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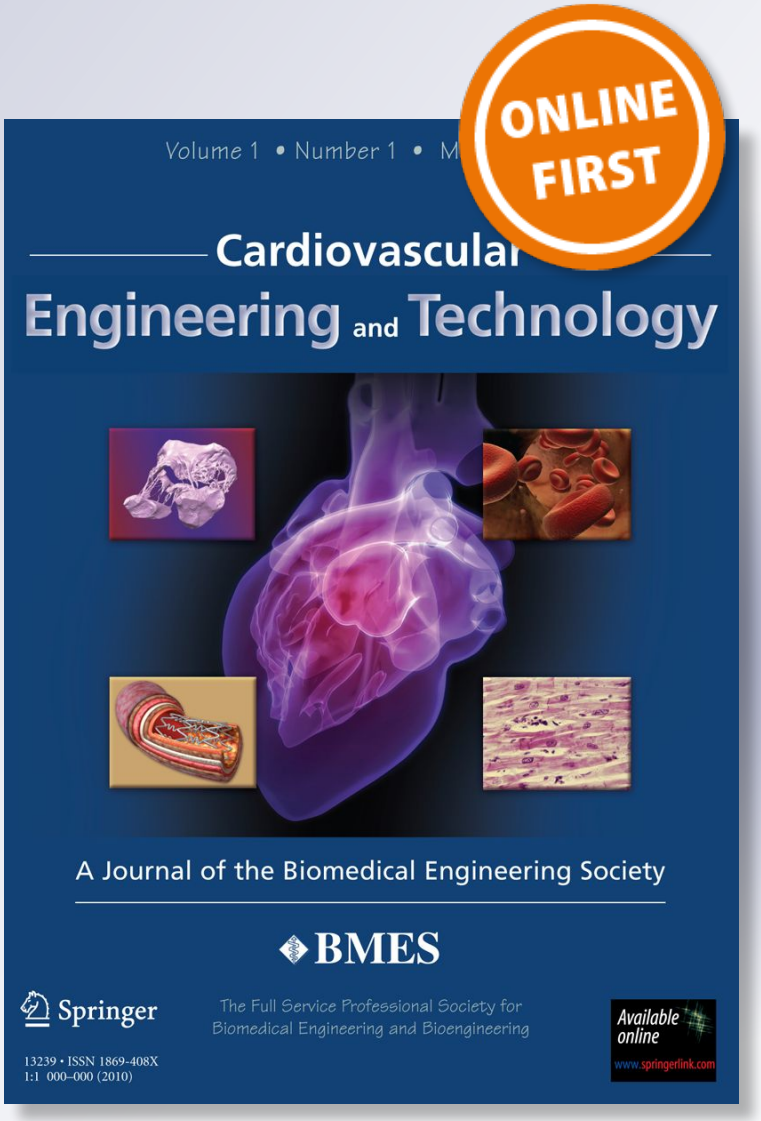

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Evaluation of Hemodynamic Autonomic Control in an Animal Model of Acute Heart Failure Induced by High Dose of Halothane

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Abstract—Acute animal models of cardiac failure are necessary to study new therapeutic options and should be thoroughly characterized from the hemodynamic point of view, including the response of the autonomic nervous system. Thus, the aim of this work was to characterize the pathophysiological adaptation of the autonomic nervous system to acute cardiac failure induced by high doses of halothane (4%). In six sheep, electrocardiogram, aortic pressure and flow were obtained and calculation of systemic vascular resistances was done. Variability analyses in the time and frequency domains were also performed. In the time domain, after heart failure induction using halothane 4%, a significant decrease of both aortic blood flow variability (from 0.13 ± 0.05 to 0.09 ± 0.02 L min^{-1}, $p < 0.05$) and the broad band spectra (from 1.80 ± 0.66 to 1.25 ± 0.57 L^2 min^{-2}, $p < 0.005$) was observed. Both mean RR (472 ± 44 to 567 ± 68 ms, $p < 0.01$), and low frequency band of RR intervals (from 6.2 ± 0.9 to 7.7 ± 1.5 ms^2, $p < 0.05$), showed a significant increase, and no change in systemic vascular resistance (from 54.9 ± 29.5 to 50.3 ± 38.4 mmHg min L^{-1}), all of them after heart failure induction. We conclude that in this model of heart failure the autonomic nervous system activity is still functioning, the combination of increased mean and RR low frequency band, with no change in systemic vascular resistance suggest an increase in the sympathetic control (due to maintained SVR), in an attempt to compensate the depression in the cardiac activity and hemodynamic alterations after severe myocardial depression induced by halothane.

Keywords—Heart rate variability, Sympathetic control, Parasympathetic control, Cardiac failure.

INTRODUCTION

Heart failure syndrome is the last stage of almost all cardiopathies having a significant high rate of mortality. Besides, there are an increasing and dramatic number of hospitalizations due to acute decompensations.[1,14]

Therapeutic options include not only pharmacological treatments but also the use of mechanical assist devices, developed to obtain a hemodynamic improvement of acute heart failure. To this scope, in 1967, Dr. Kantrowitz introduced the intra-aortic balloon pump that, for the first time, allowed the recovery of patients with left ventricular failure post myocardial infarction.[21] However, in spite of all pharmacological options and newer devices developments, reversion of very severe circulatory failure still remains a significant cause of mortality. As the consequence of the above described, several models of acute heart failure were developed as a tool to test new treatments.[11,19,26]

More recently, with the aim to develop an acute animal model of severe cardiac failure, without the undesirable characteristics previously described,[11,16,19,26,35] the authors reported studies performed in dogs and sheep in which high doses of halothane (3 or 4%) were administered.[4–6] The myocardial depression resulting from this pharmacological intervention was due to the well-known negative inotropic effect of halothane.[34] So this model was used to study different techniques of cardiovascular assistance.[7] In addition, it must be mentioned that therapeutic doses of halothane are able to decrease the barorreflex function, as it was previously described.[3,10,12] However, to the best of our knowledge, no studies of the barorreflex control and variability in experimental animals in which severe cardiac failure were induced with high doses of halothane (4%) have been yet reported.

Thus, the aim of this study was to characterize the pathophysiological hemodynamical changes produced by the autonomic nervous system in acute cardiac failure induced by high doses of halothane.

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MATERIAL AND METHODS

Six adult Corriedale sheep weighing 30 ± 3 kg and aged 12 ± 3 months were included in this study. All animals were quarantined, vaccinated, treated for skin and intestinal parasites, before the experimental session. An overnight fast was mandatory for each animal before surgery. Anesthesia was induced with sodium thiopentone (20 mg kg^{-1}, intravenously) and later maintained with halothane (1%). In all cases, respiratory mechanical assistance was performed using a ventilator (Neumovent 910, Tecme S. A., Cordoba, Argentina). A pulse oximeter was used in all animals (Pulse Oximeter 515A, Novametrix Medical Systems Inc., Wallingford, USA). Fine adjustments were made in the tidal volume and respiratory rate to maintain PO_2 (oxygen partial pressure) within physiological limits.

Surgery and Sensor Placement

A thoracotomy was performed at the level of the fourth intercostal space and an ultrasonic flowmeter (Model T206, filter setting: 100 Hz, Transonic Systems Inc., 16A/20A Probes, Ithaca, NY, USA) was positioned in the ascending aorta (Fig. 1). Afterwards, a pressure microtransducer (Millar microtip catheter) was positioned in the aortic arch near ultrasonic flowmeter. Finally, the operator confirmed that the quality of biological signals were optimal.

All animals received Heparin 300 units kg^{-1}. Besides, flush solutions used during the experimental session were heparinized. To maintain the temperature of the animals at 37.5 °C, an electrical heating blanket was used.[7]

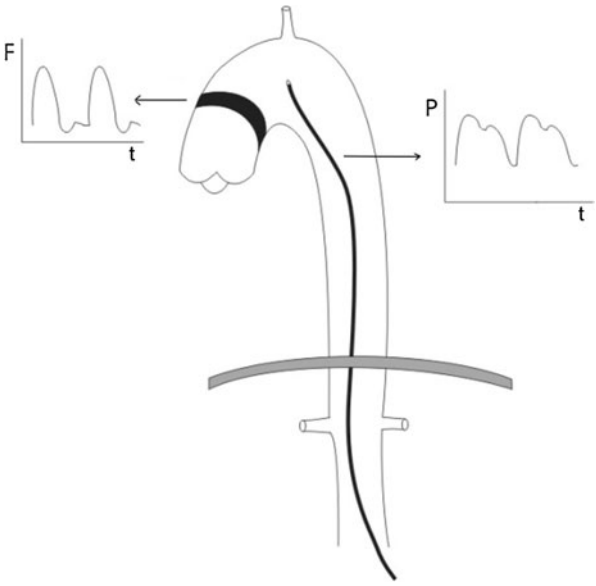

FIGURE 1. Sensors placement. Aortic pressure (*P*) was measured by a microtip catheter and aortic blood flow (*F*) was monitored by an ultrasonic flowmeter placed around ascending aorta.

Measurements

Along all experiments a surface electrocardiogram and mean aortic flow were visualized in order to monitor the hemodynamic state. The former together with the pressure signal, were electronically amplified (Model 4600 Conditioner Cage Gould Inc., Cleveland, OH, USA) in all cases.[5,7]

Electrocardiogram, pressure and flow signals digitization were performed using a data acquisition board (PCI 1200, National Instruments, Austin, TX, USA), and a LabView 8.0 software (National Instruments, Austin, TX, USA), specifically developed in our Laboratory. All signals were stored during the administration of halothane at 1% and after heart failure induction using halothane 4%. The sampling frequency was set at 500 Hz.

Control state was defined as that in which pressure and blood flow signals remained stable at least 5 min during halothane 1% administration, and cardiac failure was considered the state in which depressed systolic aortic pressures remained at least 5 min in steady state. Storage of all signals in control and cardiac failure state was performed during 15 min in each experiment. As can be seen in Fig. 2, aortic pressure decrease and remain stable after around 15 min of halothane 4% administration and remain stable for 1.5 min (steady state).

Each animal was euthanized with an overdose of sodium thiopentone after the experimental session, in compliance with the "Guide for the care and use of laboratory animals" published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985).

Data Analysis

The systemic vascular resistance (SVR) was calculated as:

$$SVR = \frac{MAP}{MAF}$$

where MAF was the mean aortic blood flow and MAP is the mean aortic pressure.[27]

The study of the autonomic control was performed by a variability analysis of RR interval and mean aortic pressure. In addition, mean aortic flow and SVR variability was evaluated. When aortic pressure and flow signals remained stable for a period of 15 min after the administration of halothane 4%, data acquisition for heart failure state was begun. To determine the RR interval, a specific detection algorithm through R wave

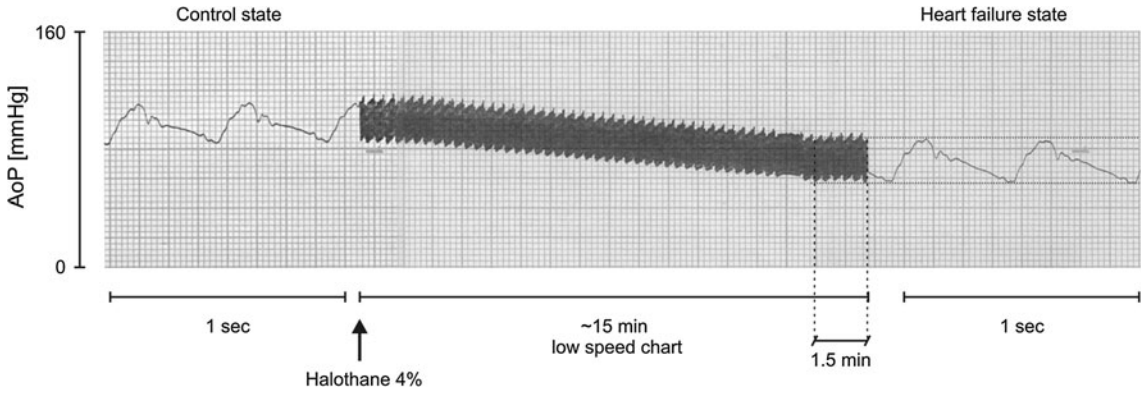

FIGURE 2. Aortic pressure. Aortic pressure (AoP) measured before and after a prolonged halothane administration. Note that the quick decrease of aortic pressure is maintained during halothane 4% administration; however in the last 1.5 min, AoP remains stable.

identification was designed. Electrical artifacts and cardiac arrhythmias recorded along each experimental session were corrected. Abnormal RR intervals, defined as those who differ more than 15% of the previous RR interval, were completely removed.

Our analysis includes that for each beat recorded; we calculated the values of RR interval, mean aortic pressure, mean aortic blood flow, and SVR. Then, beat series of all signals were constructed.[31]

Hemodynamical beat series were sampled at a constant rate of 4 Hz for each animal for temporal series generation, and then de-trending was performed. The variability analysis was performed in both, the time and frequency domain. The biological signals obtained were analyzed in the time domain that includes calculus of the mean and the standard deviation (SD). These calculations allow us to know the dispersion of the recorded values (variability) around the average of each variable analyzed.

We utilized the power spectral density analysis of the temporal series of signals for the study in the frequency domain. The method used to estimate power spectral density was based on Fast Fourier Transformation. The heart rate and MAP variability in the frequency domain were obtained using the following spectral measurements: (a) low frequency band (LF), which represents the sympathetic and parasympathetic nervous system activity (range 0.04 and 0.15 Hz) and (b) high frequency band (HF), which represents the parasympathetic nervous system activity (range 0.15 and 0.4 Hz). Variability of SVR and MAF was evaluated using broad band spectra (BBS), energy in the heart period power spectrum up to 0.4 Hz.[20] To normalize the distributions of the LF and HF indexes, we used a natural logarithm transformation.

Values are expressed as mean ± SD. All data were analyzed using two-tailed paired Student t tests. A $p < 0.05$ was considered as an indicator of statistical significance. Statistical analysis was performed using R language.[30]

TABLE 1. Hemodynamic data in control and induced heart failure (n = 6).

	Control	Heart failure	p
Mean AoP (mmHg)	92.09 ± 21.07	69.25 ± 20.04	0.001
Mean RR (ms)	472.98 ± 44.42	567.07 ± 67.87	0.008
Mean AoF (L min^{-1})	2.02 ± 0.80	1.93 ± 1.10	0.699
SVR (mmHg min L^{-1})	54.90 ± 29.47	50.28 ± 38.42	0.304

Values are mean ± SD. RR: temporal interval between R waves. AoF: aortic flow. AoP: aortic pressure. SVR: systemic vascular resistance. p values determined by two-tailed paired t test.

RESULTS

There were no animal deaths or cardiac arrhythmias during any experimental session, so all signals could be included in the data analysis.

As can be seen in Table 1, cardiac failure induced by halothane 4% was characterized by a significant decrease in MAP ($p < 0.005$), accompanied by a significant increase of RR intervals ($p < 0.01$). MAF and SVR failed to show any change.

Very few artefacts and ectopic beats were detected. The analysis in the time domain (Table 2), showed a significant decrease of aortic flow variability ($p < 0.05$) without changes in the other parameters evaluated.

The frequency domain analysis is shown in Table 3. LF values of RR intervals increased significantly ($p < 0.05$) in acute heart failure. In accordance to that observed in the time domain, MAF BBS showed a significant decrease ($p < 0.005$), while no significant differences were found in the variability analysis of MAP and SVR.

TABLE 2. Time domain results in control and induced heart failure ($n = 6$).

	Control	Heart failure	p
SD mean AoP (mmHg)	0.95 ± 0.29	0.86 ± 0.35	0.573
SD RR (ms)	2.08 ± 0.72	2.32 ± 0.65	0.541
SD mean AoF (L min^{-1})	0.13 ± 0.05	0.09 ± 0.02	0.022
SD SVR (mmHg min L^{-1})	3.74 ± 3.08	2.88 ± 2.96	0.088

Values are mean ± SD. RR: temporal interval between R waves. AoP: aortic pressure. AoF: aortic flow. SVR: systemic vascular resistance. p values determined by two-tailed paired t test.

TABLE 3. Frequency domain results in control and induced heart failure ($n = 6$).

	Control	Heart failure	p
LF AoP ($mmHg^2$)	2.48 ± 1.25	3.66 ± 0.74	0.144
HF AoP ($mmHg^2$)	6.23 ± 0.72	5.95 ± 0.81	0.473
LF RR (ms^2)	6.16 ± 0.93	7.65 ± 1.45	0.044
HF RR (ms^2)	7.09 ± 0.80	7.32 ± 0.41	0.353
BBS AoF (L^2 min^{-2})	1.80 ± 0.66	1.25 ± 0.57	0.001
BBS SVR ($mmHg^2$ min^2 L^{-2})	8.24 ± 1.55	7.15 ± 2.60	0.075

Values are mean ± SD. RR: temporal interval between R waves. AoP: aortic pressure. AoF: aortic flow. SVR: systemic vascular resistance. LF: natural logarithm of the low frequency spectral band. HF: natural logarithm of the high frequency spectral band. BBS: natural logarithm of the broad band spectra. p values determined by two-tailed paired t test.

DISCUSSION

An acute model of heart failure should mimic as possible a well-defined cardiovascular lesion, for instance, myocardial depression, and produce a significant circulatory impairment accompanied by a low cardiac output and arterial pressure.

In this pharmacological model of heart failure, the high dose of halothane administered produced, as expected, a significant reduction of aortic pressure, higher than 20%. In this scenario, an increase of heart rate would be expected if the baroreflex response was preserved. However, the reduction of aortic pressure was associated with a widening of the RR intervals (bradycardia).

The model of experimental heart failure used in this study was previously validated; nevertheless, it is noteworthy that typical clinical presentation of circulatory failure is not completely mimicked as occurred with others reported in the literature. Anyway, the selected model is the most adequate to fulfill our purposes and have no limitation to use left ventricular assist devices that need to be synchronized with the native cardiac rhythm. This is not possible in ischemic model of acute heart failure in which cardiac arrhythmias could difficult the catheter balloon pump synchronization along the circulatory assistance.[4–7]

It is well known that halothane produces myocardial impairment and induces rapid severe cardiac failure in animals.[34] Furthermore, halothane reduces myocardial contractility in a dose-dependant way; at maximum concentrations of halothane myocardial performance was severely compromised.[15] A comparative study performed in rabbits that evaluate the cardiovascular effects of volatile halogenated agents anesthetics, reported that halothane affects myocardial contractility more than isoflurane.[24] In addition, anesthetics influence the central control of the circulation. Evaluated by steady state relationship between blood pressure and heart rate in humans, it was shown that baroreflex sensitivity was consistently diminished during anesthesia with halothane (combined with nitrous oxide in 6 patients and alone in 2 patients), allowing the combination of reduced blood pressure and bradycardia.[3]

Neurohormonal change is an established component of the hemodynamic alteration in chronic heart failure; patients express higher mean heart rate and a lower variability.[8,20] Defective parasympathetic control and sympathetic activation have been documented in chronic heart failure.[9,13,17]

In this experimental series, the reduced heart rate originated by over-doses of halothane could be ascribed to a sympathetic inhibition or an increased vagal tone that could be related to the vagotonic effect of halothane.[12,25] However, spectral measurements of RR intervals showed a significant increase of LF spectral band with a non significant change in HF spectral band, indicating an increase in sympathetic activity. This could be related to the fact that the predominant action of halothane seems to be at the effector site, the heart, but not on the baroreflex activity.[32] Thus, suggesting that the myocardial depression and hemodynamic changes observed can be ascribed to the halothane effects.

This was further supported by the fact that halothane seems to have a dual effect on baroreflexes. In lower concentrations (<1.25%) the baroreflex activity seems to be reduced while in higher concentrations no actions were observed.[2] By this way, the fact that the RR sympathetic activity, represented by the LF band in the frequency domain analysis, is increased seems to be coherent with the fact that the cardiac depressive effects of halothane trigger a reflex sympathetic response to compensate a reduction of cardiac output. In addition, the data also supports the idea that the arterial baroreflexes seems to be able to sense the decrease in blood pressure and respond with an increase in sympathetic activity in an attempt to antagonize the depressive myocardial actions of halothane that, instead to be unable to revert the condition, is able to maintain a compensative steady state.

This situation is analogous to that observed in compensated cardiac failure where the increased sympathetic activity tends to maintain "normalized" the hemodynamic in normal activities. However, when an additional effort must be performed, this compensatory response is blunted and symptomatology appears.

Respect to halothane concentration, it has been reported that lower doses of halothane (0.8, 1.6, and 2.4%) do not produce bradycardia, or inclusively, increment heart rate in anesthetic dogs respect to awake state. Additionally, with these halothane concentrations, it was found that time (SD) and frequency (LF and HF) domain variability indexes were concentration-related reduced.[29]

Time domain results show that only MAF (cardiac output) variability was significantly diminished during induced heart failure with high doses of halothane. This result was also confirmed with the spectral measurements of BBS that reflects overall variability. Time and frequency domain parameters of SVR variability in induced heart failure instead of being reduced, were not statistically different from control state. In addition, Liu *et al*. analyze the relationship between heart rate variability and stroke volume variability (ultrasound based technique) in healthy subjects. They found no significant correlation of LF, HF and LF/HF between them, suggesting that stroke volume variability provides different information about autonomic nervous system activity than heart rate variability.[22]

Respective to MAP variability measurements, no statistical differences was found. LF oscillations reflect the vascular sympathetic modulation[28]; so the non-significant increment of LF AoP could suggest an increase in aortic distensibility. Related to this, Hettrick *et al*., using direct aortic measurements in mongrel dogs, demonstrated that halothane produces an increase in aortic distensibility but does not alter characteristic aortic impedance.[18]

Frequency ranges of power spectral densities components used in this study were identical to those measured in human subjects.[33] Other authors have evaluated LF and HF components in sheep with different cut-off frequencies, selected taken into account the high respiratory frequencies observed.[23] Regarding to this is important to note that in our experiments all sheep were assisted by a ventilator with respiration rates of around 12 breaths per minute.

In conclusion, in this model of heart failure, the myocardial depression induced by halothane 4%, is associated to changes in myocardial function related to a depressed myocardial activity, blood pressure, heart rate and cardiac output variability. The baroreflex activity seems to be active and able, through an increased sympathetic activity, to compensate the halothane altered cardiac hemodynamic.

LIMITATIONS OF THE STUDY

The lack of significant statistical differences in the variability of some hemodynamical parameters could be ascribed to the high variability of the data measured, the short term time of evaluation and/or the low number of animals studied. In addition, it could be attributed to the acute and open chest animal preparation.

Another limitation found in this short experimental series was the non-significant reduction of cardiac output in induced heart failure, meanwhile the aortic pressure was significantly decreased. Additionally, the presence of bradycardia in this particular animal model is far from the situation observed in clinical heart failure.

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CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

REFERENCES

[1]Arnold, J. M. O., J. G. Howlett, A. Ducharme, J. A. Ezekowitz, M. J. Gardner, N. Giannetti, H. Haddad, G. A. Heckman, D. Isaac, P. Jong, P. Liu, E. Mann, R. S. McKelvie, G. W. Moe, A. M. Svendsen, R. T. Tsuyuki, K. O'Halloran, H. J. Ross, E. J. Sequeira, and M. White. Canadian Cardiovascular Society Consensus 2008 Conference guidelines on heart failure-2008 update: best practices for the transition of care of heart failure patients, and the recognition, investigation and treatment of cardiomyopathies. *Can. J. Cardiol.* 24(Suppl 1):21–40, 2008.

[2]Behnia, R., and E. Koushampour. Local versus central effects of halothane on carotid sinus baroreceptor function. *Anesthesiology* 61:161–168, 1984.

[3]Bristow, J. D., C. Prys-Roberts, A. Fisher, T. G. Pickering, and P. Sleight. Effects of anesthesia on baroreflex control of heart rate in man. *Anesthesiology* 31(Suppl 5):422–428, 1969.

[4]Cabrera Fischer, E. I., D. Bia, Y. Zócalo, and R. Armentano. Smooth muscle-dependent changes in aortic wall dynamics during intra-aortic counterpulsation in an animal model of acute heart failure. *Int. J. Artif. Organs* 32(Suppl 6):354–361, 2009.

[5]Cabrera Fischer, E. I., J. C. Chachques, A. García, R. H. Pichel, M. C. Morales, and A. Carpentier. Temporary mechanical circulatory support for severe cardiac failure: experimental study. *Int. J. Artif. Organs* 14(Suppl 8):466–472, 1991.

[6]Cabrera Fischer, E. I., A. I. Christen, M. R. Risk, F. M. de Pessana, and E. Forteza. A new approach to assist post-operative heart failure in an animal model: juxta-aortic counterpulsation. *Artif. Organs* 26(Suppl 10):819–826, 2002.

[7]Cabrera Fischer, E. I., E. de Forteza, M. R. Risk, G. Nicolini, J. M. Camus, and F. M. Pessana. Juxtaaortic counterpulsation: comparison with intraaortic counterpulsation in an animal model of acute heart failure. *ASAIO J.* 50:311–315, 2004.

[8]Casolo, G., E. Balli, T. Taddei, J. Amuhasi, and C. Gori. Decreased spontaneous heart rate variability in congestive heart failure. *Am. J. Cardiol.* 64:1162–1167, 1989.

[9]Cohn, J. N., T. B. Levine, and M. T. Olivari. Plasma norepinepherine as a guide to progonosis in patients with chronic congestive heart failure. *N. Eng. J. Med.* 311:819–823, 1984.

[10]Constant, I., M. C. Dubois, V. Piat, M. L. Moutard, M. McCue, and I. Murat. Changes in electroencephalogram and autonomic cardiovascular activity during induction of anesthesia with sevoflurane compared with halothane in children. *Anesthesiology* 91:1604–1615, 1999.

[11]Doggrell, S. A., and L. Brown. Rat models of hypertension, cardiac hypertrophy and failure. *Cardiovasc. Res.* 39:89–105, 1998.

[12]Duke, P. C., D. Fownes, and J. G. Wade. Halothane depresses baroreflex control of heart rate in man. *Anesthesiology* 46:184–187, 1977.

[13]Eckberg, D., M. Drabinsky, and E. Braunwald. Defective cardiac parasympathetic control in patients with heart disease. *N. Eng. J. Med.* 285:877–883, 1971.

[14]Fang, J., G. A. Mensah, J. B. Croft, and N. L. Keenan. Heart failure-related hospitalization in the U.S., 1979 to 2004. *J. Am. Coll. Cardiol.* 52(Suppl 6):428–434, 2008.

[15]Hamilton, W. K., P. Larson, J. D. Bristow, and E. Rapaport. Effect of cyclopropane and halothane on ventricular mechanics; a change in ventricular diastolic pressure-volume relationships. *J. Pharmacol. Exp. Ther.* 154:566–574, 1966.

[16]Hasenfuss, G. Animal models of human cardiovascular disease, heart failure and hypertrophy. *Cardiovasc. Res.* 39: 60–76, 1998.

[17]Hasking, G. J., M. D. Esler, G. L. Jennings, D. Burton, and P. I. Korner. Norepinephrine spillover to plasma in patients with congestive heart failure: evidence of increased overall and cardiorenal sympathetic nervous activity. *Circulation* 73:615–621, 1986.

[18]Hettrick, D. A., P. S. Pagel, and D. C. Warltier. lsoflurane and halothane produce similar alterations in aortic distensibility and characteristic aortic impedance. *Anesth. Analg.* 83:1166–1172, 1996.

[19]Hongo, M., T. Ryoke, and J. Ross. Animal models of heart failure. Recent developments and perspectives trends. *Trends Cardiovasc. Med.* 7:161–167, 1997.

[20]Kahn, M. F., and M. R. Risk. Cardiac variability and heart failure. In: Cardiovascular Failure: Pathophysiological Bases and Management, edited by E. C. Fischer, A. I. Christen, and J. C. Trainini. Buenos Aires, Argentina: Fundación Universitaria Dr. René G. Favaloro, 2001, pp. 167–185.

[21]Kantrowitz, A. Introduction of left ventricular assistance. In: Cardiovascular Failure: Pathophysiological Bases and Management, edited by E. C. Fischer, A. I. Christen, and J. C. Trainini. Buenos Aires, Argentina: Fundación Universitaria Dr. René G. Favaloro, 2001, pp. 255–274.

[22]Liu, H., T. Yambe, H. Sasada, S. Nanka, A. Tanaka, R. Nagatomi, and S. Nitta. Comparison of heart rate variability and stroke volume variability. *Auton. Neurosci.* 116: 69–75, 2004.

[23]Lumbers, E. R., and Z. Y. Yu. A method for determining baroreflex-mediated sympathetic and parasympathetic control of the heart in pregnant and non-pregnant sheep. *J. Physiol.* 515(Suppl 2):555–566, 1999.

[24]Marano, G., R. Formigari, M. Grigioni, and A. Vergari. Effects of isoflurane versus halothane on myocardial contractility in rabbits: assessment with transthoracic two-dimensional echocardiography. *Lab. Anim.* 31:144–150, 1997.

[25]Marshall, B. E., and D. A. Longnecker. General anesthetics. In: Goodman and Guilman's: The Pharmacological Basis of Therapeutics9th ed., edited by P. B. Molinoff, and R. W. Ruddon. New York: Mc Graw-Hill, Health Professions Division, 1996, pp. 308–313.

[26]Muders, F., and D. Elsner. Animal models of chronic heart failure. *Pharmacol. Res.* 41(Suppl 6):605–612, 2000.

[27]Nichols, W. W., and M. F. O'Rourke. Properties of the arterial wall: practice. In: McDonald's Blood Flow in Arteries, edited by W. W. Nichols, and M. F. O'Rourke. Sidney: Arnold, 1998, pp. 73–97.

[28]Pagani, M., N. Montano, A. Porta, A. Malliani, F. Abboud, C. Birkett, and V. Somers. Relationship between spectral components of cardiovascular variabilities and direct measures of sympathetic nerve activity in humans. *Circulation* 95:1441–1448, 1997.

[29]Picker, O., T. W. Scheeren, and J. O. Arndt. Inhalation anaesthetics increase heart rate by decreasing cardiac vagal activity in dogs. *Br. J. Anaesth.* 87(5):748–754, 2001.

[30]R Development Core Team. A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing, 409 pp., 2010.

[31]Risk, M. R., A. J. Ramírez, and E. I. Cabrera Fischer. Hemodynamic improvement and heart rate variability during aortic counterpulsation. *Comput. Cardiol.* 31:453–455, 2004.

[32]Skovsted, P., M. L. Price, and H. L. Price. The effects of halothane on arterial pressure preganglionic sympathetic activity and barostatic reflexes. *Anesthesiology* 31:507–514, 1969.

[33]Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *Eur. Heart J.* 17: 354–381, 1996.

[34]Van Trigt, P., C. C. Christian, L. Fagraeus, T. L. Spray, R. B. Peyton, G. L. Pellom, and A. S. Wechsler. Myocardial depression by anesthetic agents (halothane, enflurane and nitrous oxide): quantification based on end-systolic pressure-dimension relations. *Am. J. Cardiol.* 53:243–247, 1984.

[35]Vanoli, E., S. Bacchini, S. Panigada, F. Pentimalli, and P. B. Adamson. Experimental models of heart failure. *Eur. Heart J. Suppl.* 6(Suppl F):7–15, 2004.