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

Hedvig Takács [a], Péter Kui [b], Attila S. Farkas [a], Annamária Sarusi [b], Tamás Forster [a], Julius Gy. Papp [b,c], András Varró [b], Michael J. Curtis [d], Michael J. Shattock [d], András Farkas [a,*]

[a] Second Department of Medicine and Cardiology Centre, Faculty of Medicine, University of Szeged, Szeged, Hungary
[b] Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged, Szeged, Hungary
[c] MTA-SZTE Research Group for Cardiovascular Pharmacology, Hungarian Academy of Sciences, Hungary
[d] King's College, London, UK

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ABSTRACT

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. Hypokalemic Krebs solution facilitated spontaneous development of arrhythmias. The ECG, left ventricular and perfusion pressures were recorded, and the 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.

1. 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

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.

* Corresponding author at: 2nd Department of Medicine and Cardiology Centre, Faculty of Medicine, University of Szeged, Semmelweis utca 6., H-6725 Szeged, Hungary.
E-mail address: farkas.andras@med.u-szeged.hu (A. Farkas).

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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.

2. Methods

2.1. 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, and 11.1 mM glucose. Langendorff perfusion was initiated with solution delivered at 37°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°C.

2.2. 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.

2.3. 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 analyzed 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 s of the 30-min-long control perfusion period. The percent 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-s-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 interval durations and N is the total number of intervals) and calculating $RMSSD = \sqrt{E([\Delta d]^2)}$, 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) (Fig. 1). The beat-to-beat variability of the cycle length (RMSSD), the percent 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.

2.4. 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 min 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-s-long interval during the 60-min-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: $R =$