An Optimal Physiologic Model for Study of Murine Cardiac Function Under Inhalational Anesthesia

C. Constantinides[1], R. Mean[1], and L. W. Hedlund[2]
[1]Mechanical and Manufacturing Engineering, University of Cyprus, Nicosia, Cyprus, [2]Radiology, Duke University Medical Center, Durham, NC, United States

Introduction: While cardiac mechanical functional studies initially focused on large mammals and the human, the mouse emerged as the preferred animal species for research in recent years [1]. Albeit evidence supports that bioenergetically and hemodynamically the mouse scales linearly with larger mammals and humans [2; 3], important physiological questions still remain for the appropriateness of this model for extrapolation of conclusions to man [4, 5]. Since the complete characterization of the mouse and human genomes in 2002 and 2003 respectively [1], there has been a plethora of transgenic mouse studies targeting the cardiovascular system [6; 7]. Equally important were non-invasive imaging studies of such animals for phenotypic and genotypic screening, often conducted under inhalational anesthesia [8-12]. Anesthetics, however, are known to cause severe cardio-depression [9] with adverse physiological effects on hormonal release, centrally to the heart and peripherally to the vasculature [10; 11], at the cellular level, affecting calcium entry through L-type Ca^{2+} channels, the calcium binding sensitivity of the contractile proteins to calcium, and on conduction and excitability [10]. The objective of this study was to determine the isoflurane dose in normal mice for optimal physiological status (respiration, cardiac function, and metabolism) for a period of 1-2 hours post-induction, facilitating migration of such work to the non-invasive imaging platform of MRI, with tremendous potential for future basic science towards the phenotypic screening of transgenic mice and translational research.

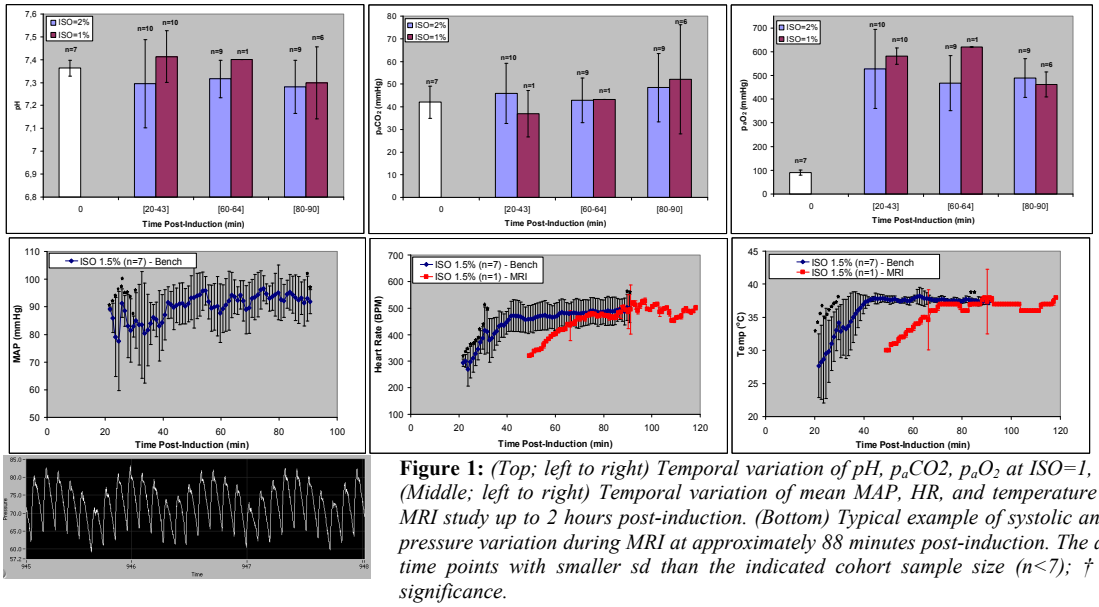

Figure 1: *(Top; left to right) Temporal variation of pH, p_aCO2, p_aO_2 at ISO=1, 2% post-induction. (Middle; left to right) Temporal variation of mean MAP, HR, and temperature for bench-top and MRI study up to 2 hours post-induction. (Bottom) Typical example of systolic and diastolic arterial pressure variation during MRI at approximately 88 minutes post-induction. The asterisk (*) denotes time points with smaller sd than the indicated cohort sample size (n<7); † denotes statistical significance.*

Methods: *Physiology Bench Studies*: Twenty-one C57BL/6 mice were initially induced with 4-5% ISO for 2-3 min. They were anesthetized with isoflurane (ISO) doses (1, 1.5, 2%) mixed with 100% oxygen throughout a 90-minute period post-induction, using a nose cone. ECG and breathing flowrate were monitored using a BIOPAC system (BIOPAC Inc., USA) recording system. Mean arterial pressure (MAP) was monitored using a catheter (PE10 tubing, Access Technologies) in the left carotid, attached to a pressure transducer and connected to the BIOPAC system. Periodic 50 μl blood samples were extracted from the carotid for blood gas analyses (pH, p_aCO2, p_aO_2, Na^+, K^+, Hct) using a BG Analyzer (Rapidlab, Bayer, USA). Proper mouse transportation and laboratory acclimation was assessed with steroid stressor hormone assays (cortisol, adrostenedione) from 1 ml arterial blood aliquots extracted at t=0 min. post-induction in a separate cohort of mice (n=5). Mouse metabolism was assessed with separate glucose and insulin assays from the same blood extracts at t=0 (n=5), 20 (n=4), 40 (n=4), 60 (n=4), and 80 (n=4) min. post-induction at each of the two target ISO doses (1, 2%) followed by euthanasia, in separate mouse cohorts. A rectal probe was used to maintain stable body temperature.
Physiology MRI Studies: The bench physiology protocol was reproduced in the MRI scanner. Work was performed at a 7T GE EXCITE scanner, with an automated heater-controlled air-blower system to maintain stable mouse body temperature. Two male C57BL/6J mice (weight±sd, 25.5±0.7g) were anesthetized using ISO at 4-5% and maintained at 1.5%. A fiber optic pressure catheter (Samba Inc., Sweeden) was positioned in the left carotid. The animals was placed in an imaging cradle and positioned in the bore of the MRI scanner. The mice were allowed to breathe freely throughout the study. ECG and breathing rates were monitored using an SA Instruments Inc. system (Stony Brook, NY, USA). A rectal probe was used to monitor and maintain stable body temperature in the magnet.
Physiological Data Processing: Physiological data from the bench and MRI studies (BIOPAC, SA, SAMBA) were exported in text files and read in Matlab (Matlab Inc., Natick, MA, USA) for processing. Custom written routines with an end-front graphical user interface allowed temporal synchronization of recorded data, selection of time bins at 1 minute intervals, and signal analyses that included computation of descriptive statistical and autocorrelation indices.
Results: Mean (n=5) cortisol and androstenedione levels were 160.4±13.7nmol/l and 0.017±0.006ng/ml immediately after induction. Glucose and insulin values ranged between 139.4±30.1–192±11.92 mg/dl/3.54±0.3–4.24±0.6 ng/dl [2% ISO], and between 176.3±26.1–188.8±11.1 mg/dl/3.4±0.5–4.2±0.9 mg/dl at 1% ISO, respectively. Figure 1 shows the temporal variation of BG analyses (pH, p_aCO_2) at 1 and 2%. Mean heart rates during the 90 min. period of 1, 1.5, and 2% ISO were 396.1±31.8, 449.2±56.5, and 463.4±37.6 bpm, respectively ($p<0.0001$) and 458.8±51.8 during the MRI study. Mean MAP was 83.1±6.9, 90.1±4.6, and 75.2±8.7mmHg ($p<0.0001$) for bench studies (0-90min). For the MRI study, systolic and diastolic pressures ranged during the last hour of the study between 70-85 and 65-40mmHg, respectively. Breathing rates were (80-150bpm) in all studies. Body temperature was kept at 36.4±2.1/36.3±2.6/36.6±1.5°C for bench studies and at 35.8±2.1 for the MRI study.
Discussion: Glucose values were significantly higher in the ISO=1.5 and 2% groups compared to the 1% group ($p<0.0001$). No significant temporal change was noted in glucose values at the 3 ISO doses. Insulin concentrations did not differ significantly between groups. BG results exhibit no statistically significance from normal baseline values (t=0) for both the ISO 1 and 2% groups at the 1% significance level. The results confirm that ISO levels ≥1.5% produce a mild hyperglycemic effect, prominent at later time periods post-induction. Temporal recordings at 90 minutes post-induction show a disruption of glucose metabolism and altered respiration patterns with a mild hyperglycemic anesthesia-induced effect. Mean heart rates are significantly different at 1 compared to 1.5 and 2% ISO ($p<0.0001$). Mean MAP values at 1.5% differ significantly from values at 1.0% ($p<0.0001$) and at the 2% ($p<0.0001$). Physiological recordings of HR and MAP indicate, in conjunction with biochemical results, that 1.5% is the optimal dose of anesthesia. Constancy of physiological indices under the current protocol support optimal and stable conditions for experimental studies, reproducible in the MRI scanner, establishing in this way a good platform for phenotypic screening and pharmacological studies of cardiac function in mice with MRI under anesthesia.

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