Title page

Impacts of different anesthetic agents on left ventricular systolic function in mice assessed by echocardiography

Chen Li[1], Jing Dai[2], Fan Wu[1], Huaxing Zhang[3*]

[1]Department of Physiology, Hebei Medical University, Shijiazhuang, Hebei, China
[2]Department of Clinical diagnostics, Hebei Medical University, Shijiazhuang, Hebei, China
[3]School of Basic Medical Sciences, Hebei Medical University, Shijiazhuang, Hebei, China

*Corresponding author: Huaxing Zhang
E-mail address: hxzhang2012@126.com
Address: School of Basic Medical Sciences, Hebei Medical University, 361 Zhongshan East Road, Shijiazhuang, Hebei 050017, China.

Short title: Impacts of anesthetics on cardiac function assessed by echocardiography

Summary

The present experiments were performed to study the effects and time trends of different anesthetic agents on the left ventricular (LV) systolic function and heart rate by high-resolution echocardiography in mice. Ten male C57BL/6J mice were submitted to echocardiography imaging separated by 72-hour intervals under the following conditions: 1) conscious mice, 2) mice anesthetized with isoflurane (ISO, inhaled), 3) mice anesthetized with tribromoethanol (TBE, intraperitoneal), 4) mice anesthetized with chloral hydrate (CH, intraperitoneal), and 5) mice anesthetized with pentobarbital sodium (PS, intraperitoneal). The effect of ISO, TBE, CH, and PS on LV systolic function was measured at 0, 1, 2, 3, 4, 6, 8, and 10 minutes after anesthesia. The results showed that LV systolic function and heart rate (HR) of anesthetized mice were reduced significantly ($P < 0.05$), compared with results in the same mice studied in the conscious state. In addition, the results indicated that the anesthetic with the least effect on LV function was CH, and followed by TBE, PS, ISO. We conclude that different anesthetic agents always depressed the HR and LV systolic function of mice, and, furthermore, the effects and time trends of different anesthetics on LV function are different. In echocardiographic experiments, we should choose proper anesthetic agents according to the experimental requirements.

Key words: echocardiography; mice; heart rate; left ventricular systolic function; anesthetic agents.

Introduction

Murine models for cardiovascular diseases have become more and more important during the last years, mainly because of the expanding availability of genetic models and gene knockout models, as well as the advantages of low cost and easy to control (Tarnavski *et al.* 2004, Yutzey and Robbins 2007, Houser *et al.* 2012, Hinton *et al.* 2008, Zhang *et al.* 2003). Echocardiography is a well-established non-invasive technology with the advantages of no radiation, dynamic observation, high repeatability, etc., and has been widely used to assess cardiac morphology and function in mice (Gardin *et al.* 1995, Hoit *et al.* 1995, Pollick *et al.* 1995, Tanaka *et al.* 1996, Prendiville *et al.* 2014).

To ensure sufficient sedation and immobilization for assessment of cardiac function by echocardiography, various anesthetic agents were used. However, it is well known that these agents have effects on cardiac function which have been investigated in several studies (Vatner and Braunwald 1975, Kiatchoosakun *et al.* 2001, Takuma *et al.* 2001, Yang *et al.* 1999, Rottman *et al.* 2003, Zuurbier *et al.* 2002).

In previous animal studies, various anesthetic agents have been used to achieve sedation for echocardiography in small rodents, such as isoflurane (Wu *et al.* 2010), pentobarbital sodium (Kawahara *et al.* 2005), chloral hydrate (Tanaka *et al.* 1996), thiopental (Lukasik and Gillies 2003), halothane (Chaves *et al.* 2001), tribromoethanol (Kiatchoosakun *et al.* 2001) and the combination of ketamine and xylazine (Hart *et al.* 2001, 23 Xu *et al.* 2007), meanwhile, the significant differences in echocardiographic parameters measured under the influence of different anesthetics were found (Akuma *et al.* 2001, Yang *et al.* 1999).

Riha *et al.* showed the effects of inhalation anesthesia with isoflurane on echocardiographic parameters in mice (Riha *et al.* 2012). Songsak *et al.* reported the effects of tribromoethanol anesthesia on echocardiographic assessment of left ventricular function in mice (Kiatchoosakun *et al.* 2001). Furthermore, the effects of different anesthetic agents on cardiac function and heart rate have been reported in previous studies (Yang *et al.* 1999, Pachon *et al.* 2015, Tanaka *et al.* 2016, Droogmans *et al.* 2008, Schaefer *et al.* 2005, Roth *et al.* 2002). In recent years, there were some controversy about the anesthetic agent which exerted the least depressant effects on left ventricular function and heart rate (Pachon *et al.* 2015, Tanaka *et al.* 2016). In addition, few studies have demonstrated the function characteristics and time trends of these anesthetic agents on left ventricular function (Rottman *et al.* 2003, Schaefer *et al.* 2005).

To our knowledge, various kinds of anesthetic agents have been used to sedate small rodents during echocardiography (as above), but the most frequently used anesthetic was isoflurane, in addition, tribromoethanol and pentobarbital sodium were commonly used intraperitoneal anesthetics in mice (Pachon *et al.* 2015). However, that does not mean that isoflurane exerts the least effects on cardiac function. The researchers reported that chloral hydrate had no significant effects on children's cardiac function, and concluded that chloral hydrate was a safe and successful anesthetic for use in children for transthoracic echocardiography (Napoli *et al.* 1996, Coskun *et al.* 2001). To see if we can get this kind of response in mice, we performed echocardiography in mice with and without anesthesia using a high-frequency, high-resolution digital imaging system (Vevo® 2100 Imaging System, FUJIFILM VisualSonics Inc., Toronto, Canada). Four anesthetics, isoflurane, tribromoethanol, chloral hydrate and pentobarbital sodium, were tested. We assessed the effects and time trends of different anesthetic agents on the left ventricular function and heart rate by the high-resolution echocardiography in mice.

Methods

Animals

A total of 10 healthy male wild-type C57BL/6J mice (8-10 weeks old, Vital River Laboratories, Beijing, China) weighing 20 to 26 g were used in this study. The animals were kept in standard conditions (12h light/dark cycle) under constant temperature (22°C ± 2°C) and humidity (60%), and given free access to food and water.

All our animal experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) of the United States and approved by the Ethics Committee for Laboratory Animals Care and Use of Hebei Medical University.

Experimental Protocols

Before the study, conscious mice were trained for echocardiographic measurements over a period of 3 days using the method described below (Pachon *et al.* 2015). We picked up the mouse by the nape of the neck and held it firmly in the palm of one hand in the supine position, with the tail held tightly between the last two fingers of the examiner, the probe of the ultrasound transducer was placed lightly on the mouse left hemithorax. Before echocardiography, the left hemithorax hair was carefully removed by using a commercial human depilatory cream.

The mice were randomly anesthetized by isoflurane, tribromoethanol, chloral hydrate, or pentobarbital sodium, followed by another measurement using a different anesthetic 72 hours later until all anesthetics were used (Pachon *et al.* 2015). Echocardiographic measurements were performed on mice at 0, 1, 2, 3, 4, 6, 8, and 10 minutes after anesthesia. The depth of anesthesia was determined by immobility and assessing the absence of the withdrawal reflex of the right paw.

Anesthesia and sedation

Isoflurane was used at 3% concentration for ~2 min for the induction of anesthesia and was then inhaled through a sealed nose cone at 1%-1.5% concentration for the maintenance of anesthesia (Ríha *et al.* 2012); 2.5% tribromoethanol was used at a dose of 290 mg/kg *ip* (Pachon *et al.* 2015, Roth *et al.* 2002); 3% chloral hydrate was used at a dose of 400 mg/kg *ip* (Wise and Munn 1995, Cao *et al.* 2018); and 1% pentobarbital sodium was used at a dose of 50 mg/kg *ip* (Rottman *et al.* 2003). The induction time which was defined as the time from injecting anesthetic agents to mice to sedation and effectual time which was defined as the time from sedation to awake were also recorded.

Echocardiographic Method

Transthoracic echocardiography was performed using a high-frequency, high-resolution digital imaging system (Vevo® 2100 Imaging System, FUJIFILM VisualSonics Inc., Toronto, Canada) with a high-frequency transducer probe (VisualSonics MS400, FUJIFILM VisualSonics, Inc., Toronto, Canada with a frequency range of 18-38 MHz).

After sedation, the mouse was placed in a supine position on a warming pad (38°C) to maintain normothermia. The gold-plated electrodes of the warming pad were attached to the mouse's legs by the gummed paper tape, and pre-warmed ultrasound gel was applied to the left hemithorax. Heart rate (HR) and respiratory physiology were continuously monitored by ECG electrodes. All echocardiograms were performed by one trained individual. Images were captured on cine loops at the time of the study and afterward measurements were done off-line.

The probe of the ultrasound transducer was placed slightly left of the sternum over the fourth

and sixth ribs by hand or by the use of a rail system. The parameters of the left ventricular end-diastolic dimension (LVEDD) and the LV end-systolic dimension (LVESD) are measured from M-mode images at the level of the papillary muscles. Each of these captured image loops included 10 to 20 cardiac cycles, and data were averages from at least 3 cycles per loop. Other parameters such as the LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), ejection fraction (EF), fractional shortening (FS), stroke volume (SV), and cardiac output (CO) were derived automatically by the Vevo® 2100 high-frequency, high-resolution digital imaging system from the following formulas:

(1) $LVEDV = [7.0/(2.4 + LVEDD)] \times LVEDD^3$;

(2) $LVESV = [7.0/(2.4 + LVESD)] \times LVEDD^3$;

(3) $EF\ (\%) = 100 \times [(LVEDV - LVESV)/LVEDV]$;

(4) $FS\ (\%) = 100 \times [(LVEDD - LVESD)/LVEDD]$;

(5) $SV = LVEDV – LVESV$;

(6) $CO = SV \times HR$.

Statistical Analysis

Results were expressed as mean ± SEM. Statistical analysis was performed using an SPSS software package, version 13.0 (SPSS, Inc., Chicago, IL, United States). Comparisons between two time points from one mouse were made using paired samples t-test. Comparisons between two groups were made using Student’s t-test. The results for three or more groups were compared using one-way ANOVA followed by Student Newman-Keuls t-test. $P < 0.05$ was considered statistically significant.

Results

Effects of anesthetics on left ventricular function and heart rate.

After sedation, the ejection fraction (EF), fractional shortening (FS), heart rate (HR), and cardiac output (CO) of anesthetized mice were reduced significantly ($P < 0.05$), compared with results in the same mice studied in the conscious state (Fig. 1A, 1B, 1E, and 1F). Nevertheless, there were no significant differences in the stroke volume (SV) between anesthetized mice and conscious mice (Fig. 1C). Regarding the left ventricular end-diastolic dimension (LVEDD), conscious animals had lower values than animals anesthetized with isoflurane (ISO) and pentobarbital sodium (PS) in Figure 1D. The results showed that chloral hydrate (CH) alone exerted the least depressant effects on left ventricular (LV) function, with tribromoethanol (TBE) second (Fig. 1A and 1B).

Time trends of the different anesthetic agents

Figures 2, 3, 4, and 5 showed time trends of the effects on cardiac function, induction time and effectual time of the four anesthetic agents.

Mice anesthetized with ISO showed a significant reduction in HR over the 10-minute study period, the LVEDD and the left ventricular end-systolic dimensions (LVESD) increased firstly then decreased with anesthesia time, while time trends of EF and FS were smooth (Fig. 2A, 2B and 2C). The induction time and effectual time of ISO were 2.4 minutes and 1.8 minutes, respectively (Fig. 2D).

In the study of mice anesthetized with TBE, it was found that the EF, FS and HR declined firstly and then rose with the anesthesia time, where as LVEDD and LVESD were of the opposite trend (Fig. 3A, 3B and 3C). The induction time and effectual time of TBE were 1.4 minutes and 10.8 minutes, respectively (Fig. 3D).

For mice anesthetized with CH, there was no obvious change of LV function and HR in the first 5 minutes, and then EF and FS decreased significantly, whereas HR, LVEDD and LVESD increased in the second 5 minutes (Fig. 4A, 4B and 4C). The induction time and effectual time of CH were 2.1 minutes and 63.4 minutes, respectively (Fig. 4D).

Administration of PS resulted in a significant decrease of HR, whereas EF, FS, LVEDD and LVESD remained unchanged (Fig. 5A, 5B and 5C). The induction time and effectual time of PS were 2.0 minutes and 59.1 minutes, respectively (Fig. 5D).

Discussion

Our study demonstrated that the use of any of the tested anesthetic agents induced a reduction in left ventricular (LV) function and heart rate (HR) compared to the same animals in the conscious state. The extent of these effects depended on the type of anesthesia and anesthetic timing.

A few studies have compared the effects of different anesthetic agents used for transthoracic echocardiography on echocardiographic parameters in rats (Pachon *et al.* 2015, Tanaka *et al.* 2016, Plante *et al.* 2006, Stein *et al.* 2007). Ronald *et al.* reported that ketamine exerted the least depressant effects on LV function and heart rate, with TBE second (Pachon *et al.* 2015). A previous study indicated that the combination of ketamine and xylazine might be the best anesthetic option for the in vivo assessment of cardiac systolic function in hamsters (Tanaka *et al.* 2016). As shown in Figures 1A and 1B, we observed that the anesthetic with the least effect on LV function was chloral hydrate (CH), and followed by tribromoethanol (TBE), pentobarbital sodium (PS), isoflurane (ISO). This suggested that CH or TBE could be used when it is not feasible to study mice in the conscious state. Even more important, there were no significant differences in the strake volume (SV) between anesthetized mice and conscious mice (Fig. 1C).

The present results also showed that the timing of echocardiographic measurements after anesthesia could affect echocardiographic parameters in mice. For more detailed measurements, we chose a shorter time interval (2 minutes) between echocardiographic measurements than others (Xu *et al.* 2007, Roth *et al.* 2002, Schaefer *et al.* 2005, Droogmans *et al.* 2008).

Our results showed that LV systolic function of mice anesthetized with PS was stable in 10 minutes after anesthesia, as well the left ventricular end-diastolic dimension (LVEDD) and the left ventricular end-systolic dimensions (LVESD). This suggested that PS can be used for the long-time echocardiographic measurements. Compared with other anesthetic agents, CH alone exerted the least depressant effects on LVEF and LVFS in 5 minutes after anesthesia, with TBE second, and it was consistent with the results above.

Inhalation anesthesia with ISO has currently been considered ideal and useful for experimental studies in the mouse because of its rapid conduction, safety, easy control of the depth of anesthesia (Collins *et al.* 2003, Butterfield *et al.* 2004, Szczesny *et al.* 2004, Matsuda *et al.* 2007). Previous research showed that over 75% of studies in mice with anesthesia used ISO (Pachon *et al.* 2015). A previous study found that less variability overtime in FS with ISO compared with TBE and ketamine-midazolam (Roth *et al.* 2002). ISO in our study showed less variation overtime in EF and FS compared with CH and TBE.

Anesthetic agent, timing of echocardiographic measurements, and genetic background are all critical variables during echocardiography in mice (Roth *et al.* 2002). Any anesthetic can affect the heart function and heart rate of mice, but it is comparable in the same group. In echocardiographic experiments, appropriate anesthesia should be selected according to the actual needs and the characteristics of anesthetics, and the consistency of anesthesia conditions in the whole experiment should be maintained. In the present study, the results indicate that chloral hydrate alone exerts the least depressant effects on LV function of wild-type C57BL/6J mice, but in view of good stability, PS is suited for the long-time echocardiographic measurements. Moreover, ISO is doubtlessly the safest anesthetic for experimental studies in the mouse.

In the present study, all anesthetic agents reduced heart rate and cardiac output significantly,

compared with results in the same animals studied in the conscious state (Fig. 1E and 1F), that may result in decreases in blood pressure (Droogmans *et al.* 2008). However, on the other hand, anesthetics also can inhibit the output of sympathetic nerves by inhibiting cardiovascular center, and then dilate blood vessels to lower blood pressure.

Conversely, decreased blood pressure can also affect cardiac function through baroreceptor reflex. In any case, our research only focused on the overall impact of different anesthetics on cardiac function in mice.

In addition, the effect of timing of echocardiographic measurement after anesthesia could be also affected by activation of both stress axes during experiment. Stress is associated with an increase in the activity of the hypothalamic-pituitary-adrenal axis. It has been shown that different anaesthetic/analgesic protocols may stimulate a stress response (Gil *et al.* 2010, González *et al.* 2001, González *et al.* 2006). Korneyev et al. also reported that anesthetic doses of ethanol, chloral hydrate, and urethane induce sharp and sustained dose-dependent increase in rat brain pregnenolone and progesterone content. In contrast, other general anesthetics such as ketamine, pentobarbital, and the sedative/hypnotic clonazepam decrease brain pregnenolone and progesterone content. Meanwhile, they found that blood-borne steroids fail to contribute to the anesthetic effect during anesthetic-induced activation of hypothalamic pituitary adrenal axis (Korneyev *et al.* 1993).

There were several limitations in this study. One was that we did not evaluate effects of different anesthetic agents' concentration on LV diastolic function in mice, and additionally, whether this protocol is also applicable to sick mice remains unknown. Another limitation was that we examined only one dose of each anesthetic, although doses of anesthetic agents were chosen to be in agreement with published reports. Future studies will be necessary to solve these limitations.

In summary, our study demonstrated that different anesthetic agents always depressed the HR and LV systolic function of mice, and, furthermore, these effects were dependent on anesthetic agent and the timing of echocardiographic measurements after anesthesia. From our study we concluded that for echocardiographic experiments in mice, we should choose proper anesthetic agents according to the demand, and more importantly, we should keep the timing of echocardiographic measurements after anesthesia consistent during the whole experiment.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors would like to thank Dr. Sheng Jin (Hebei Medical University, Shijiazhuang, Hebei, China) for his kindly guidance in statistics.

References

BUTTERFIELD NN, GRAF P, RIES CR, MACLEOD BA: The effect of repeated isoflurane anesthesia on spatial and psychomotor performance in young and aged mice. *Anesth Analg* **98**: 1305-1311, 2004.

CAO XY, HUA X, XIONG JW, ZHU WT, ZHANG J, CHEN L: Impact of Triclosan on Female Reproduction through Reducing Thyroid Hormones to Suppress Hypothalamic Kisspeptin Neurons in Mice. *Front Mol Neurosci* **11**: 6, 2018.

CHAVES AA, WEINSTEIN DM, BAUER JA: Non-invasive echocardiographic studies in mice: influence of anesthetic regimen. *Life Sci* **69**: 213-222, 2001.

COLLINS KA, KORCARZ CE, LANG RM: Use of echocardiography for the phenotypic assessment of genetically altered mice. *Physiol Genomics* **13**: 227-239, 2003.

COSKUN S, YUKSEL H, ONAG A: Chloralhydrate in children undergoing echocardiography. *Indian J Pediatr* **68**: 319-322, 2001.

DROOGMANS S, LAUWERS R, COSYNS B, ROOSENS B, FRANKEN PR, WEYTJENS C, BOSSUYT A, LAHOUTTE T, SCHOORS D, VAN CAMP G: Impact of anesthesia on valvular function in normal rats during echocardiography. *Ultrasound Med Biol* **34**: 1564-1572, 2008.

GARDIN JM, SIRI FM, KITSIS RN, EDWARDS JG, LEINWAND LA: Echocardiographic assessment of left ventricular mass and systolic function in mice. *Circ Res* **76**: 907-914, 1995.

GIL AG, MARTÍNEZ-MATEOS MM, LORENZO-GARCÍA P, ILLERA JC: Serum glucocorticoid concentrations after three different anaesthetic/analgesic protocols in rabbits. *Vet Rec* **166**: 562-563, 2010.

GONZÁLEZ GIL A, ILLERA JC, SILVÁN G, ILLERA M: Plasma glucocorticoid concentrations after fentanyl-droperidol, ketamine-xylazine and ketamine-diazepam anaesthesia in New Zealand white rabbits. *Vet Rec* **148**: 784-786, 2001.

GONZÁLEZ GIL A, SILVÁN G, GARCÍA-PARTIDA P, ILLERA JC: Serum glucocorticoid concentrations after halothane and isoflurane anaesthesia in New Zealand white rabbits. *Vet Rec* **159**: 51-52, 2006.

HART CY, BURNETT JC JR, REDFIELD MM: Effects of avertin versus xylazine-ketamine anesthesia on cardiac function in normal mice. *Am J Physiol Heart Circ Physiol* **281**: H1938-H1945, 2001.

HINTON RB JR, ALFIERI CM, WITT SA, GLASCOCK BJ, KHOURY PR, BENSON DW, YUTZEY KE: Mouse heart valve structure and function: echocardiographic and morphometric analyses from the fetus through the aged adult. *Am J Physiol Heart Circ Physiol* **294**: H2480-H2488, 2008.

HOIT BD, KHOURY SF, KRANIAS EG, BALL N, WALSH RA: In vivo echocardiographic detection of enhanced left ventricular function in gene-targeted mice with phospholamban deficiency. *Circ Res* **77**: 632-637, 1995.

HOUSER SR, MARGULIES KB, MURPHY AM, SPINALE FG, FRANCIS GS, PRABHU SD, ROCKMAN HA, KASS DA, MOLKENTIN JD, SUSSMAN MA, KOCH WJ: Animal models of heart failure: A scientific statement from the American heart association. *Circ Res* **111**: 131-150, 2012.

KAWAHARA Y, TANONAKA K, DAICHO T, NAWA M, OIKAWA R, NASA Y, TAKEO S: Preferable anesthetic conditions for echocardiographic determination of murine cardiac function. *J Pharmacol Sci* **99**: 95-104, 2005.

KIATCHOOSAKUN S, KIRKPATRICK D, HOIT BD: Effects of tribromoethanol anesthesia

on echocardiographic assessment of left ventricular function in mice. *Comp Med* **51**: 26-29, 2001.
KORNEYEV AY, COSTA E, GUIDOTTI A: During anesthetic-induced activation of hypothalamic pituitary adrenal axis, blood-borne steroids fail to contribute to the anesthetic effect. *Neuroendocrinology* **57**: 559-565, 1993.
LUKASIK VM, GILLIES RJ: Animal anaesthesia for in vivo magnetic resonance. *NMR Biomed* **16**: 459-467, 2003.
MATSUDA Y, OHSAKA K, YAMAMOTO H, NATSUME K, HIRABAYASHI S, KOUNOIKE M, INOUE M: Comparison of newly developed inhalation anesthesia system and intraperitoneal anesthesia on the hemodynamic state in mice. *Biol Pharm Bull* **30**: 1716-1720, 2007.
NAPOLI KL, INGALL CG, MARTIN GR: Safety and efficacy of chloral hydrate sedation in children undergoing echocardiography. *J Pediatr* **129**: 287-291, 1996.
PACHON RE, SCHARF BA, VATNER DE, VATNER SF: Best anesthetics for assessing left ventricular systolic function by echocardiography in mice. *Am J Physiol Heart Circ Physiol* **308**: H1525-H1529, 2015.
PLANTE E, LACHANCE D, ROUSSEL E, DROLET MC, ARSENAULT M, COUET J: Impact of anesthesia on echocardiographic evaluation of systolic and diastolic function in rats. *J Am Soc Echocardiogr* **19**: 1520-1525, 2006.
POLLICK C, HALE SL, KLONER RA: Echocardiographic and cardiac Doppler assessment of mice. *J Am Soc Echocardiogr* **8**: 602-610, 1995.
PRENDIVILLE TW, MA Q, LIN Z, ZHOU P, HE A, PU WT: Ultrasound-guided transthoracic intramyocardial injection in mice. *J Vis Exp* **90**: e51566, 2014.
RIHA H, PAPOUŠEK F, NECKÁŘ J, PIRK J AND OŠŤÁDAL B: Effects of isoflurane concentration on basic echocardiographic parameters of the left ventricle in rats. *Physiol Res* **61**: 419-423, 2012.
ROTH DM, SWANEY JS, DALTON ND, GILPIN EA, ROSS J JR: Impact of anesthesia on cardiac function during echocardiography in mice. *Am J Physiol Heart Circ Physiol* **282**: H2134-H2140, 2002.
ROTTMAN JN, NI G, KHOO M, WANG Z, ZHANG W, ANDERSON ME, MADU EC: Temporal changes in ventricular function assessed echocardiographically in conscious and anesthetized mice. *J Am Soc Echocar diogr* **16**: 1150-1157, 2003.
SCHAEFER A, MEYER GP, BRAND B, HILFIKER-KLEINER D, DREXLER H, KLEIN G: Effects of anesthesia on diastolic function in mice assessed by echocardiography. *Echocardiography* **22**: 665-670, 2005.
STEIN AB, TIWARI S, THOMAS P, HUNT G, LEVENT C, STODDARD MF, TANG XL, BOLLI R, DAWN B: Effects of anesthesia on echocardiographic assessment of left ventricular structure and function in rats. *Basic Res Cardiol* **102**: 28-41, 2007.
SZCZESNY G, VEIHELMANN A, MASSBERG S, NOLTE D, MESSMER K: Long-term anaesthesia using inhalatory isoflurane in different strains of mice: the haemodynamic effects. *Lab Anim* **38**: 64-69, 2004.
TAKUMA S, SUEHIRO K, CARDINALE C, HOZUMI T, YANO H, SHIMIZU J, MULLIS-JANSSON S, SCIACCA R, WANG J, BURKHOFF D, DI TULLIO MR, HOMMA S: Anesthetic inhibition in ischemic and nonischemic murine heart: Comparison with conscious echocardiographic approach. *Am J Physiol Heart Circ Physiol* **280**: H2364-H2370, 2001.

TANAKA N, DALTON N, MAO L, ROCKMAN HA, PETERSON KL, GOTTSHALL KR, HUNTER JJ, CHIEN KR, ROSS J JR: Transthoracic echocardiography in models of cardiac disease in the mouse. *Circulation* **94**: 1109-1117, 1996.

TANAKA DM, ROMANO MM, CARVALHO EE, OLIVEIRA LF, SOUZA HC, MACIEL BC, SALGADO HC, FAZAN-JÚNIOR R, SIMÕES MV: Effect of different anesthetic agents on left ventricular systolic function assessed by echocardiography in hamsters. *Braz J Med Biol Res* **49**: e5294, 2016.

TARNAVSKI O, MCMULLEN JR, SCHINKE M, NIE Q, KONG S, IZUMO S: Mouse cardiac surgery: comprehensive techniques for the generation of mouse models of human diseases and their application for genomic studies. *Physiol Genomics* **16**: 349-360, 2004.

VATNER SF, BRAUNWALD E: Cardiovascular control mechanisms in the conscious state. *N Engl J Med* **293**: 970-976, 1975.

WISE RA, MUNN E: Withdrawal from chronic amphetamine elevates baseline intracranial self-stimulation thresholds. *Psychopharmacology (Berl)* **117**: 130-136, 1995.

WU J, BU L, GONG H, JIANG G, LI L, MA H, ZHOU N, LIN L, CHEN Z, YE Y, NIU Y, SUN A, GE J, ZOU Y: Effects of heart rate and anesthetic timing on high-resolution echocardiographic assessment under isoflurane anesthesia in mice. *J Ultrasound Med* **29**: 1771-1778, 2010.

XU Q, MING Z, DART AM, DU XJ: Optimizing dosage of ketamine and xylazine in murine echocardiography. *Clin Exp Pharmacol Physiol* **34**: 499-507, 2007.

YANG XP, LIU YH, RHALEB NE, KURIHARA N, KIM HE, CARRETERO OA: Echocardiographic assessment of cardiac function in conscious and anesthetized mice. *Am J Physiol* **277**: H1967-H1974, 1999.

YUTZEY KE, ROBBINS J: Principles of genetic murine models for cardiac disease. *Circulation* **115**: 792-799, 2007.

ZHANG T, MAIER LS, DALTON ND, MIYAMOTO S, ROSS J JR, BERS DM, BROWN JH: The deltaC isoform of CaMKII is activated in cardiac hypertrophy and induces dilated cardiomyopathy and heart failure. *Circ Res* **92**: 912-919, 2003.

ZUURBIER CJ, EMONS VM, INCE C: Hemodynamics of anesthetized ventilated mouse models: Aspects of anesthetics, fluid support, and strain. *Am J Physiol Heart Circ Physiol* **282**: H2099-H2105, 2002.

Figure legends

Fig. 1. A, Ejection fraction of conscious and anesthetized mice; B, Fractional shortening of conscious and anesthetized mice; C, Stroke volume of conscious and anesthetized mice; D, Left ventricular internal dimension diastole of conscious and anesthetized mice; E, Heart rate of conscious and anesthetized mice; F, Cardiac output of conscious and anesthetized mice. Con: conscious; ISO: isoflurane; TBE: tribromoethanol; CH: chloral hydrate; PS: pentobarbital sodium. Repeated measures ANOVA was used for statistical analysis.

Fig. 2. Measured and derived echocardiographic parameters of C57BL/6J mice with isoflurane anesthesia at 0, 1, 2, 3, 4, 6, 8, and 10 min after anesthesia. A, ejection fraction and fractional shortening. B, heart rate, strake volume, and cardiac output. C, left ventricular internal dimension (diastole and systole). D, induction time and effectual time of isoflurane. **Fig. 3.** Measured and derived echocardiographic parameters of C57BL/6J mice with tribromoethanol anesthesia at 0, 1, 2, 3, 4, 6, 8, and 10 min after anesthesia. A, ejection fraction and fractional shortening. B, heart rate, strake volume, and cardiac output. C, left ventricular internal dimension (diastole and systole). D, induction time and effectual time of tribromoethanol.

Fig. 4. Measured and derived echocardiographic parameters of C57BL/6J mice with chloral hydrate anesthesia at 0, 1, 2, 3, 4, 6, 8, and 10 min after anesthesia. A, ejection fraction and fractional shortening. B, heart rate, strake volume, and cardiac output. C, left ventricular internal dimension (diastole and systole). D, induction time and effectual time of chloral hydrate.

Fig. 5. Measured and derived echocardiographic parameters of C57BL/6J mice with pentobarbital sodium anesthesia at 0, 1, 2, 3, 4, 6, 8, and 10 min after anesthesia. A, ejection fraction and fractional shortening. B, heart rate, strake volume, and cardiac output. C, left ventricular internal dimension (diastole and systole). D, induction time and effectual time of pentobarbital sodium.

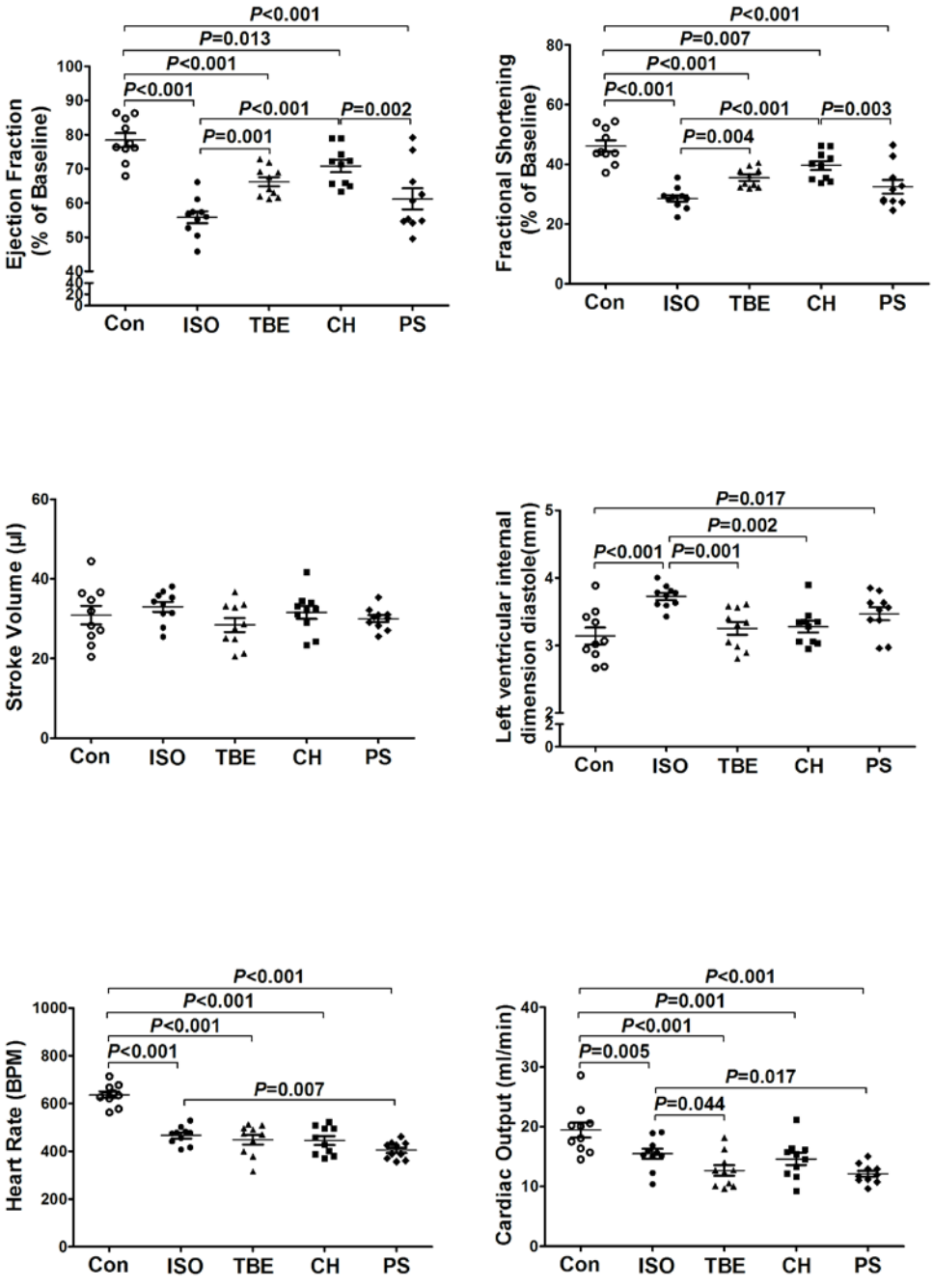
Ejection Fraction (% of Baseline)
P<0.001
P=0.013
P<0.001
P<0.001
P<0.001
P=0.002
P=0.001
Con
ISO
TBE
CH
PS
Fractional Shortening (% of Baseline)
P<0.001
P=0.007
P<0.001
P<0.001
P<0.001
P=0.003
P=0.004
Stroke Volume (μl)
Left ventricular internal dimension diastole(mm)
P=0.017
P=0.002
P<0.001
P=0.001
Heart Rate (BPM)
P<0.001
P<0.001
P<0.001
P<0.001
P=0.007
Cardiac Output (ml/min)
P<0.001
P=0.001
P<0.001
P=0.005
P=0.017
P=0.044

Fig. 1.

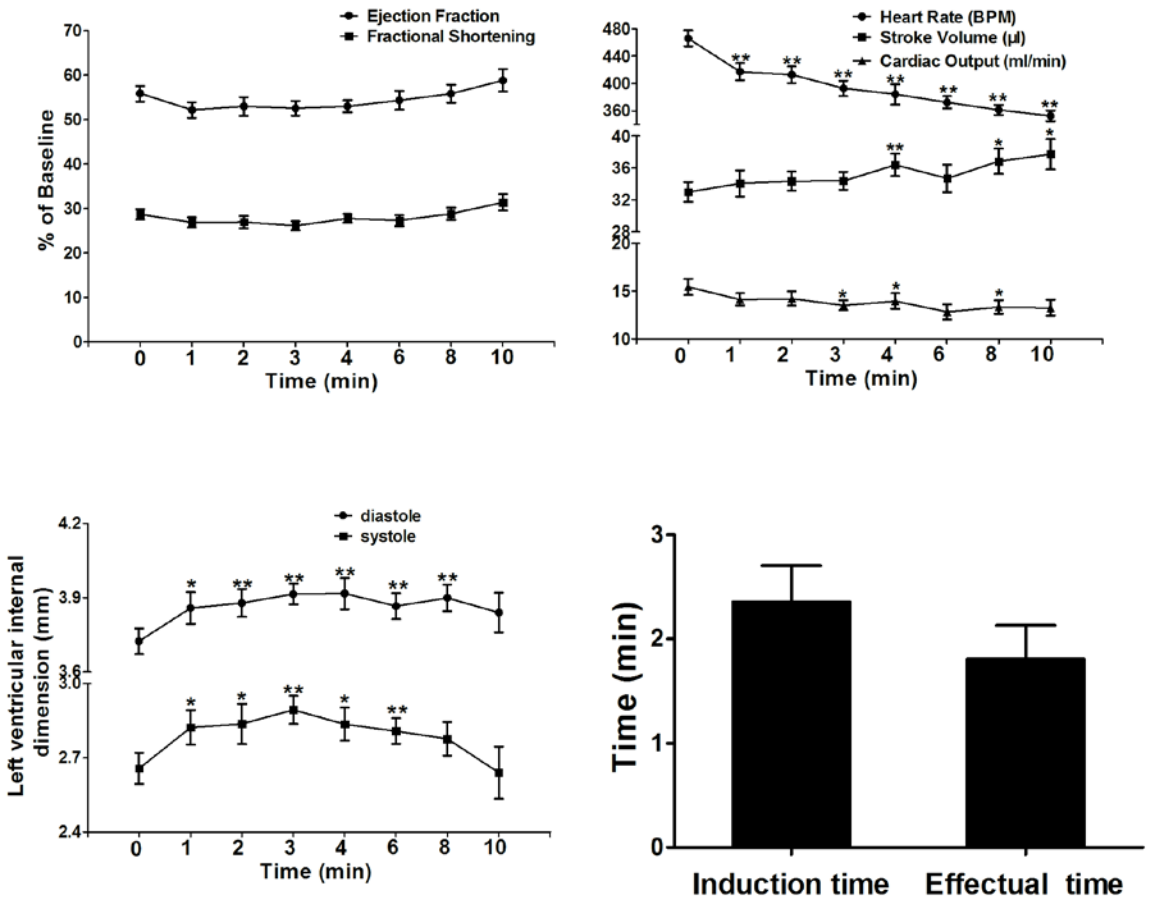
Ejection Fraction
Fractional Shortening
% of Baseline
Time (min)
Heart Rate (BPM)
Stroke Volume (μl)
Cardiac Output (ml/min)
Time (min)
diastole
systole
Left ventricular internal dimension (mm)
Time (min)
Time (min)
Induction time
Effectual time

Fig. 2.

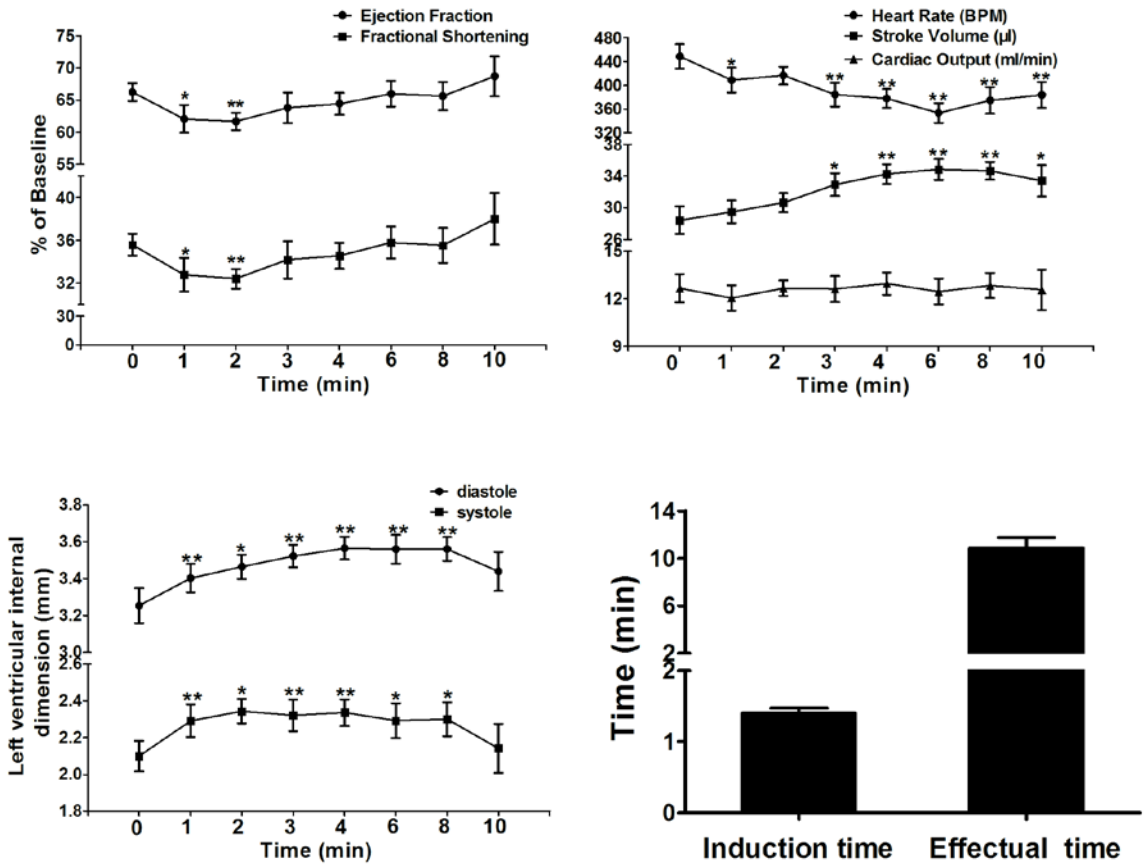
Ejection Fraction
Fractional Shortening
% of Baseline
Time (min)
Heart Rate (BPM)
Stroke Volume (μl)
Cardiac Output (ml/min)
Time (min)
diastole
systole
Left ventricular internal dimension (mm)
Time (min)
Time (min)
Induction time
Effectual time

Fig. 3.

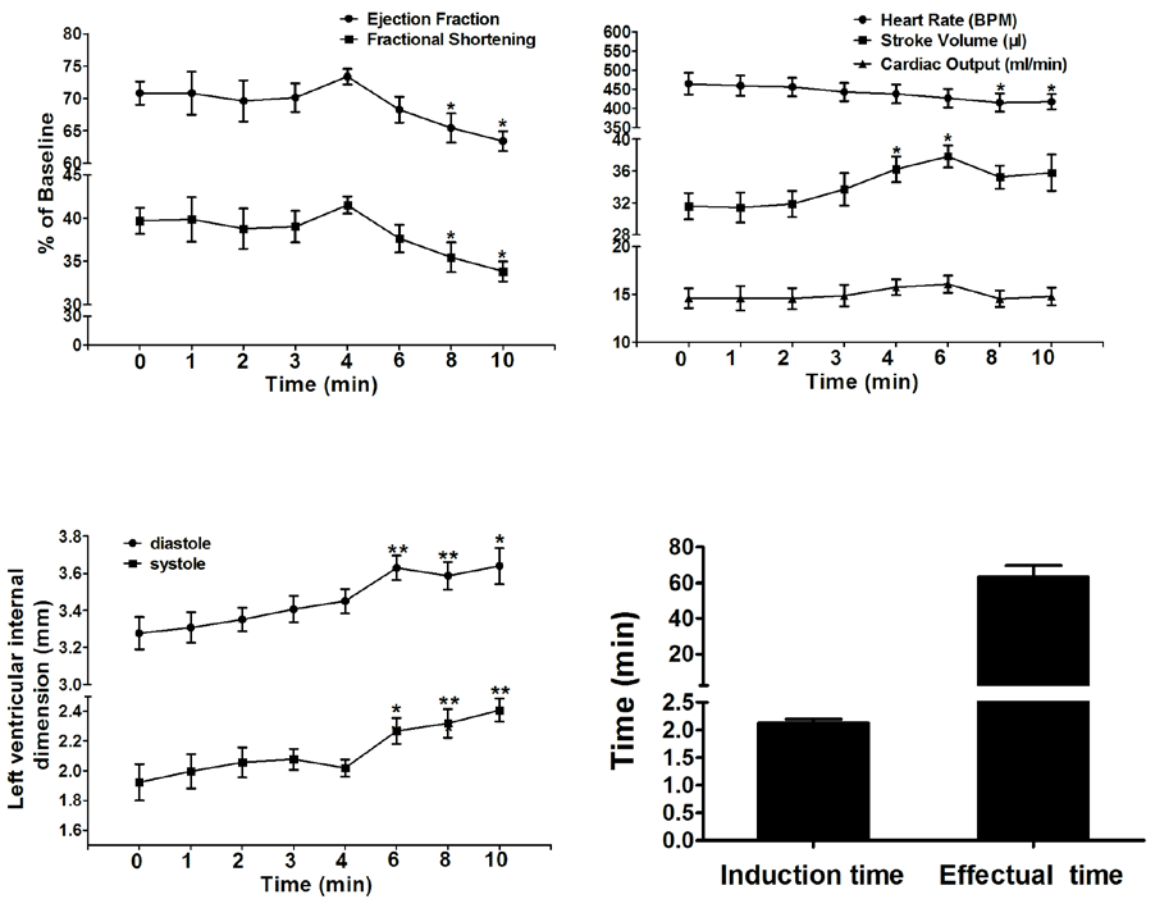
Ejection Fraction
Fractional Shortening
% of Baseline
Time (min)
Heart Rate (BPM)
Stroke Volume (μl)
Cardiac Output (ml/min)
Time (min)
diastole
systole
Left ventricular internal dimension (mm)
Time (min)
Time (min)
Induction time
Effectual time

Fig. 4.

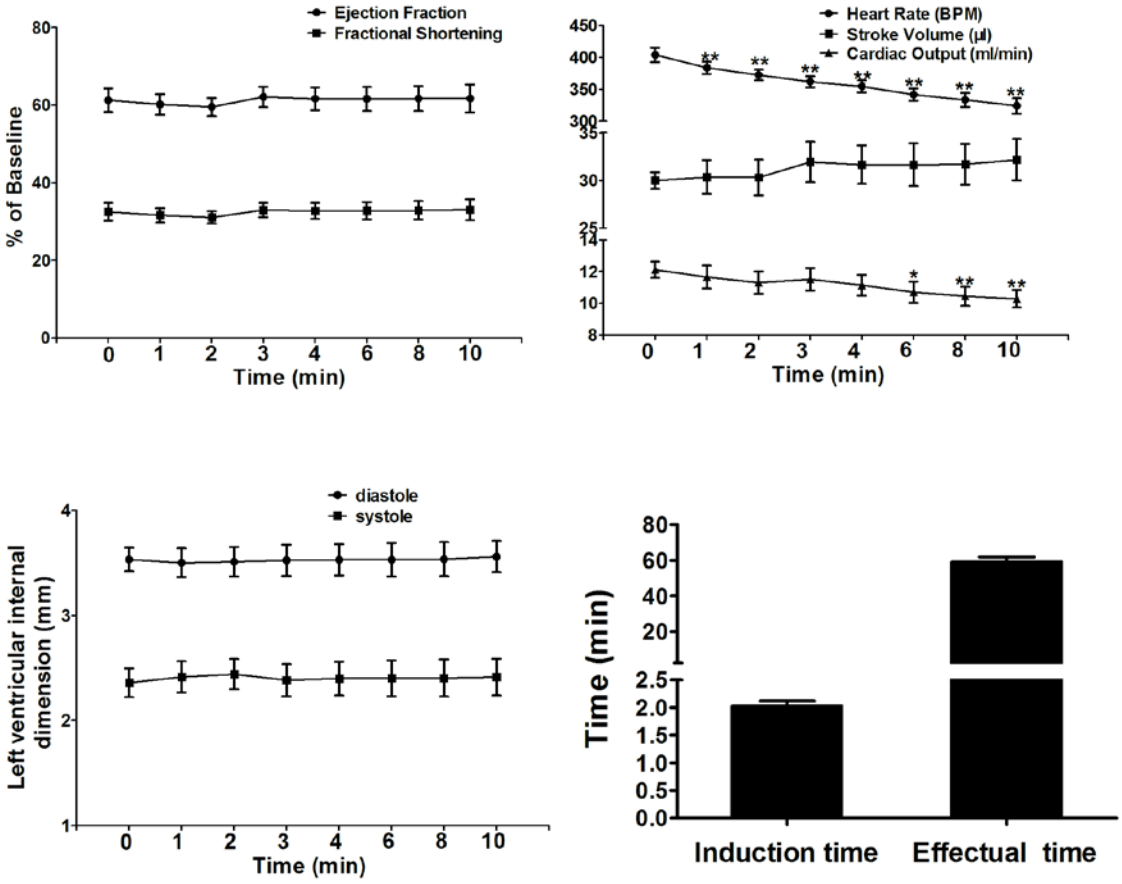
Ejection Fraction
Fractional Shortening
% of Baseline
Time (min)
Heart Rate (BPM)
Stroke Volume (μl)
Cardiac Output (ml/min)
Time (min)
diastole
systole
Left ventricular internal dimension (mm)
Time (min)
Time (min)
Induction time
Effectual time

Fig. 5.