Methodology article

Method for non-invasively recording electrocardiograms in conscious mice

Victor Chu[1], Jose M Otero[2], Orlando Lopez[2], James P Morgan[1], Ivo Amende[1] and Thomas G Hampton*[2]

Address: [1]Cardiovascular Division, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215 USA & and [2]Research and Development, Mouse Specifics, Inc., Boston, MA 02139, USA

E-mail: Victor Chu - vchu1@caregroup.harvard.edu; Jose M Otero - otero@mousespecifics.com; Orlando Lopez - or_palacio@hotmail.com; James P Morgan - jmorgan@caregroup.harvard.edu; Ivo Amende - amende.ivo@mh-hannover.de; Thomas G Hampton* - hampton@mousespecifics.com

*Corresponding author

Published: 25 June 2001

BMC Physiology 2001, **1**:6

Received: 8 May 2001
Accepted: 25 June 2001

This article is available from: http://www.biomedcentral.com/1472-6793/1/6

Abstract

Background: The rapid increase in the development of mouse models is resulting in a growing demand for non-invasive physiological monitoring of large quantities of mice. Accordingly, we developed a new system for recording electrocardiograms (ECGs) in conscious mice without anesthesia or implants, and created Internet-accessible software for analyzing murine ECG signals. The system includes paw-sized conductive electrodes embedded in a platform configured to record ECGs when 3 single electrodes contact 3 paws.

Results: With this technique we demonstrated significantly reduced heart rate variability in neonates compared to adult mice. We also demonstrated that female mice exhibit significant ECG differences in comparison to age-matched males, both at baseline and in response to β-adrenergic stimulation.

Conclusions: The technology we developed enables non-invasive screening of large numbers of mice for ECG changes resulting from genetic, pharmacological, or pathophysiological alterations. Data we obtained non-invasively are not only consistent with what have been reported using invasive and expensive methods, but also demonstrate new findings regarding gender-dependent and age-dependent variations in ECGs in mice.

Background

Although electrocardiograms (ECGs) have been obtained in conscious mice, currently reported techniques require restraint [1] or anesthesia and surgical implantation of telemetry devices [2,3,4]. Anesthesia, however, may depress cardiovascular function, and adequate recovery after transmitter implantation in mice is nearly 3 weeks [3,5]. Accordingly, we developed a non-invasive technique for obtaining ECGs in conscious mice by placing the animal on a platform embedded with paw-sized ECG electrodes connected to an amplifier. This method is much less traumatic, requires no anesthesia or surgery, and promotes rapid screening of large quantities of mice. ECG data we obtained non-invasively in conscious mice are comparable to those recently published using surgically implanted telemetry devices [2,4]. To test the efficacy of our system, we evaluated ECGs in mice of either sex, of several strains, and of different ages. Moreo-

ver, we tested whether our system could detect ECG alterations in response to pharmacological challenge by isoproterenol. The baseline heart rate data and responses to the β-adrenergic agonist isoproterenol we recorded non-invasively in mice are comparable to data published using invasive methods[6]. We developed an ECG signal processing, analyzing, and database Web portal, which we named e-MOUSE™, accessible to the biotechnology community. The advantages of the ECG recording and analyses paradigm we developed are clear, given the high cost of breeding, housing, and transporting mice, and the call for comprehensive yet widely available phenotyping tests [7].

Results

We recorded ECGs in 10 males and 10 females each of C57BL/6, 129/Sv, and FVB/N mice, three inbred strains commonly used to model human diseases. A representative ECG recording from an adult male C57BL/6 mouse is shown in Fig. 1. Since the mice are conscious, baseline artifact and noise are apparent in the unfiltered signals. Yet, the P-waves and T-waves are discernible by eye and interpretable by the software algorithmic processing of the signal digitized at 2500 samples per second. Analyses of the digitized signals via e-MOUSE™ demonstrated significantly faster heart rates in C57BL/6 female mice than in males (741 ± 2 bpm vs. 692 ± 5 bpm, $P < 0.05$, n = 10) and shorter QRS duration (7.5 ± 0.2 ms vs. 8.1 ± 0.2 ms, $P < 0.05$). Table 1 summarizes results in 129/Sv mice (10 males and 10 females), again demonstrating faster heart rate and shorter QRS duration and QT interval in female mice than in age-matched males. Rate corrected QT (QTc) in male 129/Sv mice was significantly longer than in females (66.7 ± 1.9 ms vs. 58.4 ± 1.6 ms, $P < 0.05$). Gender differences in heart rate were also apparent in the FVB/N strain [736 ± 5 bpm in males (n = 10) vs. 706 ± 7 bpm in females (n = 10), $P < 0.05$].

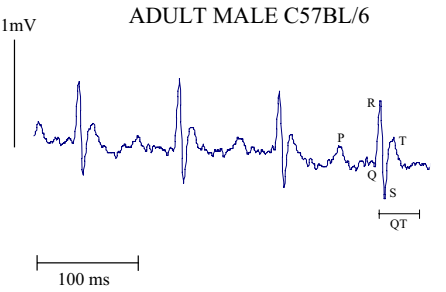

Figure 1
Electrocardiogram from a conscious adult male C57BL/6 mouse at baseline, with indication of ECG parameters.

Reducing the spacing of the electrodes allowed us to record ECGs in 6-day and again in 12-day old nursling C57BL/6 mice (8 males and 5 females) and return them to their mother. The ECG from a 6-day-old C57BL/6 nursling female is shown in Fig. 2. Heart rate variability in neonates was significantly less than in adults (2.5 ± 0.4 bpm vs. 21 ± 2 bpm, $P < 0.05$). Heart rate in nursling females was significantly slower than in adult females (655 ± 6 bpm vs. 741 ± 2 bpm, $P < 0.05$). Interestingly, the gender differences in heart rate we observed in adult mice were absent in 6-day old and 12-day old neonates. Upon weaning (21 days old), however, gender differences in heart rate became apparent [697 ± 14 bpm in males (n = 5) vs. 750 ± 8 bpm in females (n = 3), $P < 0.05$].

The acute increase in heart rate within the first minutes following one intraperitoneal injection of the β-adrener-

Table 1: Electrocardiographic parameters in male and female 129/Sv, C57BL/6, and FVB conscious mice.

	129/Sv		C57BL/6		FVB	
	Males (n = 10)	Females (n = 10)	Males (n = 10)	Females (n = 10)	Males (n = 10)	Females (n = 10)
Heart Rate (bpm)	571 ± 13	689 ± 12 *	692 ± 5	741 ± 2 *	736 ± 5	706 ± 7 *
HR var (bpm)	15 ± 4	20 ± 4	12 ± 3	15 ± 3	33 ± 2	16 ± 2 *
PR (ms)	31.9 ± 1.3	28.3 ± 0.6*	26.0 ± 0.8	24.4 ± 0.7	25.4 ± 0.6	27.7 ± 0.6 *
QRS (ms)	9.6 ± 0.3	8.5 ± 0.1*	8.1 ± 0.2	7.5 ± 0.2*	7.4 ± 0.1	7.7 ± 0.1
QT (ms)	70.2 ± 1.8	54.7 ± 2.0*	55.4 ± 0.8	53.8 ± 0.6	53.9 ± 1.2	55.7 ± 1.4
QT_c (ms)	66.7 ± 1.9	58.4 ± 1.6*	59.0 ± 0.8	58.7 ± 0.3	59.3 ± 1.2	59.9 ± 1.6

Values are mean ± SE. *, $P < 0.05$ vs. males within strain. HR var, heart rate variability.

gic agonist isoproterenol (2.5 μg/g) was significantly less in female compared to male C57BL/6 adult mice (+5 ± 2% females vs. +12 ± 2% males, $P < 0.05$, n = 5 for each group). Saline injection had no effect on heart rate. After 3 days of repeated isoproterenol injection (2.5 μg/g, once at 9AM and once at 9PM), heart rate in males prior to the 6th injection was significantly reduced compared to control heart rate in males prior to the 1st injection, whereas the heart rate in females did not change. Both male and female mice exhibited significant acute increases in heart rate (+27 ± 6% males and +22 ± 3% females) after the 6th injection of the drug on day 3 ($P < 0.05$ compared to % increases following 1st injection on day 1). Fig. 3 illustrates significant electrocardiographic alterations immediately after the 1st administration of isoproterenol on day 1 in a C57BL/6 male mouse. ST segment depression, suggestive of acute subendocardial ischemia, was consistently observed in C57BL/6 male mice but not in all female mice immediately following administration of isoproterenol. Heart weight in males and females treated with isoproterenol for 3 days was significantly increased (16%) compared to hearts from mice treated with saline for 3 days ($P < 0.05$).

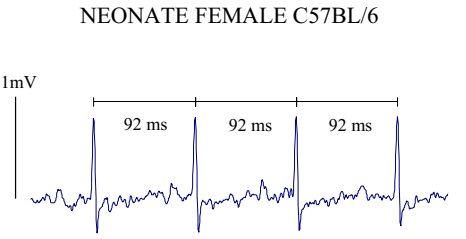

Figure 2
Electrocardiogram from a conscious neonate (6 days old) female C57BL/6 mouse. Heart rate variability in neonates was significantly less than in adult mice.

Discussion

This report describes the development of a system for non-invasively recording ECGs in conscious mice; neither attachment of wires, nor anesthesia, nor surgical implantation of devices is required. Our heart rate data in conscious mice are comparable to those obtained using sutured steel wire [8] and chronic indwelling catheters [6]. Our ECG measurements are comparable to those obtained using implantable telemetry devices [2,4]. This non-invasive method, standardized protocol, and Internet-accessible mouse ECG analyses software (e-MOUSE™) may reduce the heterogeneity in data collected from different laboratories [7].

Strain & Gender Differences in ECGs in Adult Mice

To our knowledge, this is the first study to describe gender differences in heart rate in genetically homogenous strains of conscious mice. Studies in humans [9,10] and in rats [11,12] have shown females to have faster heart rates than males, differences that disappear with age [10,13]. In C57BL/6 mice and in 129/Sv mice, we found that conscious females exhibited significantly faster heart rates than male mice. In FVB/N mice, however, we noted faster heart rates and briefer ECG time intervals in male compared to female mice. Mitchell et al., using implanted transmitters, reported slightly but insignificantly faster heart rates in male compared to female FVB mice [4]. Gender and strain differences in heart rate may reflect differences in hormone activity, which are known to affect cardiovascular autonomic regulation differentially in males and females [9] and vary among mouse strains [14]. Our strain-dependent observations agree with results of invasive studies [8,6] and support the importance of genetic factors in influencing heart rate.

Developmental Changes in ECGs in Conscious Neonatal Mice

We found that the gender differences apparent in C57BL/6 adults were absent in nursling mice. Reduced activity in newborns could contribute to reduced heart rate and heart rate variability. However, observations in quietly resting adult mice (n = 3), monitored 30 min after placement on the recording platform, of reduced heart rate (471 ± 20 bpm) and increased heart rate variability (53 ± 6 bpm) suggest that the results in neonates may be attributable to reduced sympathetic and parasympathetic signaling [15]. The time-domain heart rate variability data we obtained non-invasively are comparable to those obtained using implanted telemetry devices [2,16]. The developmental changes in heart rate we measured in mice parallel those in human neonates [13,17], lambs [18], and newborn rats [19]. Moreover, our observations in neonatal mice are in agreement with Wang et al. who reported progressive and significant shortening of R-R intervals during neonatal development [1]. Accordingly, our approach, adaptable to the study of small mice that might otherwise die from anesthesia or surgery, might be valuable in examining developmental changes and abnormalities [13,17].

Effects of Sympathetic Stimulation in Conscious Mice

The blunted heart rate increase in response to β-adrenergic stimulation in conscious C57BL/6 female mice is consistent with gender related differences in baroreflex control of heart rate in healthy humans [20]. Estrogen

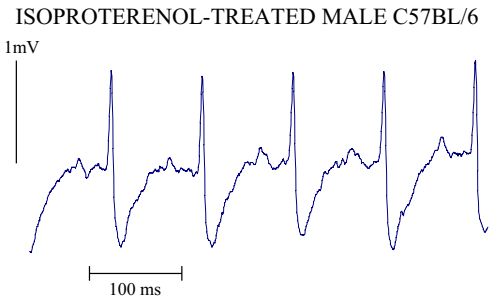

Figure 3
Electrocardiogram from the C57BL/6 male mouse of Fig. 1 recorded immediately after an intraperitoneal injection of 2.5 μg/g isoproterenol on day 1, showing ST segment depression suggestive of acute subendocardial ischemia.

has been shown to enhance baroreflex sensitivity [21] and attenuate the response of heart rate to administration of isoproterenol in rats [22]. After repeated injections of isoproterenol, heart rate decreased and the QRS duration increased significantly in male mice, but did not change in female mice. Yet, both males and females demonstrated increased sensitivity to acute isoproterenol injection after repeated administration that resulted in significant cardiac hypertrophy [23,24]. We observed a 16% increase in heart mass in both sexes after 3-days of isoproterenol treatment compared to mice treated with equivolume of saline. Although β-adrenoceptor desensitization [23] or reduced ATPase activity [25] may account for the decrease in basal heart rate in the hypertrophied male hearts, the increased sensitivity to isoproterenol may support the hypotheses of ischemia-induced activation of β-adrenoceptor kinase [26] or modulation of G-protein signaling to preserve adenylate cyclase activity [27]. To our knowledge, this is the first report that describes electrocardiographic evidence of myocardial ischemia in conscious mice, although ST segment changes have characterized isoproterenol-induced ischemia in rat [28] and man [29]. Why males consistently developed ST segment depression following isoproterenol administration and females did not remains unanswered. However, significant gender-related differences in the expression of glutathione S-transferases (GST) have been observed in mice, with female mice expressing significantly more of this antioxidant than male mice [30]. In mice, isoproterenol-induced oxidative stress may be attenuated in females due to higher levels of GST [31].

Study Limitations

Mice are sensitive to even modest handling [3,8] and transport [3,32]. The ECG indices we measured may reflect physiologic responses to the experimental environment relative to its home cage. We encourage a 10 min acclimation period prior to recording data to attenuate effects consequent to handling and transport. Usually mice establish contact between 3 electrodes and 3 limbs within 5 minutes after acclimation to record a continuous ECG for approximately 2 seconds. The duration of the ECG recording should be considered in the interpretation of heart rate variability [33]. The age, gender and strain variations in heart rate may reflect age, gender and strain variations in physiologic responses to transport [32], handling [3], and adaptation to repeated measurements [8]. Yet, the inter-strain differences in heart rate we non-invasively obtained after ECG recordings of short duration are in agreement with invasive experimental techniques intended to monitor heart rate in mice as caged [6,8]. Our measurements were made in daytime hours, disrupting the less active phases of the mouse circadian cycle. Future innovation might incorporate an array of conductive electrodes into the animals' cages to eliminate the effects of handling and transport, and perhaps enable timed recordings.

Materials and Methods

Mice

Young adult mice (9 ± 1 weeks old; 21 ± 1 g) from 3 commonly used inbred strains, C57BL/6, 129/Sv, and FVB/N (The Jackson Laboratory, Bar Harbor, ME), were housed in standard conditions within the Animal Resource Facility at the Beth Israel Deaconess Medical Center, Boston MA. Nursling C57BL/6 mice of either sex were examined at post-natal day 6 and 12 and returned to their mothers. The same mice, weaned, were examined at 3 weeks of age.

ECG recording

Mice were gently removed from their cages and positioned on the ECG recording platform. An array of gel-coated ECG electrodes (Red Dot; 3 M, St. Paul, MN) were embedded in the floor of the platform and spaced to provide contact between the electrodes and animals' paws. For adults, the spacing between electrodes was 3 cm, and for nurslings, the spacing was reduced to 2.5 cm. Filter paper, with openings for the electrodes, prevented mouse urine from short-circuiting the signals. The electrodes were connected to an amplifier (HP78901A, Hewlett-Packard, Andover, MA) by a shielded 3-electrode lead set (M1605A Snap, Hewlett-Packard, Andover, MA). Since even modest handling of mice may induce alterations in heart rate [6], each mouse was permitted to acclimatize for 10 min prior to collection of baseline data. The signals were digitized with 16-bit pre-

cision (DI-220, DATAQ Instruments, Inc., Akron, OH) at a sampling rate of 2500 samples/second. When mice were sitting or otherwise positioned such that the paws were not in contact with three electrodes, the output from the amplifier was discarded. Only data from continuous recordings of 20-30 ECG signals were used in the analyses. Data were transmitted to the *mousespecifics.com* Internet site (Mouse Specifics, Inc., Boston, MA) using standard file-transfer protocols for ECG signal analyses by e-MOUSE™.

ECG analyses

Each signal was analyzed using e-MOUSE™, an Internet-based physiologic waveform analysis portal. e-MOUSE™ incorporates Fourier analyses and linear time-invariant digital filtering of frequencies below 2 Hz and above 100 Hz to minimize environmental signal disturbances. The software uses a peak detection algorithm to find the peak of the R-waves and to calculate heart rate. Heart rate variability was calculated as the mean of the differences between sequential heart rates for the complete set of ECG signals. Subsequently, determination of 1^{st} and 2^{nd} derivatives and algebraic "if-thens" search the ECG signals for probable P-wave peaks and onset and termination of QRS complexes. Since the T-wave is not separate from the QRS in rodent ECGs [28, 34], there have been discrepancies in the definition of the QT interval and reported values [4]. In accord with Mitchell et al. [4], we routinely included the inverted and/or biphasic portions of the T-wave in our calculations of the QT interval. We defined the end of the T-wave of each signal as the point where the signal returned to the isoelectric line [1,35] [the mean voltage between the preceding P-wave and QRS interval]. The QT intervals were rate corrected (QTc) by application of the equation recommended by Mitchell et al. [4] for use in mice. The software plots its interpretation of P,Q,R,S, and T for each beat so that spurious data resulting from unfiltered noise or motion artifacts may be rejected. e-MOUSE™ then calculates the mean of the ECG time intervals for each set of waveforms.

β-adrenoceptor stimulation

To test the sensitivity of our system for describing ECG alterations in response to a drug, C57BL/6 mice were given either an intraperitoneal injection of 2.5 μg/g (-)-isoproterenol (Sigma, St. Louis, MO) (5 males, 5 females) dissolved in 0.1 ml saline or an equal volume of saline (5 males, 5 females), twice daily for 3-days. ECGs were recorded within 5 min prior to each injection and between 1 and 2 min after each injection to capture the peak of the response to the drug [6]. After the last measurement, mice were euthanized by intraperitoneal administration of pentobarbital (150 mg/Kg), consistent with the American Veterinary Medical Association Panel on Euthanasia guidelines. Excised hearts, excluding atria and blotted dry, were then weighed.

Statistics

Data are presented as the means ± SE. Comparisons between genders among strains were performed using Student's *t*-test for unpaired observations. Effects of isoproterenol or saline injections within groups were performed using Student's *t*-test for paired observations, and between group comparisons using Student's *t*-test for unpaired observations. Differences were considered significant with $P < 0.05$.

Conclusions

We developed a non-invasive technique for obtaining ECGs in conscious mice. We developed an Internet-accessible portal for analyses of mouse electrocardiograms. Using this system, we demonstrated significant strain, gender and age-dependent differences in electrocardiograms in mice. Moreover, we demonstrated significant gender-dependent differences in the cardiovascular response to β-adrenoceptor stimulation. Our results may suggest that the stimulatory effects of genes and drugs on cardiac function may be more profound in male or masked in female mice. This non-invasive and rapid ECG phenotyping technique may improve the quality and increase the quantity of data collected from mouse models.

Acknowledgements

Mr. Diego Yepes is gratefully acknowledged for his innovative design approaches to the ECG acquisition system. Ms. Jeanne Smith provided excellent administrative support for this project and manuscript submission. I. Amende received generous support from Förderkreis zur Verbesserung des Gesundheitswesen e.V.

References

1. Wang L, Swirp S, Duff H: **Age-dependent response of the electrocardiogram to K+ channel blockers in mice** *Am J Physiol Cell Physiol* 2000, **278**:C73-C80
2. Gehrmann J, Hammer PE, Maguire CT, Wakimoto H, Triedman JK, Berul CI: **Phenotypic screening for heart rate variability in the mouse** *Am J Physiol Heart Circ Physiol* 2000, **279**:H733-H740
3. Kramer K, van Acker SABE, Voss H-P, Grimbergen JA, van der Vijgh WJF, Bast A: **Use of telemetry to record electrocardiogram and heart rate in freely moving mice** *J Pharmacol Toxicol Methods* 1993, **30**:209-215
4. Mitchell GF, Jeron A, Gideon K: **Measurement of heart rate and Q-T interval in the conscious mouse** *Am J Physiol Heart Circ Physiol* 1998, **274**:H747-H751
5. Stiedl O, Spiess J: **Effect of tone-dependent fear conditioning on heart rate and behavior of C57BL/6N mice** *Behav Neurosci* 1999, **111**:703-711
6. Desai KH, Sato R, Schauble E, Barsh GS, Kobilka BK, Bernstein D: **Cardiovascular indexes in the mouse at rest and with exercise: new tools to study models of cardiac disease** *Am J Physiol Heart Circ Physiol* 1997, **272**:H1053-H1061
7. Abbott A: **Database to standardize mouse phenotyping** *Nature* 1999, **401**:833
8. Blizard DA, Welty R: **Cardiac activity in the mouse: strain differences** *J Compar Physiol Psych* 1971, **77**:337-344
9. Huikuri VH, Pikkujämsä SM, Airaksinen KEH, Ikäheimo MJ, Rantala AO, Kauma H, Lilja M, Kesäniemi A: **Sex-related differences in autonomic modulation of heart rate in middle-ages subjects** *Circulation* 1996, **94**:122-125

10. Umetani K, Singer DH, McCraty R, Atkinson M: **Twenty-four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades** *J Am Coll Cardiol* 1998, **31**:593-601
11. Chandler MP, DiCarlo SE: **Acute exercise and gender alter cardiac autonomic tonus differently in hypertensive and normotensive rats** *Am J Physiol Reg Integ Comp Physiol* 1998, **43**:R510-R516
12. El-Mas MM, Abdel-Rahman AA: **Estrogen enhances baroreflex control of heart rate in conscious ovariectomized rats** *Can J Physiol Pharmacol* 1997, **76**:381-386
13. Harper RM, Leake B, Hodgman JE, Hoppenbrouwers T: **Developmental patterns of heart rate and heart rate variability during sleep and waking in normal infants and infants at risk for the sudden infant death syndrome** *Sleep* 1982, **5**:28-38
14. Piccini N, Knopf JL, Gross KW: **A DNA polymorphism, consistent with gene duplication, correlates with high renin levels in the mouse submaxillary gland** *Cell* 1982, **30**:205-213
15. Sugihara G, Allan W, Sobel D, Allan KD: **Nonlinear control of heart rate variability in human infants** *Proc Natl Acad Sci* 1996, **93**:2608-2613
16. Jumrussirikul P, Dinerman J, Dawson TM, Dawson VL, Ekelund U, Georgakopoulos D, Schramm LP, Calkins H, Snyder SH, Hare JM, Berger RD: **Interaction between neuronal nitric oxide synthase and inhibitory G protein activity in heart rate regulation in conscious mice** *J Clin Inv* 1998, **102**:1279-1285
17. Schechtman VL, Harper RM, Kluge KA, Wilson AJ, Hoffman HJ, Southall DP: **Cardiac and respiratory patterns in normal infants and victims of the sudden infant death syndrome** *Sleep* 1988, **11**:413-426
18. Siimes AS, Välimäki IAT, Sarajas HSS, Sakoné K, Oja RT: **Heart rate variation in relation to age and sleep state in neonatal lamb** *Acta Physiol Scand* 1984, **537**:7-15
19. Hofer MA, Reiser MF: **The development of cardiac rate regulation in preweanling rats** *Psychosom Med* 1969, **31**:372-388
20. Abdel-Rahman ARA, Merrill RH, Wooles WR: **Gender-related differences in the baroreceptor reflex control of heart rate in normotensive humans** *J Appl Physiol* 1994, **77**:606-613
21. Mohamed MK, El-Mas MM, Abdel-Rahman AA: **Estrogen enhancement of baroreflex sensitivity is centrally mediated** *Am J Physiol Reg Integ Comp Physiol* 1999, **276**:R1030-R1037
22. Fregly MJ, Thrasher TN: **Response of heart rate to acute administration of isoproterenol in rats treated chronically with norethynodrel, ethinyl estradiol, and both combined** *Endocrinology* 1977, **100**:148-154
23. Kudej RK, Iwase M, Uechi M, Vatner DE, Oka N, Ishikawa Y, Shannon RP, Bishop SP, Vatner SE: **Effects of chronic β-adrenergic receptor stimulation in mice** *J Mol Cell Cardiol* 1997, **29**:2735-2746
24. Slawson SE, Roman BB, Williams DS, Koretsky AP: **Cardiac MRI of the normal and hypertrophied mouse heart** *Magn Reson Med* 1998, **39**:980-987
25. Gordon AL, Inchiosa MA, Lehr D: **Isoproterenol-induced cardiomegaly: assessment of myocardial protein content, actomyosin ATPase and heart rate** *J Mol Cell Cardiol* 1972, **4**:543-557
26. Ungerer M, Kessebohm K, Kronsbein K, Lohse MJ, Richardt G: **Activation of β-Adrenergic receptor kinase during myocardial ischemia** *Circ Res* 1996, **79**:455-460
27. Hammond HK, Roth DA, McKirnan MD, Ping P: **Regional myocardial downregulation of the inhibitory guanosine triphosphate-binding protein (Giα2) and β-adrenergic receptors in a porcine model of chronic episodic myocardial ischemia** *J Clin Invest* 1993, **92**:2644-2652
28. Bestetti RB, Oliveira JSM: **The surface electrocardiogram: a simple and reliable method for detecting overt and latent heart disease in rats** *Braz J Med Biol Res* 1990, **23**:1213-1222
29. Winsor T, Mills B, Winbury MM, Howe BB, Berger H: **Intramyocardial diversion of coronary blood flow: effects of isoproterenol-induced subendocardial ischemia** *Microvasc Res* 1975, **9**:261-278
30. Mitchell AE, Morin D, Lakritz J, Jones DA: **Quantitative profiling of tissue- and gender-related expression of glutathione S-transferase isoenzymes in the mouse** *Biochem J* 1997, **325**:207-216
31. Rathore N, John S, Kale M, Bhatnagar D: **Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues** *Pharmacol Res* 1998, **39**:297-303
32. Tuli JS, Smith A, Morton DB: **Stress measurements in mice after transportation** *Laboratory Animals* 1995, **29**:132-138
33. Tsuji H, Venditti FJ, Manders ES, Evans JC, Larson MG, Feldman CL, Levy D: **Determinants of heart rate variability** *J Am Coll Cardiol* 1996, **28**:1539-46
34. Richards AG, Simonson E, Visscher MB: **Electrocardiogram and phonogram of adults and newborn mice in normal conditions and under the effects of cooling, hypoxia, and potassium** *Am J Physiol* 1953, **174**:293-298
35. Kirchhoff S, Nelles E, Hagendorff A, Krüger O, Traub O, Willecke K: **Reduced cardiac conduction velocity and predisposition to arrhythmias in connexin40-deficient mice** *Curr Biol* 1998, **8**:299-302