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PII: S1056-8719(15)00273-7
DOI: doi: 10.1016/j.vascn.2015.10.001
Reference: JPM 6304

To appear in: *Journal of Pharmacological and Toxicological Methods*

Please cite this article as: Takács, H., Kui, P., Farkas, A.S., Sarusi, A., Forster, T., Papp, J.G., Varró, A., Curtis, M.J., Shattock, M.J. & Farkas, A., Ventricular cycle length irregularity affects the correlation between ventricular rate and coronary flow in isolated, langendorff perfused guinea pig hearts, *Journal of Pharmacological and Toxicological Methods* (2015), doi: 10.1016/j.vascn.2015.10.001

Ventricular cycle length irregularity affects the correlation between ventricular rate and coronary flow in isolated, Langendorff perfused guinea pig hearts

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Abstract (word count should not exceed 250 words)

Introduction: Heart rate affects coronary flow, but the mechanism is complex. The relationship between rhythm and flow is unclear, especially in experimental settings used for determining drug actions. The present study examined whether ventricular irregularity influences coronary flow independently of heart rate.

Methods: Guinea pig hearts were perfused (Langendorff mode) at constant pressure. Hypokalaemic Krebs solution facilitated spontaneous development of arrhythmias. The ECG, left ventricular and perfusion pressures were recorded, and coronary flow was measured. Beat-to-beat ventricular cycle length variability was quantified. Hearts were retrospectively allocated to arbitrary 'Low' or 'High' RR variability groups.

Results: A positive linear correlation was found between mean ventricular rate and coronary flow. The slope of the regression line was significantly greater in the 'High' versus 'Low' RR variability group, with greater coronary flow values in the 'High' RR variability group in the physiological heart rate range. During regular rhythm, left ventricular pressure exceeded perfusion pressure and prevented coronary perfusion at peak systole. However, ventricular irregularity significantly increased the number of beats in which left ventricular pressure remained below perfusion pressure, facilitating coronary perfusion.

Discussion: In isolated hearts, cycle length irregularity increases the slope of the positive linear correlation between mean ventricular rate and coronary flow via producing beats in which left ventricular pressure remains below perfusion pressure. This means that changes in rhythm have the capacity to influence coronary flow independently of heart rate in isolated hearts perfused at constant pressure, which should be noted in drug studies on arrhythmias performed in Langendorff hearts.

Keywords: perfusion pressure, coronary flow, guinea pig, isolated hearts, Langendorff perfusion, left ventricular pressure, methods, ventricular rate, ventricular cycle length variability, irregular rhythm

Abbreviations:

APB: atrial premature beat; PESP: post-extrasystolic potentiation; RMSSD: root mean square of the successive difference of the RR intervals; VPB: ventricular premature beat

Introduction

Understanding the relationship between ventricular rhythm and coronary flow autoregulation in experimental preparations such as the Langendorff is important for several reasons.

Many patients live with irregular ventricular rate caused by either frequent ventricular or atrial arrhythmias. Irregular ventricular rate may be harmful in the long term, e.g. it is well documented that frequent ventricular premature beats (VPB) can lead to development of cardiomyopathy (Yokokawa, et al., 2012). However, it is not known whether an effect of irregular ventricular rhythm on coronary flow may contribute to the harmful effects of irregular ventricular rhythm.

In *in vivo* conditions coronary flow is regulated by a combination of i) intramural pressure in coronary arteries caused by wall stress during the cardiac cycle, ii) the autonomic nervous system, iii) and work-induced autoregulation via local metabolites (Duncker, Bache, & Merkus, 2012; Kingma & Rouleau, 2007). Additionally, irregular rhythm affects the work of the myocardium independently of rate and load (Cooper, 1993). This implies that irregular ventricular rhythm may have an independent effect on coronary flow via modifying the work-induced autoregulation of the coronary arteries. However, this has never been examined, and data about the well-known positive correlation between ventricular rate and coronary flow have been obtained from hearts free of arrhythmias (Bernier, Curtis, & Hearse, 1989; Duncker & Merkus, 2007). As it is not known how work-induced autoregulation of coronary arteries is affected by ventricular irregularity, the approach of the present study was to determine the effect of beat-to-beat variability of ventricular cycle length on coronary flow in isolated, Langendorff-perfused guinea pig hearts.

Caval veins do not fill the right atrium in Langendorff-perfused heart, and thus when the hydrostatic pressure of the perfusion column is constant, hearts can be studied with

coronary arteries perfused under constant pressure (Curtis, 1998). Also, Langendorff-perfused hearts are normally denervated. Thus, under these conditions coronary flow is regulated independently of perfusion pressure and autonomic nervous system, and only intramural pressure during the cardiac cycle and work-dependent autoregulation determine coronary resistance. As ventricular irregularity was found to significantly affect coronary flow in the present investigation, the mechanism was examined in a second set of experiments performed in Langendorff perfused guinea pig hearts.

Methods

Animals and General Experimental Methods

Female guinea pigs (n=87 in the first set of experiments and n=33 in the second), weighing 300-400 g were used. The animal-handling protocol was in accordance with the Guidance of the Operation on the Animals (Scientific Procedures) Act 1986 and the European Community guidelines for the use of experimental animals.

The method of Langendorff perfusion we used has been described in detail (Farkas & Curtis, 2002, 2003; Farkas, Qureshi, & Curtis, 1999). Briefly, animals were anesthetized with pentobarbital (60 mg/kg i.p.) mixed with 1000 IU sodium heparin to prevent blood clot formation in the coronary vasculature. Sodium heparin (500 IU) was additionally administered i.v. Hearts were excised and placed immediately into ice-cold modified Krebs-solution containing: 118.5 mM NaCl, 25.0 mM $NaHCO_3$, 0.5 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 1.8 mM CaCl, 3.0 mM KCl, 11.1 mM glucose. Langendorff perfusion was begun with solution delivered at 37^{o}C and pH 7.4 at constant perfusion pressure (60 mmHg). In the first set of experiments, a unipolar electrogram (ECG) was recorded by implanting one stainless-steel wire electrode into the middle of the anterior wall of the left ventricle with a second

connected to the aorta. In the second set of experiments, volume conducted ECG was recorded by submerging the ventricles of the hearts in modified Krebs solution at 37 ^{o}C.

Measurement of coronary flow

Coronary flow was measured by timed collection of coronary effluent. At the end of the experiment atria were removed from the hearts and ventricles were weighed. Coronary flow values are shown in ml/min/g.

ECG analysis, measurement of the RR intervals and calculation of variability of the ventricular cycle length in the first set of experiments

The ECG was recorded and analysed by LabChart7 (ADInstruments Ltd, Oxford, UK). In the guinea pig Langendorff preparation, non-complex arrhythmias (mostly VPBs, atrial premature beats [APBs], and sinus arrhythmia) occur frequently during the initial period after mounting the heart, especially if Krebs solution contains a low concentration of K^+ (3.0 mM) and a high concentration of Ca^{2+} (1.8 mM). However, these arrhythmias spontaneously resolve within 20-30 min. The reason for these baseline arrhythmias is not known; it is a particular feature of guinea pig hearts and the same perfusion method does not cause arrhythmias in rat and rabbit hearts (Farkas & Curtis, 2002; Farkas, et al., 2006). These ventricular and atrial arrhythmic beats were defined according to the Lambeth Conventions II (Curtis, et al., 2013) and manually counted in the last 30 seconds of the 30-minute-long control perfusion period. The per cent frequency of arrhythmic beats (defined as APBs, VPBs or individual QRT complexes in a run of a salvo or tachycardia) was calculated in the sampling period as the number of ventricular arrhythmic beats divided by the total number of beats times 100.

The RR intervals were measured irrespective of rhythm even during arrhythmias in the 30-second-long sampling period. The mean ventricular rate was calculated as the total number of ventricular complexes (arrhythmic or not) times 2. The beat-to-beat variability of the RR intervals was quantified by the root mean square of the successive differences of the RR intervals (RMSSD) as described previously (Farkas, et al., 2009): taking the successive differences of the RR intervals ($\Delta d_j = d_{j+1} - d_j; 0 \le j \le N-2,$ where d_j represents the RR or QT interval durations and N is the total number of intervals) and calculating $\mathrm{RMSSD} = \sqrt{E([\Delta d]^2)},$ where E denotes the mean value. RMSSD of the RR interval during sinus rhythm in vivo is a parameter widely used for quantifying heart rate variability as a biomarker of parasympathetic activity (Farkas, et al., 2010; Vincze, et al., 2008), but in the Langendorff preparation it quantifies only the irregularity of the cycle length since parasympathetic tone is absent.

These hearts were divided into two groups based on RMSSD value as the 'Low' RR variability group (RMSSD < 3 ms; n=50 hearts), and the 'High' RR variability group (RMSSD > 3 ms; n=37 hearts) (Figure 1). The beat-to-beat variability of the cycle length (RMSSD), the per cent frequency of the arrhythmic beats, the mean ventricular rate and the coronary flow were compared between the 'Low' and 'High' RR variability groups. Note (with respect to justification of animal usage) that all hearts were entered into a separate, unrelated experimental protocol 30 min after the start of perfusion.

Measurement of the duration of perfused and non-perfused intervals in every cardiac cycle

It was found in the first set of experiments that cycle length irregularity increased the slope of the linear correlation between mean ventricular rate and coronary flow. Myocardium in vivo is perfused during diastole and is not perfused when intramural capillaries are compressed by intramural pressure during systole. In order to examine whether elevated RR

interval variability increased the ratio of durations of perfused intervals to non-perfused intervals in the Langendorff preparation, a second set of experiments was performed with 33 hearts perfused identically for 60 minutes with the same modified Krebs solution but with hearts submerged in Krebs solution in order to record volume conducted ECG. Left ventricular pressure was recorded via a thin medical needle stuck through the apex of the heart, and attached to a plastic cannula filled with saline. Perfusion pressure (aortic pressure) was recorded via a side arm at the bottom of the perfusion column where perfusion cannula was connected to the aorta. “Real-time” aortic flow was measured by an ultrasonic flow meter (T106 Animal Research Flowmeter, Transonic Systems Inc. Ithaca, NY, U.S.A) implanted to the bottom of the perfusion column, closely above the aortic stump. Krebs solution was continuously pumped to the reservoir by a pump (Peri-star Pro, World Precision Instruments, Sarasota, Florida, U.S.A.) during the experiment and a built-in overflow system kept the height of the column at a pre-set, constant level, thus the hydrostatic pressure of the perfusion column was constant (65 mmHg). Volume-conducted ECG, real-time aortic flow, aortic and left ventricular pressures were recorded continuously by using National Instruments data acquisition hardware (PC card, National Instruments, Austin, TX., U.S.A.) and SPEL Advanced Haemosys software (version 3.26, Experimetria Ltd. and Logirex Software Laboratory, Budapest, Hungary).

RR interval variability (RMSSD of the RR interval), mean ventricular rate and mean aortic flow were determined in every 30-second-long interval during the 60-minute-long perfusion period in each heart. A strong, positive, linear correlation was found between the measured coronary flow and the calculated mean aortic flow values ($y = 1.0166x + 0.5482$ where y is the mean aortic flow value (ml/min/g) and x is the measured coronary flow value (ml/min/g); regression coefficient: $R = 0.966$; $P < 0.0001$, linear regression). In 15 hearts, the ventricular rate did not match the mean ventricular rate found in the first set of experiments,

thus these hearts were excluded from the analysis. In each of the remaining 18 hearts, there was at least one 30-second-long interval in which mean ventricular rate matched the mean ventricular rate measured in the first set of experiments. One of these 30-second-long intervals was chosen for further analysis in each heart. These 18 hearts thus replicated the RR interval variability and mean ventricular rate of the 'Low' and 'High' RR variability groups in the first set of experiments, with 9 hearts allocated into a 'Low' RR interval variability group (RMSSD < 3 ms), and the other 9 to a 'High' RR interval variability group (RMSSD > 3 ms).

An analysis of the durations of perfused and non-perfused intervals in the 18 hearts was performed in a blinded manner. The number of beats in which left ventricular pressure did not exceed perfusion pressure during systole, and the duration of the non-perfused intervals during each systole were determined. The non-perfused interval (i.e. the interval where intramural pressure prevents coronary perfusion) was defined as the interval in which left ventricular pressure was greater than perfusion pressure during systole. Perfused interval (i.e. the interval where intramural pressure allows coronary perfusion) was defined as the interval in which left ventricular pressure was lower than perfusion pressure. The ratio of the perfused to non-perfused intervals was calculated by dividing the cumulative duration of the perfused intervals by the cumulative duration of the non-perfused intervals in the 30-second-long sampling interval at the time point of the measurement.

Statistical analysis

Continuous data were expressed as mean ± standard deviation (SD) and the 'Low' and 'High' RR variability groups were compared using the non-parametric Mann-Whitney U test. Within group comparison of continuous data was performed using the non-parametric Wilcoxon test. The coronary flow values were plotted against the mean ventricular rate values. The correlation between mean ventricular rate and coronary flow was examined with

analysis of covariance, in which variables of coronary flow (as the continuous dependent variable), mean ventricular rate (as the continuous predictor variable, i.e. covariate) and group ('Low' versus 'High' RR variability group, as the categorical grouping variable) were tested. $P<0.05$ was taken as indicative of a statistically significant difference between values.

Results

First set of experiments, irregular rhythm affects coronary flow

First, the beat-to-beat variability of the ventricular cycle length was tested as an independent variable influencing coronary flow. RMSSD values were significantly greater in the 'High' RR variability group than in the 'Low' RR variability group (Figure 2a). This reflects the intensity of arrhythmias; the per cent frequency of arrhythmic beats was significantly greater in the 'High' RR variability group versus the 'Low' RR variability group (per cent frequency in the 'High' RR variability group: mean 14, median 2.7, range 0-53 versus per cent frequency in the 'Low' RR variability group: mean: 0; median 0 range: 0-0.8; $P<0.05$). RR interval variability was inevitably increased by spontaneously occurring arrhythmias during the observed 30-second-long period in the 'High' RR variability group: VPBs occurred in 25 hearts (an average of 21 beats, median 5 beats, range 1-58 beats), however VPBs with short coupling interval (R-on-T VPBs) occurred very rarely (in 6 hearts, an average of 3 beats, median 2.5 beats, range 1-5 beats); APB-s occurred in 9 hearts (an average of 12 beats, median 4 beats, range 2-47 beats); increased variability of RR intervals was caused only by sinus arrhythmia in 10 hearts. Hearts in the 'Low' RR variability group remained in regular sinus rhythm.

Importantly, the mean ventricular rate did not differ significantly between the two groups (Figure 2b), whereas the coronary flow was significantly greater in the 'High' RR variability group (Figure 2c).

The relationship between mean ventricular rate and coronary flow was tested in a further analysis. A significant positive linear correlation was found between these variables in the 'Low' RR variability group, but with a low correlation coefficient (Figure 3). A similar relationship was seen in the 'High' RR variability group (Figure 3). However, the slope of the regression line was significantly greater in the 'High' versus 'Low' RR variability group, with consequentially greater coronary flow values in the 'High' RR variability group in the physiological heart rate range for guinea pig (210-280 1/min) (Figure 3).

Second set of experiments; duration of perfused and non-perfused intervals

The RMSSD, mean ventricular rate and coronary flow values measured in the first set of experiments were reproduced in the second set of experiments. Accordingly, the beat-to-beat variability of the ventricular cycle length was significantly greater in the 'High' RR variability group than in the 'Low' RR variability group (RMSSD: 29.4±16.2 ms vs. 1.0±0.5 ms, respectively; $P<0.05$). Mean ventricular rate did not differ between the 'High' and 'Low' RR variability groups (240±7 vs. 236±4 1/min, respectively). Mean aortic flow (which strongly correlated with coronary flow; see methods) was significantly greater in the 'High' RR variability group than in the 'Low' RR variability group (8.9±1.8 ml/min/g vs. 6.8±1.1 ml/min/g, respectively; $P<0.05$).

The analysis of the real time aortic flow signal, the perfusion pressure and the left ventricular pressure in each cardiac cycle revealed that left ventricular pressure exceeded perfusion pressure in most beats in hearts with 'Low' RR variability, associated with a transient reversal of the direction of aortic flow (Figure 4). The average (per beat) duration of

the non-perfused interval (in which left ventricular pressure exceeded perfusion pressure) did not differ significantly between the 'High' and 'Low' RR variability groups (37±10 ms vs. 38±8 ms, respectively). However, cumulative number of beats that lacked non-perfused interval was greater in the 'High' RR variability group versus in the 'Low' RR variability group (Figure 5a). Consequently, the cumulative duration of the perfused intervals was significantly greater in the 'High' RR variability group versus in the 'Low' RR variability group (Figure 6a). Thus, ventricular irregularity significantly increased the ratio of the perfused to non-perfused intervals (Figure 6c). Usually, the beats that lacked non-perfused interval were atrial or ventricular premature beats and beats that follow 'post-extrasystolic potentiation' (PESP) beats (Figure 7).

Discussion

There is a positive linear correlation between mean ventricular rate and coronary flow in Langendorff perfused guinea pig hearts. However, the slope of the regression line is steeper when beat-to-beat variability of ventricular cycle length increases, which results in an increase in coronary flow in the physiological heart rate range independently of mean RR interval.

In the present investigation with Langendorff perfused guinea pig hearts, left ventricular pressure at peak systole exceeded perfusion pressure. The positive effect of increased beat-to-beat variability of the cycle length on coronary flow resulted from the preponderance of beats in which left ventricular pressure did not exceed perfusion pressure during systole, facilitating coronary perfusion.

Positive, linear correlation between the ventricular rate and coronary flow

The linear correlation between mean ventricular rate and coronary flow reflects the established relationship between coronary blood flow in vivo and myocardial oxygen and

nutrient demand (Duncker, et al., 2012; Kingma & Rouleau, 2007). One interesting implication from the present study is confirmation that autoregulation is largely autonomic-independent (Langendorff hearts are denervated). In vivo, the autonomic nervous system may influence autoregulation (Kingma & Rouleau, 2007), but the relationship is preserved in autonomically denervated dogs (Kingma & Rouleau, 2007; Rouleau, Simard, Rodrigue, Blouin, & Kingma, 2002), and in isolated, denervated, Langendorff-perfused rat hearts (Bernier, et al., 1989). The present data confirm that autoregulation is intrinsic to the heart.

The relationship between cardiac work and the oxygen supply is likely to be of critical importance to this. Oxygen extraction is nearly maximal from the circulating blood in the heart, therefore an increased oxygen supply can be achieved only by increasing coronary flow via coronary vasodilatation (Duncker, et al., 2012). Exercise-induced tachycardia results in an increased coronary blood flow due to decreased coronary vascular resistance (Duncker, et al., 2012), as shown in several species (Duncker, Stubenitsky, & Verdouw, 1998; Khouri, Gregg, & Rayford, 1965; Laughlin, Klabunde, Delp, & Armstrong, 1989; Manohar, 1988; Vatner, Higgins, Millard, & Franklin, 1974; von Engelhardt, 1977). Increased production of adenosine and other endogenous vasodilator substances, such as bradykinin, atrial natriuretic peptide or nitric oxide have been reported to contribute to this (Achs, Garfinkel, & Opie, 1982; Berne, 1963; Duncker, et al., 2012; Vial, Owen, Opie, & Posel, 1987). The rate-dependence of flow that we observed is in agreement with published data (Bernier, et al., 1989), and in this respect validates the method used.

Mechanism by which ventricular irregularity increases coronary flow in the physiological heart rate range in Langendorff perfused hearts

Beat-to-beat variability of the ventricular cycle length is referred to '*heart rate variability*' when measured *in sinus rhythm in vivo*, and *heart rate variability* is a biomarker

of vagal nerve activity *in vivo* (Koizumi, Terui, & Kollai, 1985). However, the present experiments were performed in isolated, Langendorff perfused hearts (denervated, in the absence of the rest of the animal). Thus, the effect of cycle length variability on coronary flow was not mediated by parasympathetic activity or any effect of the autonomic nervous system.

The results of the second set of experiments showed that the positive effect of ventricular irregularity on coronary flow in the physiological heart rate range was mediated via changes in left ventricular pressure, presumably via ventricular compression of coronary arterioles. In regular rhythm, left ventricular (and intramural) pressures overcame perfusion pressure in systole, reversing the direction of aortic flow for a short period during systole in each beat. In contrast, a single premature beat produced at least two beats, in which left ventricular pressure remained below the perfusion pressure in systole. This suggests that the positive effect of ventricular irregularity on coronary flow is specific to the isolated heart preparation perfused at constant pressure, and it does not necessary involve any change in work-induced autoregulation.

Balance between left ventricular load and ejection in Langendorff perfused hearts

Real-time aortic flow, perfusion pressure and left ventricular pressure signals imply that left ventricle ejected during systole in regular rhythm in the present experiments with Langendorff perfused guinea pig hearts. Real-time aortic flow values showed that there was an apparent balance between left ventricular filling and ejection in each beat in regular rhythm. A crucial question emerges: how does the perfusion fluid enter into the left ventricle in Langendorff perfused hearts? According to the original description by Langendorff, when perfusion solution flows retrogradely to the aorta, the aortic valve is closed (Bell, Mocanu, & Yellon, 2011; Langendorf, 1895), therefore the left ventricle should not be filled with perfusion solution. One explanation is that the aortic valve was incompetent in the present

experiments. Technical failure (e.g., valve damage during insertion of the aortic cannula) is unlikely as special care was taken during preparation to avoid this. Also, the dicrotic notch in the aortic pressure signal (Figure 4) implies that aortic valves were functioning normally. A more plausible explanation is that that the intact (not disrupted) aortic valve does not close properly in isolated hearts perfused with constant pressure, and there is a considerable leakage of perfusion fluid into the left ventricle in normal circumstances (Wiggers, 1909). Wiggers used dog, cat and rabbit hearts on a constant pressure perfusion system, and measured the outflow from the filled right atrium, and the fluid entering the left ventricle was drained by a cannula pushed through the left ventricle wall (Wiggers, 1909). Massive leakage was recorded from the left ventricle in most of the experiments when the perfusion cannula was in the aorta. However when the perfusion cannula was inserted directly into the coronaries, the leakage decreased significantly, but a small amount of fluid still entered into the left ventricle through the Thebesian vessels (Wiggers, 1909). Our results accord with Wiggers' data and show that the Langendroff perfused guinea pig heart is loaded just like the isolated, perfused dog, cat and rabbit hearts (Wiggers, 1909). Furthermore, results of the present investigation imply that ventricular irregularity disturbs the balance between load and ejection, which consequently affects left ventricular and intramural pressures, and thus, influences coronary flow in isolated hearts.

Pharmacological relevance

The proarrhythmic liability of almost every newly developed drug has to be evaluated (Farkas & Nattel, 2010). Isolated Langendorff-perfused hearts are frequently used for proarrhythmia investigations (Farkas, et al., 2006; Farkas, et al., 2009), and ancillary readout such as coronary flow helps form an integrated risk assessment (Pugsley, Authier, & Curtis, 2008). The present results indicate that drug-induced arrhythmias may affect coronary flow

independently of heart rate in isolated hearts, meaning any observed flow changes will include a component that is entirely independent of direct coronary vascular actions and rate-dependent vascular tone changes. Also, a drug that inhibits arrhythmias may be expected to evoke rate-independent decremental effects on coronary flow that could be falsely interpreted as a coronary vasoconstrictor adverse drug action. These possibilities may now be anticipated and accounted for when coronary flow data are interpreted in any such drug study in which rhythm alterations occur.

Conclusions

There is a positive, linear correlation between mean ventricular rate and coronary flow in isolated Langendorff perfused guinea pig hearts, but variability of the ventricular cycle length influences this correlation independently of mean ventricular rate. When cycle length variability increases, the slope of the regression line gets steeper. Thus, in the physiological heart rate range, an autonomic-independent increase in coronary flow occurs in guinea pig hearts, which is mediated by cycle length irregularity via producing beats that have left ventricular pressure reduced below perfusion pressure. Changes in ventricular rhythm will therefore affect coronary flow independently of heart rate in isolated hearts perfused at constant pressure, and this fact should be noted in drug studies on arrhythmias performed in Langendorff perfused hearts.

Acknowledgements

This work was supported by Heptagon Foundation, Burton Fund, Hungarian Scientific Research Fund [OTKA PD 105882] and National Development Agency and European Regional Fund [TÁMOP-4.2.2-08/1-2008-0013, TÁMOP-4.2.1/B-09/1/KONV-2010-0005].

Dr. Attila S. Farkas is a holder of János Bolyai fellowship (BO/00794/12) of the Hungarian Academy of Sciences.

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Figure legends

Figure 1. The beat-to-beat variability of the ventricular cycle length quantified by the root mean square of the successive differences of the RR intervals (RMSSD) in 87 Langendorff perfused guinea pig hearts in the first set of experiments. Experiments are sorted by increasing order of the RMSSD. The 87 hearts were retrospectively divided into two groups based on the value of the RMSSD parameter. That is, a 'Low' RR variability group (RMSSD < 3 ms; n=50 hearts, dark squares), and a 'High' RR variability group (RMSSD > 3 ms; n=37 hearts, light circles) were arbitrarily defined.

Figure 2. Beat-to-beat variability of the ventricular cycle length (RMSSD) (part a), mean ventricular rate (part b) and coronary flow (part c) in the 'Low' and 'High' RR variability groups in the first set of experiments. The dark columns illustrate the 'Low' RR variability group; light columns illustrate the 'High' RR variability group. *P<0.05 vs. 'Low' RR variability group. For further details, see Figure 1.

Figure 3. Correlation between mean ventricular rate and coronary flow in the 'Low' and 'High' RR variability groups in the first set of experiments. The slope of the regression line of the 'High' RR variability group is significantly greater than that of the 'Low' RR variability group (0.08 vs. 0.04, respectively; *P<0.05, analysis of covariance).

Figure 4. The volume conducted electrocardiogram (ECG), real-time aortic flow (Flow), the perfusion pressure (PP) and the left ventricular pressure (LVP) signals in regular sinus rhythm in an isolated, Langendroff perfused guinea pig heart. D: dicrotic notch in the perfusion pressure signal. A short segment of the flow, perfusion pressure and left ventricular pressure

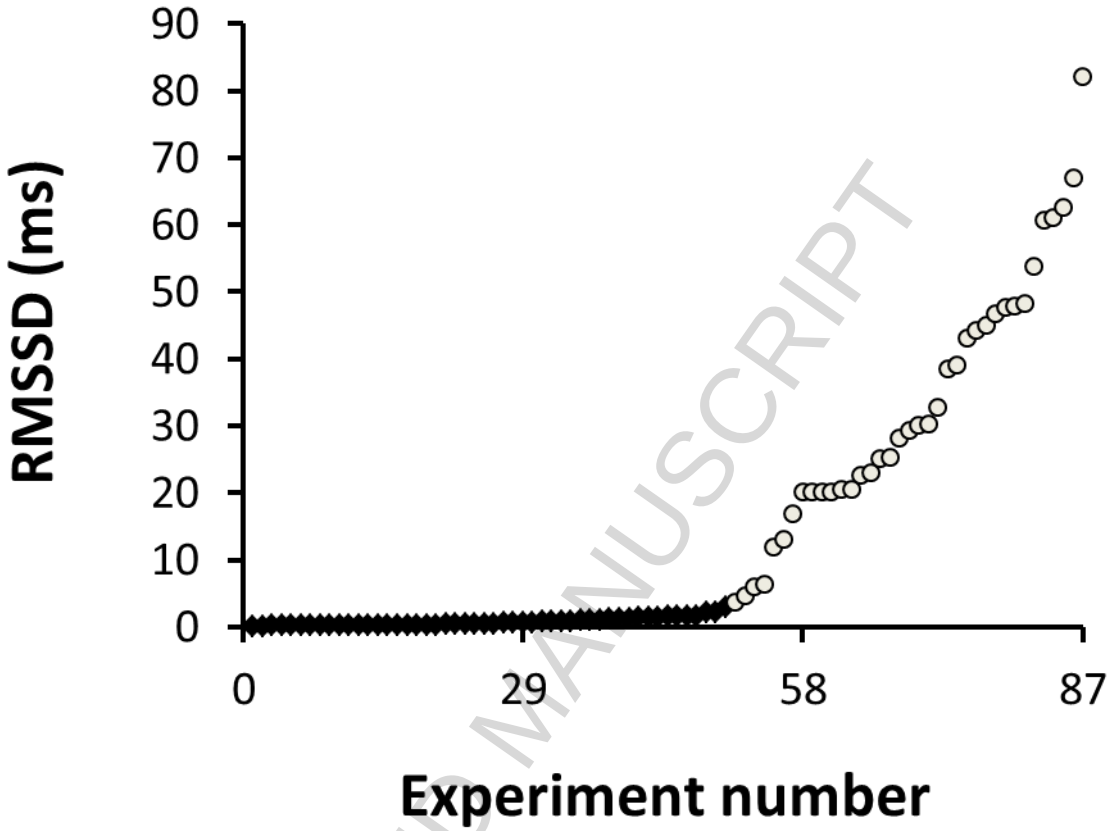
signals in the box is magnified in the right hand site. Table shows the corresponding values of the flow, perfusion pressure and left ventricular pressure at the dashed, vertical marker lines in the magnified segment. Note that left ventricular pressure exceeds perfusion pressure during systole in the grey area between marker lines 2-3 meaning that coronary artery perfusion is compromized by intramural pressure during this period. Also, real-time aortic flow signal shows negative values during this non-perfused period meaning that left ventricle ejects into the aortic stump against the perfusion column, when left ventricular pressure overcomes perfusion pressure. Closure of the aortic valve at the end of ejection is shown by the dicrotic notch in the perfusion pressure signal, when left ventricular pressure becomes lower than perfusion pressure immediately after marker line 3. Note that atrial activity (P wave) is not recognizable in the volume conducted ECG signal as only ventricles were submerged.

Figure 5. The number of perfused and non-perfused beats during the examined 30-second-long interval in the 'Low' and 'High' RR variability groups in the second set of experiments (n=9 hearts per group); box and whisker plots. Part a: the number of perfused beats, that is, the number of beats, in which left ventricular pressure remained below the perfusion pressure during systole. Part b: the number of non-perfused beats, that is, the number of beats, in which left ventricular pressure exceeded perfusion pressure during systole. *P<0.05 vs. 'Low' RR variability group.

Figure 6. The cumulative duration of the perfused (part a) and non-perfused (part b) intervals in the examined 30-second-long period, and the ratio of the perfused to non-perfused intervals (part c) in the second set of experiments. The dark columns illustrate the 'Low' RR variability

group; light columns illustrate the 'High' RR variability group. *P<0.05 vs. 'Low' RR variability group. For further details, see Figure 5.

Figure 7. The effect of an atrial premature beat (APB) on the aortic flow (Flow), the perfusion pressure (PP) and the left ventricular pressure (LVP) in an isolated, Langendroff perfused guinea pig heart. ECG: volume conducted electrocardiogram, D: dicrotic notch in the perfusion pressure signal. Table shows the corresponding values of the flow, perfusion pressure and left ventricular pressure at the dashed, vertical marker lines. For further details see Figure 4. Note that the maximal left ventricular pressure value of the premature beat (at marker line 2) is lower than the corresponding value of the perfusion pressure. Premature beat is followed by a 'post-extrasystolic potentiation' (PESP) beat, in which left ventricle produces a high peek ventricular pressure and a massive ejection (see values at marker line 3). PESP beat is followed by 3 beats in which the peek left ventricle pressure remains under the perfusion pressure, though left ventricular pressure increases gradually (see values at marker lines 4-6). Also note that coronary flow remains in the positive range in all 4 beats in which left ventricular pressure remains below the perfusion pressure (see values at marker lines 2, 4-6). This means that left ventricle does not eject in these 4 beats, and this is confirmed by the lack of the dicrotic notch (the sign of closure of the aortic valve) in the perfusion pressure signal in the beats at marker lines 2, 4, 5 and 6; coronary artery perfusion is not compromized by the intramural pressure during the entire duration of these 4 beats.



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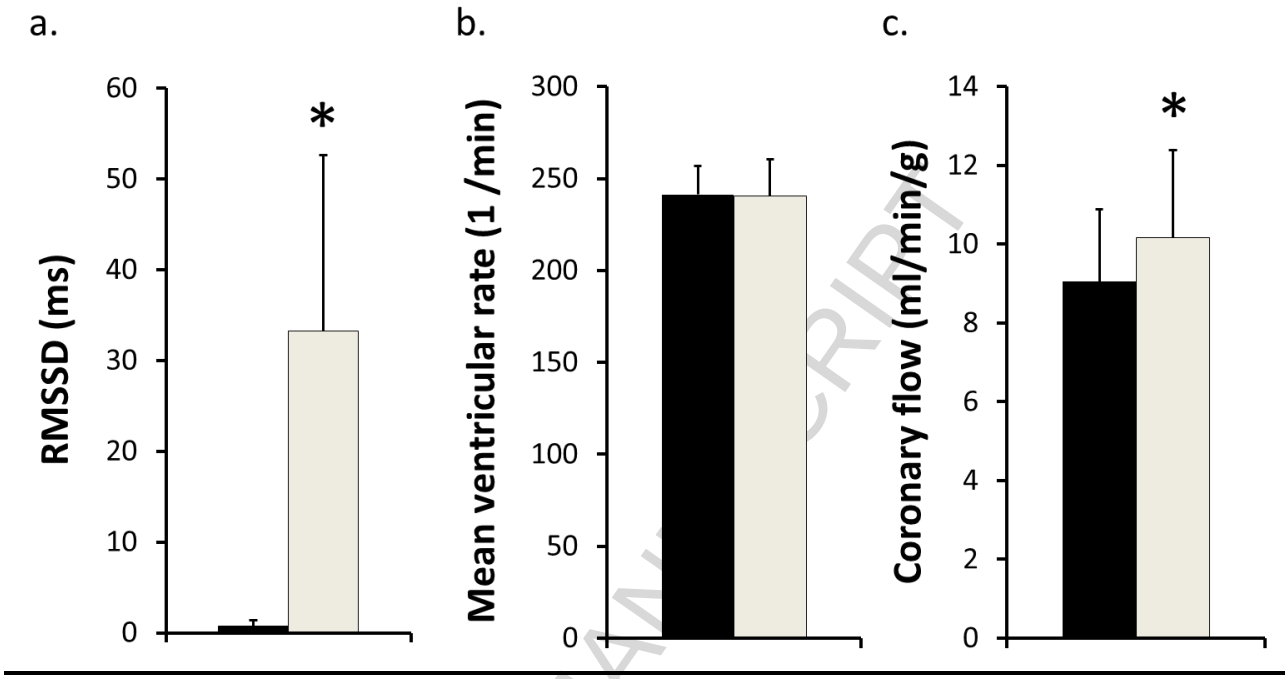

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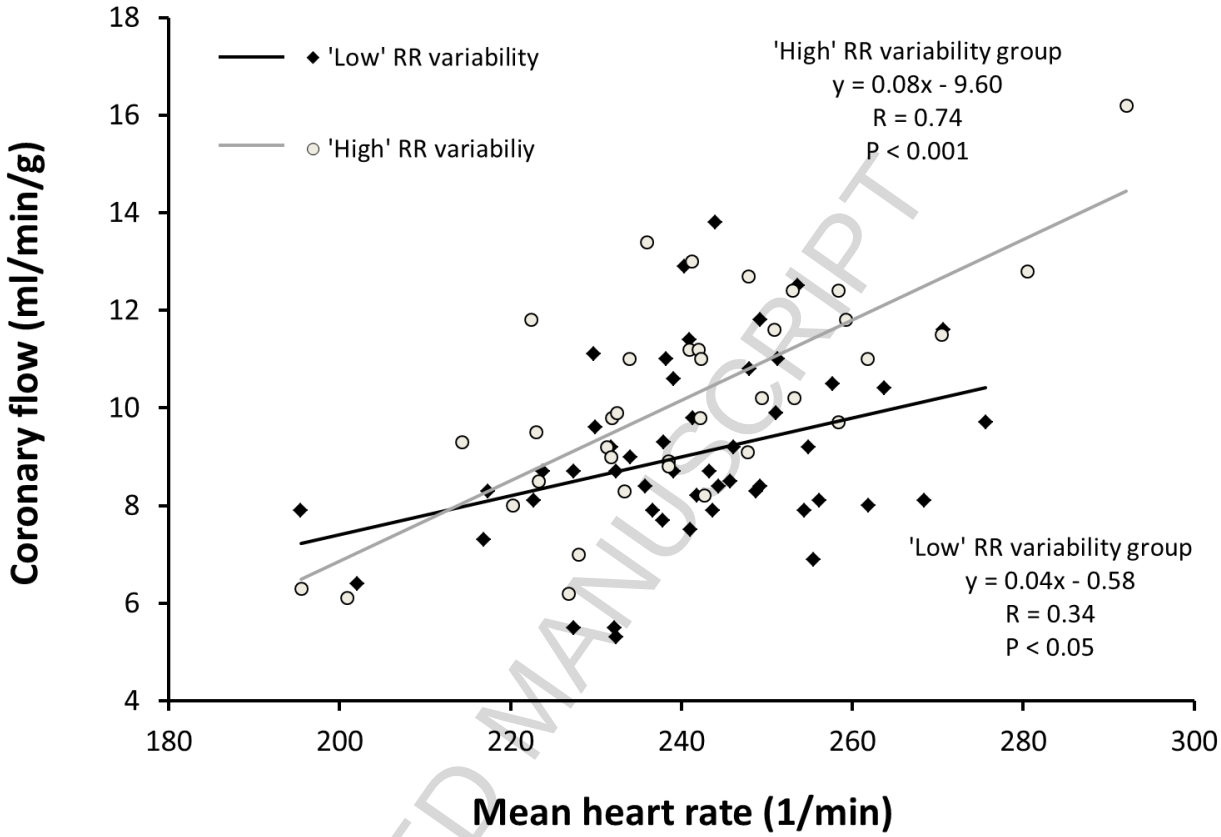

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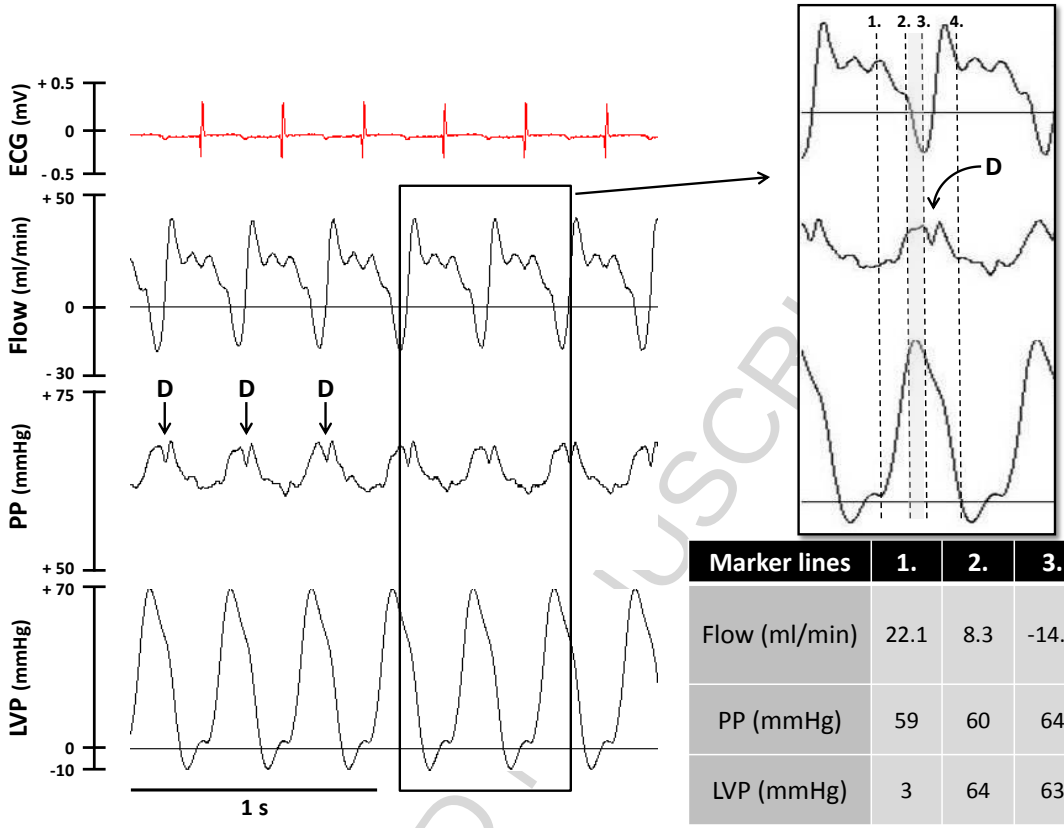
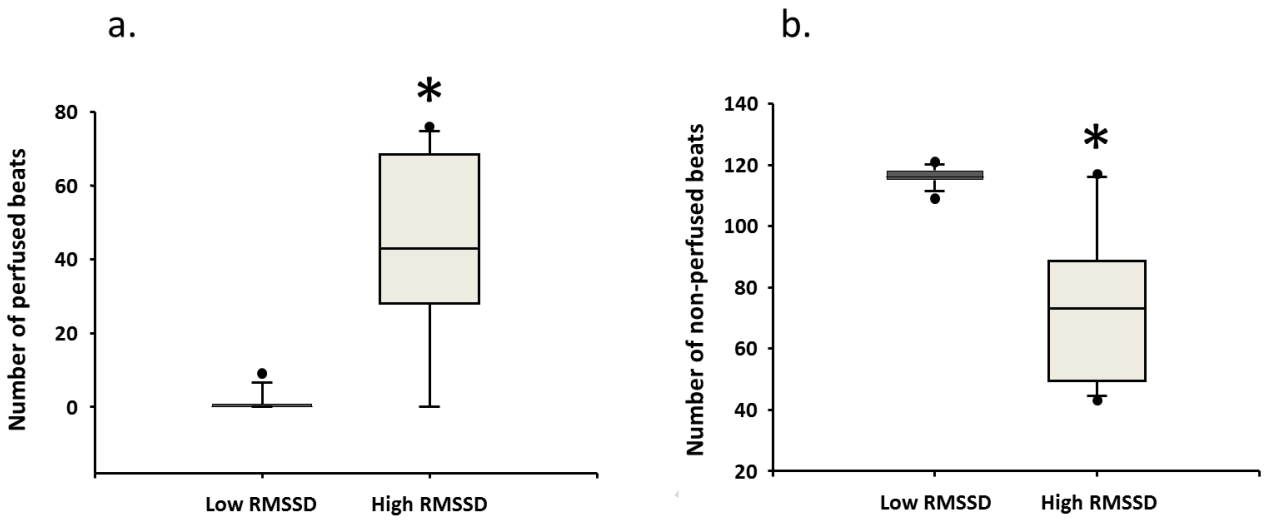


Marker lines	1.	2.	3.	4.
Flow (ml/min)	22.1	8.3	-14.9	18.7
PP (mmHg)	59	60	64	61
LVP (mmHg)	3	64	63	0

Figure 4. The volume conducted electrocardiogram (ECG), real-time aortic flow (Flow), the perfusion pressure (PP) and the left ventricular pressure (LVP) signals in regular sinus rhythm in an isolated, Langendroff perfused guinea pig heart. D: dicrotic notch in the perfusion pressure signal. A short segment of the flow, perfusion pressure and left ventricular pressure signals in the box is magnified in the right hand site. Table shows the corresponding values of the flow, perfusion pressure and left ventricular pressure at the dashed, vertical marker lines in the magnified segment. Note that left ventricular pressure exceeds perfusion pressure during systole in the grey area between marker lines 2-3 meaning that coronary artery perfusion is compromized by intramural pressure during this period. Also, real-time aortic flow signal shows negative values during this non-perfused period meaning that left ventricle ejects into the aortic stump against the perfusion column, when left ventricular pressure overcomes perfusion pressure. Closure of the aortic valve at the end of ejection is shown by the dicrotic notch in the perfusion pressure signal, when left ventricular pressure becomes lower than perfusion pressure immediately after marker line 3. Note that atrial activity (P wave) is not recognizable in the volume conducted ECG signal as only ventricles were submerged.



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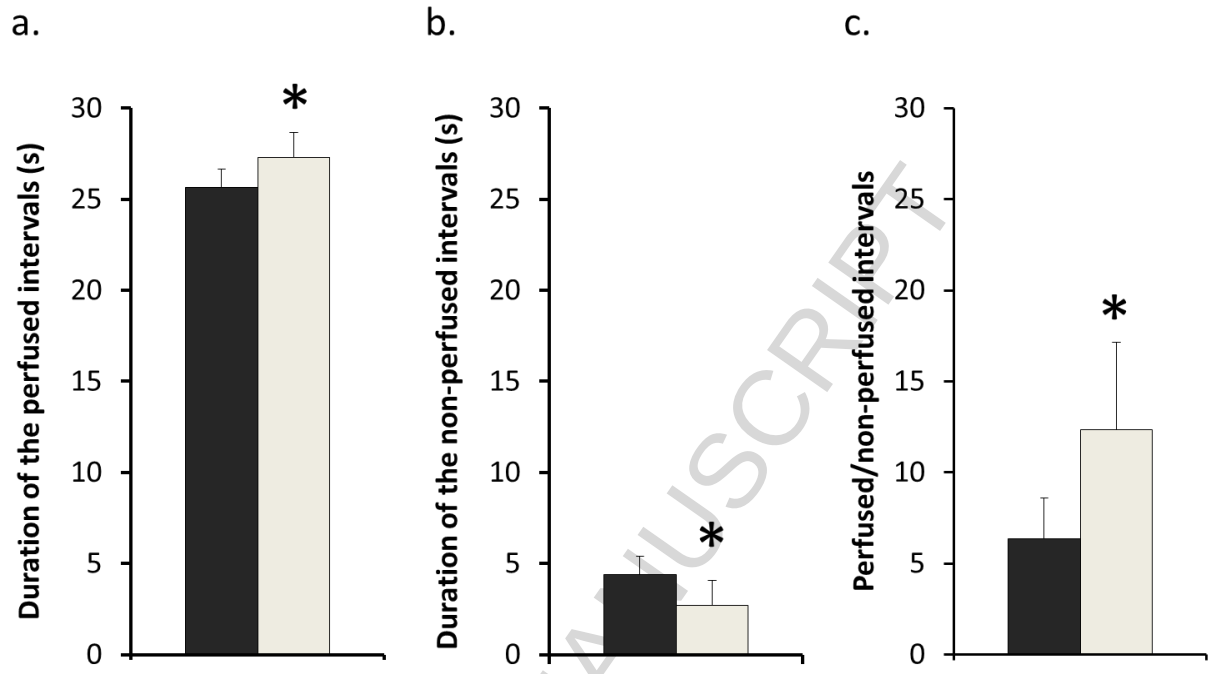

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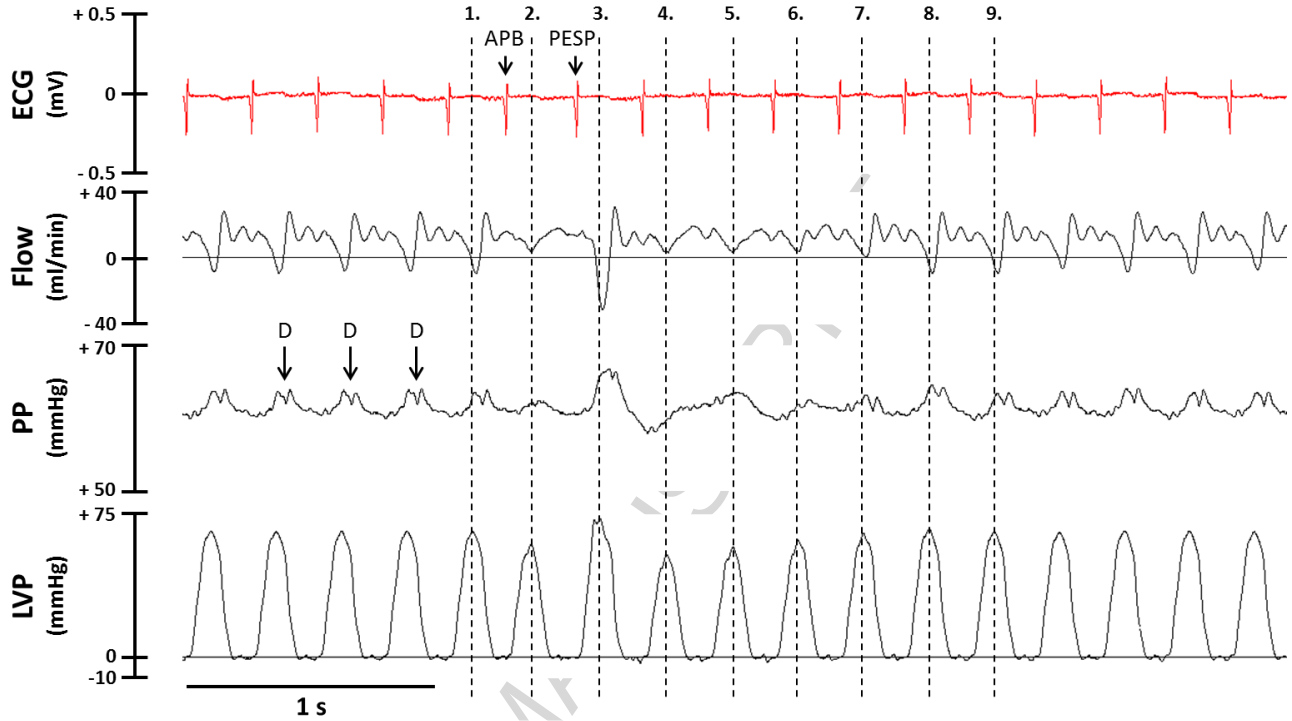

Marker lines	1.	2.	3.	4.	5.	6.	7.	8.	9.
Flow (ml/min)	-4.5	2.9	-27.9	2.9	2.9	3.4	1.6	-4.3	-5.3
PP (mmHg)	65	64	68	62	65	64	65	66	65
LVP (mmHg)	67	60	73	54	58	62	66	68	67

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