BL (HR: 350–380 beats per minute [bpm]; MAP: 95–10 mm Hg) and pulse pressure was regular and 20–35 mm Hg. Data collected between 10.00 and 14.00, and between 22.00 and 2.00, were used for day and nighttime analyses, respectively. For discussion of data, hypotension was defined as a MAP < 65 mm Hg.

Histopathology

Immediately after animals were killed, the injured region of the thoracic spinal cord was dissected, post-fixed in 4% paraformaldehyde for <48 h, cryoprotected in 30% sucrose in PBS for 48–72 h, and then frozen at –80°C until sectioning. The lesioned region (10 mm) was sectioned transversely at 20 µm on a cryostat and sections were stained with luxol fast blue for myelin and cresyl echt violet for Nissl substance. The amount of spared white matter was determined at the lesion epicenter and reported as percentage of the area of a section rostral to the lesion. The more rostral section used for comparison was a section within 5 mm of the lesion epicenter, containing only mild damage and no cord collapse. The extent of loss of the intermediolateral cell column was determined by examining stained sections at every 60 µm throughout the lesion.

Statistical analysis

Data are presented as means ± standard error of the mean (SE). A repeated measures analysis of variance (ANOVA) was used to analyze all behavioral data. The null hypothesis was rejected at $\alpha=0.05$. A one-way ANOVA was used to determine significant differences between BL and end point data and for analysis of perioperative data. Significant differences identified by the ANOVA were isolated using the Holm-Sidak procedure for pairwise multiple comparison post-hoc test. The statistical computations were performed with software packages (Sigmastat 3.0, SPSS, Chicago, IL).

Results

Rats weighed 234 ± 4 g at the time of transducer implantation, 237 ± 3 g at the time of SCI, and 240 ± 4 g at the time of death. Because of poor quality, the cardiovascular telemetry data from two rats (one from Iso-50 and one from Pent-50) were not used.

Anesthetic affected locomotor recovery but not white matter sparing

All injured animals showed partial recovery of locomotor function, demonstrated by a significant increase in the BBB locomotor score over time ($p < 0.001$) (Fig. 1A). BBB locomotor scores at 14 days after SCI were 11.3 ± 0.2 (Iso-25), 10.0 ± 0.8 (Pent-25), 9.4 ± 0.6 (Iso-50), and 8.3 ± 1.1 (Pent-50). Animals subjected to a 25-mm SCI improved to a significantly higher level than did animals subjected to a 50-mm SCI ($p = 0.012$). Recovery of locomotor function was best in the Iso-25 group; that group recovered better than did animals in the Iso-50 group ($p = 0.056$), and significantly better than did animals in the Pent-50 group ($p = 0.006$). Animals in the Pent-25 group recovered better than did those in the Pent-50 group ($p = 0.064$). There was no significant difference in recovery between the Pent-25 and Iso-50 groups ($p = 0.62$).

Histopathological analysis of lesion centers showed that white matter sparing was significantly larger in animals that

FIG. 1. Hindlimb locomotor recovery (**A**) and white matter sparing at the lesion center (**B**). In **A**, recovery of hindlimb motor function is shown. More severe injuries (50 g-cm) resulted in significantly more severe locomotor deficits in both isoflurane- and pentobarbital-anesthetized animals (α : $p=0.012$). In addition, all groups showed significant recovery of function over time ($***$: $p<0.001$). Differences related to anesthetic are shown by the difference in recovery of the Iso-25 group compared to the Iso-50 ($p=0.056$) and Pent-50 groups ($p=0.006$). In addition, animals in the Pent-25 group recovered better than those in the Pent-50 group ($p=0.064$), but there was no significant difference in recovery between the Pent-25 and Iso-50 groups ($p=0.62$). **B** shows that lesion severity resulted in significant differences in white matter sparing ($***$: $p<0.001$), however, this was not significantly affected by the anesthetic used during the injury. Color image is available online at www.liebertonline.com/neu

were subjected to the milder injury (Iso-25: $17.6 \pm 1.5\%$ and Pent-25: $18.9 \pm 3.8\%$ vs. Iso-50: $7.3 \pm 2.2\%$ and Pent-50: $5.7 \pm 1.4\%$; $p<0.001$) (Fig. 1B). There was no significant effect of anesthetic on white matter sparing ($p=0.97$). However, there was a strong correlation between white matter sparing and BBB locomotor scores ($R^2=0.61$; isoflurane: $R^2=0.73$; and pentobarbital: $R^2=0.6$). The extent of intermediolateral cell column loss in the gray matter was significantly smaller in animals that received the milder injury (Iso-25: 5.04 ± 0.4 mm and Pent-25: 5.56 ± 0.3 mm vs. Iso-50: 6.98 ± 0.4 mm and Pent-50: 7.41 ± 0.5 mm; $p<0.001$). No significant effect of anesthetic on extent of intermediolateral cell column loss was seen ($p=0.28$).

Anesthetic did not affect cardiovascular parameters at BL

Telemetric derived data taken at BL is shown in Figure 2. Figure 2A shows a representative tracing used to determine

FIG. 2. Representative pressure tracing from a telemetric pressure transducer catheter placed in the aortic arch shown for a period of 5 sec (**A**) and 24 h (**B**). The arterial blood pressure tracing is shown in **A** with arrows indicating the systolic and diastolic pressures. The 24-h recording shows circadian rhythm fluctuation of heart rate and moving average, and to a lesser extent of arterial blood pressure. Time=0 h equals 8 a.m. HR, heart rate; BPM, beats per minute; SBP, systolic blood pressure; MAP, mean arterial blood pressure; DBP, diastolic blood pressure; MA, moving average; cnt/min, counts per minute $\times 10$. Day=light time, Night=dark time.

quality of recording on a daily basis. Recordings with pulse pressures of >20 – 25 mm Hg were considered good quality. Figure 2B is a 24-h recording in which all parameters that were telemetrically obtained by the pressure transducer are indicated: HR, systolic and diastolic blood pressure, MAP, and activity. Circadian rhythm of HR and activity (moving average) are noticeable, with both those parameters increasing during the night/dark cycle. During anesthesia prior to SCI, average HR and MAP were 306 ± 12 bpm and 75 ± 4 mm Hg, respectively, and no significant group differences were present prior to SCI ($p=0.8$ and $p=0.7$, respectively) (Fig. 3). From rats that were allowed to move freely in their home cages, an average HR of 374 ± 9 bpm and MAP of 105 ± 2 mm Hg were recorded, before SCI; no significant group differences were present at BL ($p=0.3$ and $p=0.8$, respectively) (Fig. 4).

Anesthetic affected MAP acutely after SCI

MAP was elevated in pentobarbital-anesthetized animals but decreased in isoflurane-anesthetized animals during the acute period at the time of SCI. SCI resulted in a significant elevation of MAP (~ 20 mm Hg) during a 1-min period at the time of impact in the Pent-25 and Pent-50 animals ($p<0.001$;

FIG. 3. Cardiovascular changes during the SCI surgical procedure. A 60-min intra-operative interval in which at time = 5 min SCI was induced (arrow) showing mean arterial blood pressure (MAP) (A) and heart rate (HR) (B). In C and D MAP and HR are shown in more detail for three time points around the time of impact (t = 5 min) for the four study groups. In all animals, MAP significantly increased over time (A) (***: $p < 0.001$). In addition, after SCI, isoflurane-anesthetized animals had significantly higher MAPs than did pentobarbital-anesthetized animals (A) (α : $p < 0.001$). Significant differences in MAP changes after SCI were seen between isoflurane- and pentobarbital-anesthetized animals during the impact (C) (***: $p < 0.001$). In the Iso-25, Iso-50, Iso-control, and Pent-25 groups, HR increased significantly over time after SCI (B) (***: $p < 0.001$). In addition, HR in the isoflurane-anesthetized animals and the Pent-25 animals was significantly higher than in the Pent-control group (B) (β : $p < 0.001$). In the isoflurane-anesthetized animals, HR increased significantly when compared to HR at time = 0 (B) (χ : $p < 0.001$). Color image is available online at www.liebertonline.com/neu

Fig. 3A and 3C), whereas, the MAP in the Iso-25 and Iso-50 animals decreased by ~ 20 mm Hg at the time of impact (Fig. 3C). After this initial sharp rise in MAP in the pentobarbital-anesthetized animals, MAP decreased. In isoflurane-anesthetized animals, MAP started to increase after the initial decline. In animals anesthetized with pentobarbital, MAP continued to decrease by 30–40 mm Hg after SCI (following the brief increase at the time of impact), whereas in animals anesthetized with isoflurane, MAP continued to rise immediately after SCI (following the brief decline at the time of impact). Apart from this initial difference in the immediate post-impact phase, the MAP response during the remainder of the recovery from anesthesia after SCI was not significantly different among the individual groups ($p = 0.99$); in all groups MAP increased over time ($p < 0.001$).

MAPs increased significantly after SCI. MAPs recorded > 25 min after SCI were significantly higher than MAPs recorded from 0 to 12 min after SCI ($p < 0.001$; Fig. 3A). In addition, at time points > 2.5 min after SCI, MAPs in isoflurane-anesthetized animals were significantly higher than those in pentobarbital-anesthetized animals (Iso-25 and Iso-50 vs. Pent-25 and Pent-50; $p = 0.015$). A significant interaction between anesthetic and time was present, resulting from significant differences of MAP at t = 5 min (time of impact) and t = 16–48 min ($p < 0.001$; Figs. 3A and C).

Hypotension occurred in both pentobarbital-anesthetized groups but only in the isoflurane-anesthetized group that received the most severe SCI. MAPs stayed > 65 mm Hg only in the control (Iso and Pent) and Iso-25 animals. In the

FIG. 4. Mean arterial blood pressure (MAP) (A) and heart rate (HR) (B) shown for a period of 14 days after SCI (time=0). **A.** MAP was significantly lower on the day of SCI surgery than at all other time points in all injured groups (***: $p < 0.001$). **B.** There was no significant difference in HR over time in the Iso-control group. In the Pent-control group, HR was significantly lower on the day of SCI surgery than at all other time points ($p < 0.001$). SCI resulted in a significant increase in HR in all groups: For all groups, on day 3, HR was significantly higher than on 12 and 13 days after SCI (***: $p < 0.001$). Furthermore, from 1 to 13 days after SCI, HR was significantly higher than at baseline (BL) and the day of surgery for the Iso-50, Pent-25, and Pent-50 groups (***: $p < 0.001$). For the Iso-25 group, HR was significantly higher from 2 to 10 days after SCI compared to BL and the day of SCI surgery. Furthermore, for the Iso-50 and Pent-50 groups, HR was consistently higher than in the Iso-control group from 2 to 13 days after SCI ($p < 0.001$). Injury severity significantly affected HR; HR was significantly lower in the moderately injured animals than in the severely injured animals (Iso-25 and Pent-25 vs. Iso-50 and Pent-50) ($\alpha = p < 0.001$). Color image is available online at www.liebertonline.com/neu

other three groups, animals were hypotensive (MAP < 65 mm Hg) immediately after SCI for 2 min (Pent-25), 4 min (Iso-50), and 10 min (Pent-50). However, the effect of lesion severity was not statistically significant ($p = 0.7$).

Anesthetic affected HR acutely after SCI

In pentobarbital-anesthetized animals, SCI resulted in acute elevation of HR, although HR remained low. During SCI surgery, HR in the uninjured Pent-control group was significantly different from in all the other groups over time ($p < 0.001$; Fig. 3B); HR remained low throughout the 60 min period. SCI resulted in mild elevation of HR in the Pent-25 and Pent-50 animals, which was most noticeable 5 min following impact (Fig. 3D). This change in HR was not seen in the Pent-

control group or in isoflurane-anesthetized animals (Iso-control, Iso-25, Iso-50).

HR determined >10–15 min after SCI were significantly higher than those recorded before SCI and until 5 min after SCI. HR did not significantly change throughout the 60 min in the Pent-control and Pent-50 groups ($p > 0.05$). In the four other groups, HR significantly increased over time with HR >10–5 min after SCI being significantly higher than those recorded before SCI and until 5 min after SCI ($p < 0.001$; Fig. 3B). From 15 min after SCI onward, HR in the Pent-control group was significantly lower than in all other groups except for the Pent-50 group ($p < 0.001$). In addition, from 35 min after SCI onward, HR in the Pent-control group was significantly lower than in the Pent-50 group ($p < 0.001$; Fig. 3B). When groups were combined (Iso-25 and Iso-50 vs. Pent-25 and Pent-50), there was a significant interaction between anesthetic and time resulting in significant differences of HR from $t = 7$ –10 min and $t = 29$ –59 min ($p < 0.001$).

Recovery from pentobarbital anesthesia was significantly slower when compared to that from isoflurane

Recovery from anesthesia was rapid in animals anesthetized with isoflurane (~5–15 min) and slow in animals anesthetized with pentobarbital (~6–9 h). During recovery from anesthesia, in the uninjured Iso-control group, MAP increased from 70–80 mm Hg (anesthesia) to 100–110 mm Hg (BL) over a 5 min period, and HR increased from 350 bpm (anesthesia) to 420 bpm (BL) over a 10–15 min period (Figs. 3A and B). Pentobarbital anesthesia resulted in more prolonged and severe cardiovascular depression. In the uninjured Pent-control group, the increase of MAP from 70–80 mm Hg (anesthesia) to 100–110 mm Hg (BL) took 380 min (6 h, 20 min; data not shown) and the increase of HR from 350 bpm (anesthesia) to 420 bpm (BL) took 580 min (9 h, 40 min; data not shown).

In the injured Iso-25 and Iso-50 groups, MAP increased rapidly to BL (100–110 mm Hg) during recovery from anesthesia after SCI (Fig. 3A; 5 min). In the Pent-25 and Pent-50 groups, however, this increase of MAP to BL occurred more gradually over a period of 540–570 min (9–9.5 h; data not shown) and was much longer than the time it took for this to happen in the Pent-control group (see previous text; 6 h, 20 min).

Similarly, for the Iso-25 and Iso-50 groups, it took 15 min for HR to return to BL (380 bpm) after SCI (Fig. 3B), which was not significantly different than in the Iso-control group ($p > 0.05$). For the Pent-25 and Pent-50 groups, return of HR to BL (380 bpm) took 700 min (11 h, 40 min), much longer than in Pent-control animals (see previous text; 9 h, 40 min).

Severe SCI at T3 produced long-term chronic tachycardia

Postoperatively, MAP was not significantly different over time in the uninjured Iso- and Pent-control groups ($p > 0.1$). In all other groups, MAP was significantly lower on the day of SCI than at all other time points ($p < 0.001$; Fig. 4A). In the uninjured Iso-control group, there was no significant difference in HR over time ($p > 0.1$), but in the uninjured Pent-control group, HR was significantly lower on day 0 (day of

anesthesia) compared to all other time points ($p < 0.001$; Fig. 4B). SCI resulted in chronic HR elevation in all groups (Fig. 4B). In the Iso-50, Pent-25, and Pent-50 groups, HR was significantly higher during days 1–13 after SCI when compared to BL and day 0 (SCI; $p < 0.001$). In the Iso-25 group, HR was significantly higher during days 2–10 after SCI when compared to BL and day 0 (SCI; $p < 0.001$). Furthermore, in all groups, on day 3 after SCI, HR was significantly higher than on days 12 and 13 after SCI ($p < 0.001$). HR was consistently higher in the Iso-50 and Pent-50 groups, than in the Iso-control group during days 2–13 after SCI ($p < 0.001$). Moreover, injury severity significantly affected HR. HR was significantly lower in the moderately injured animals (combined Iso-25 and Pent-25) compared to HR in the severely injured animals (combined Iso-50 and Pent-50; $p < 0.001$).

Discussion

This study shows that the anesthetic used during experimentally induced SCI can affect short- and long-term cardiovascular and locomotor outcome measurements and should be an important consideration in experimental designs and data analyses. Pentobarbital is a barbiturate anesthetic that is commonly used in rodents because it is an easily (i.p.) administered nonirritant solution that produces a rapid onset anesthesia and has a relatively long half-life (14–133 min) (Ossenberg et al., 1975). However, pentobarbital impairs the dynamics of respiratory and cardiovascular systems (Torbaty et al., 1999; Walker et al., 1986; Webber and Peiss, 1979; Yamada et al., 1983). In prolonged anesthesia, pentobarbital has also been shown to impair myocardial contractility (Segel and Rendig, 1986), and to reduce myocardial blood flow and ejection fraction (Iltis et al., 2005). In addition, pentobarbital can progressively decrease body temperature through inhibition of brain metabolic activity (Kiyatkin and Brown, 2005) and decrease renal blood flow and glomerular filtration rate, probably mediated through the renin-angiotensin system (Walker et al., 1986). Appropriate animal support, including temperature and hydration regulation, is recommended when using pentobarbital anesthesia. Isoflurane is an inhalation anesthetic, producing rapid induction and recovery from anesthesia (half-life: 0–13 min) (Weightman, 2004). Similar to pentobarbital, isoflurane produces depression of the cardiovascular and respiratory systems (Fee and Thompson, 1997), however, this typically is mild and most studies report isoflurane to be a safe and reliable anesthetic with little negative effect on the cardiovascular and respiratory systems (Iltis et al., 2005; Szczesny et al., 2004). Compared to pentobarbital, in rats, isoflurane produces less deleterious cardiac effects, demonstrated by a higher mean coronary blood flow caused by a larger ejection fraction and higher cardiac output (Iltis et al., 2005). As far as the authors are aware, this is the first study in which pentobarbital and isoflurane are compared and hemodynamic measurements are performed perioperatively in anesthetized animals, and chronically (2 weeks) in conscious spinal cord injured animals. We show that pentobarbital-anesthetized animals had more severe locomotor and cardiovascular derangements than isoflurane-anesthetized animals.

An older study investigating effects of inhaled halothane versus pentobarbital after a mid-thoracic SCI demonstrated improved neurological scores (modified Tarlov and somatosensory-evoked potentials) and increased serotonin distal to

the lesion across all injury severities 1 week after injury in the groups anesthetized with halothane (Salzman et al., 1990). In that study, MAP from the femoral artery was calculated from systolic and diastolic blood pressure measurements, and measurements were only made in the acute period of injury (BL, and 15–30 min after injury). In addition, the spread of these calculated means is large, making these hemodynamic data difficult to interpret. A more recent study investigating different anesthetics in a high thoracic transection model, showed that acute hemodynamic changes (up to 60 min after transection) were anesthetic agent dependent (Leal et al., 2007). The authors investigated four different anesthetics (ether, 20% urethane, tri-bromide-ethanol, and chloral hydrate/urethane) and found that the hypertensive peak, which typically is seen immediately after spinal cord transection, did not always occur. Its presence was dependent upon the anesthetic used during the transection procedure. In addition, the degree to which the MAP dropped after spinal cord transection was also anesthesia dependent. The authors proposed a mechanism for these differences that included different degrees of inhibition of the autonomic nervous system and different levels of sympathetic hyperactivity. Functional outcomes were not reported (Leal et al., 2007).

O'Connor and associates (2003) examined effects of pentobarbital, halothane, and isoflurane in a rodent model of traumatic brain injury. Their group found that pentobarbital was associated with increased mortality in female rats and that isoflurane had neuroprotective effects in females, as demonstrated by improved performance in cognitive tests. No hemodynamic testing was done in these experiments. Grissom and associates (1994) demonstrated deleterious effects of ketamine after SCI when compared to isoflurane and fentanyl/nitrous oxide on functional outcome in rats that were anesthetized with different anesthetics 7–8 days after an implant had been inserted at T12 to produce progressive neurologic deficits from which recovery would occur.

Similar to the findings in the present report, Mayorov and associates (2001) showed that, in rats, after clip compression at T5, a decrease in blood pressure and elevation of HR occurs (Mayorov et al., 2001). This group used telemetric pressure transducers implanted in the distal aorta to measure hemodynamic parameters in conscious rats. The hemodynamic changes reported are consistent with our findings, with the exception that the recovery of MAP to baseline in our study was much more rapid. In our most severely affected rats that were anesthetized with pentobarbital, recovery of MAP to baseline took ~9–10 h, whereas Mayorov and associates (2001) report that MAP was decreased for 48 h. This may have been because these investigators used a different injury model (severe clip compression lasting 1 min vs. our rapid contusion model) and in Mayorov's study, telemetric transducers were implanted in the abdominal aorta (vs. the aortic arch in our study). Similar to our findings, HR was elevated (425–450 bpm) for the duration of their study (42 days) after SCI. Hemodynamic measurements were not reported for the perioperative period (Mayorov et al., 2001). After complete spinal cord transection at T4, Laird and associates (2006) reported a slight MAP decrease and elevation of HR using telemetric pressure transducers in the distal aorta. MAP was lowest on day 3 after transection (71 mm Hg) and increased thereafter, but remained slightly lower (95 mm Hg) than baseline measurements (117 mm Hg). In addition, this group

found that basal core body temperature in injured animals was lower than in intact animals, another indicator of autonomic nervous system dysfunction (Laird et al., 2006). Two other studies also showed a significant elevation of HR following T4–5 transection (Baldridge et al., 2002; Rodenbaugh et al., 2003) and elevation of HR was also found after T9 transection (Guizar-Sahagun et al., 2004). Transection at the level of T2 in rats resulted in bradycardia in two studies (Baldridge et al., 2002; Guizar-Sahagun et al., 2004). This suggests that in the rat, most of the sympathoexcitatory input to the heart originates from rostral to T2 and that (partial) sparing of these supraspinal pathways is one of the mechanisms for the tachycardia seen in T4–5 lesions. Second, the reduction of peripheral resistance will activate the sympathetic system through arterial baroreceptors, and third, sympathetic input from below the lesion may be relayed through spinal interneurons (Rodenbaugh et al., 2003). Another cause of tachycardia that should be mentioned is related to the toxic effects of anesthetics on cardiac myocytes and performance. Although our control groups did not develop tachycardia, the combination of anesthesia and injury may result in a different susceptibility to anesthetic stressors such as cardiac histamine that is released subsequent to ischemia-reperfusion injury by toxic oxygen metabolites (Valen et al., 1993).

Previous studies have demonstrated that severity of hemodynamic derangements is related to injury severity (Guha et al., 1989; Maiorov et al., 1998), similar to the present findings. In addition, more severe injuries are also associated with more severe local (spinal cord) perfusion abnormalities. One study demonstrated a more pronounced decrease of spinal cord blood flow and inability to normalize the flow with adrenaline in more severe SCI, most likely a consequence of loss of autoregulation of spinal cord blood flow (Guha et al., 1989). The effect of injury severity on the magnitude of cardiovascular derangements can be explained by partial preservation of the bulbospinal sympathoexcitatory input to sympathetic preganglionic neurons of the cord below the level of injury in milder lesions. Although, restoration of arterial pressure can occur in spinal animals because of the facilitation of neurohumoral feedback mechanisms and/or changes in sodium and water homeostasis (Osborn et al., 1989), injury severity dependence implies that recovery of the bulbospinal neurogenic drive also contributes to this restoration of blood pressure. In addition to (partial) removal of the sympathetic input to the cardiovascular system, there is evidence for a role of the parasympathetic component of the autonomic nervous system in the pathogenesis of cardiovascular derangements after SCI. Bravo and associates (2001, 2002) used ganglionic and sympathetic blockers, adrenalectomy, and vagotomy to demonstrate that early cardiovascular alterations result from increased parasympathetic activity in combination with a sympathetic withdrawal (Bravo et al., 2001, 2002). In one of the studies, they demonstrate an additional role of endothelial-derived nitric oxide, probably released after cholinergic stimulation (Bravo et al., 2001). Furthermore, it has been shown that choline, administered intracerebroventricularly, can reverse hypotension in spinal cord transected rats by activating central nicotinic receptors via presynaptic mechanisms in rats in spinal shock. An increase in plasma vasopressin was seen associated with this pressor response (Savci and Ulus, 1998).

In our study we found a brief elevation of MAP at the time of impact in pentobarbital-anesthetized animals. A similar

finding was reported by Leal Filho and associates (2005), who demonstrated doubling of the systolic blood pressure in animals that underwent SCI at T8 under pentobarbital anesthesia followed by an immediate decrease in systolic blood pressure. Similar changes were seen in diastolic blood pressure. In contrast, in animals anesthetized with ketamine-xylazine in that study, only a mild elevation of systolic blood pressure and no change of diastolic blood pressure were seen. Hemodynamic parameters in that study were only determined before, during, and for 1 min after SCI. Interestingly, the study demonstrates that neurogenic pulmonary edema is more pronounced in rats anesthetized with pentobarbital than in rats anesthetized with ketamine-xylazine (Leal Filho et al., 2005).

Recovery of function is a critical outcome measurement in experimental SCI research. Our study demonstrates that cardiovascular function was depressed for a longer duration in animals anesthetized with pentobarbital than in animals anesthetized with inhalant isoflurane, and that this was associated with reduced recovery of locomotor function. This study adds to other studies and supports the hypothesis that ischemic/hypoxic conditions are detrimental to recovery after SCI (Yanagawa et al., 2001) and that aggressive resuscitation protocols in human and veterinary clinical medicine are justified (Vale et al., 1997). This also implies that in experimental SCI, more monitoring and acute care should be provided to bring the level of care of rodents used in these experiments to a level that is comparable to that provided for human and veterinary patients. The choice of anesthetic to be used during the SCI procedure is a critical factor in the experimental design, as it influences systemic DO_2 to tissues including the nervous system, and may affect lesion development and functional outcome measurements. We suggest that critical care monitoring should be part of a comprehensive program in experimental SCI.

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Address correspondence to:

Yvette S. Nout, D.V.M., Ph.D.

College of Agriculture

California State Polytechnic University

3801 W. Temple Avenue

Pomona, CA 91768

E-mail: yvette.nout@ucsf.edu