

Left coronary pressure–flow relations of the beating and arrested rabbit heart at different ventricular volumes

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Abstract

Objective: To study the effect of cardiac contraction on left coronary artery pressure–flow relations at different vascular volumes and to compare these relations in the beating heart with those in the heart arrested in systole and diastole. **Methods:** Maximally vasodilated, Tyrode perfused, rabbit hearts ($n=6$) with an intra-ventricular balloon were used. The left coronary artery was separately perfused via a cannula in the left main coronary artery. The slopes and the intercepts of left coronary pressure–flow relations were determined in the beating and arrested heart at different chamber volumes. A 3-factor design with repeated measures was used to compare the effect of three factors: phase of contraction (systole and diastole), chamber volume (V_0 and V_1 , left ventricular end-diastolic pressure 1.4 and 20 mm Hg, respectively) and the type of contraction (beating and arrested; a measure of capacitive effects). **Results:** The phase of contraction has a significant effect on the intercepts (>40 mmHg, $p=0.00032$) but not on the slopes of the pressure–flow relations. Chamber volume had a small effect on the intercepts (<5 mm Hg, $p=0.037$), but not on the slopes of the pressure–flow relations. The type of contraction has a significant effect on the slopes ($\sim 10\%$, $p=0.00021$) but not on the intercepts of the pressure–flow relations. **Conclusions:** In the isolated Tyrode perfused rabbit heart left coronary pressure–flow relations are mainly determined by contraction, while left ventricular chamber volume and capacitive effects contribute little. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Diastolic arrest; KCl; Barium contracture; Isolated heart; Left ventricular pressure; Rabbit

1. Introduction

Coronary arterial inflow is impeded in systole as a result of cardiac muscle contraction. Previously, we have hypothesized that this diminished flow is the result of the varying mechanical properties of the cardiac muscle during the heart cycle [1–6]. In the ventricular cavity, contraction results in a time varying pressure–chamber volume relation, i.e., the time varying elastance [7,8]. In the coronary vessels, we assume cardiac contraction to have the same effect, resulting in a time varying pressure–vascular volume or pressure–vascular cross-sectional area relation [9]. The decrease in cross-sectional area of the coronary vessels during systole results in an increase in resistance, and in a ‘pumping action’ or capacitive effect [1]. Left

ventricular pressure, as used by the waterfall model [10] and the intra myocardial pump model [11–13], play a minor role in our concept.

Earlier, we studied the effect of cardiac contraction on coronary arterial inflow in the maximally dilated [5] and autoregulating bed [4], while keeping constant arterial and venous pressures. In these studies, variation of left ventricular pressure by changing left ventricular chamber volume had little effect on the impeding of coronary flow. These findings are in line with the varying elastance concept. However, only one single perfusion pressure was used. Coronary pressure–flow relations in diastole were determined in several studies [14–16]. However, to the best of our knowledge, no studies have been performed where coronary pressure–flow relations were measured simultaneously both in systole and diastole both in the

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beating and in the arrested heart at different volumes. The changes in vessel diameters during the cardiac contraction result not only in vascular resistance changes but also in vascular volume changes (capacitive effects) and tend to decrease arterial inflow mainly in early contraction [1,17]. These capacitive effects can be derived by comparing diastolic and systolic left coronary pressure–flow relations in the beating with those obtained in the arrested heart.

In the present study we test the hypothesis that tissue stiffness is the main determinant of coronary flow. The stiffness change due to contraction is much larger than the change in stiffness resulting from volume changes. Therefore contraction (i.e., the difference between diastolic and systolic muscle) is predicted to have a larger effect on flow than stretch (i.e., an increase in ventricle volume).

Altogether three factors that determine coronary pressure–flow relations were studied: phase of contraction (systole and diastole), left ventricular chamber volume (V_0 and V_1) and type of contraction (beating and arrested).

2. Methods

The investigation conforms with the *Guide for the care and use of laboratory animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised in 1985) and is performed according to the guidelines of the D.E.C. (Animal Experiments Committee of the Free University of Amsterdam, The Netherlands).

2.1. Heart preparation

Male Flemish Giant rabbits ($n=6$), weighing 5.92 ± 0.35 kg (mean \pm sem), were anaesthetized with Fluanisone (9 mg/kg) and fentanyl citrate (0.3 mg/kg) (Hypnorm, Janssen Pharmaceutica B.V., Tilburg, The Netherlands), injected intramuscularly. Subsequently, sodium pentobarbital (30 mg) with benzyl alcohol (4.5 mg, Nembutal, Sanofi B.V., Maassluis, The Netherlands) was injected in an ear vein and the heart was exposed. Heparin (2500IU, Leo Pharmaceutical Products B.V., Weesp, The Netherlands) was administered to avoid blood coagulation. The aorta was cannulated, the heart was excised quickly and the aorta cannula was connected to the set up. The perfusion of the heart was discontinued only for a few seconds.

The heart was perfused (not recirculated) with a Tyrode solution (128.3 mM NaCl, 4.7 mM KCl, 1.36 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.05 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 20.2 mM NaHCO_3 , 0.42 mM $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 11.1 mM $\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$) containing adenosine (10 μM) to maximally dilate the coronary vascular bed, in order to establish constant vasomotor tone. The perfusate was bubbled with a mixture of 95% O_2 and 5% CO_2 at 28°C and pH 7.3–7.4. Before entering the heart the Tyrode solution was passed through a 3 μm filter (Millipore S.A., Molsheim, France). To drain

the Thebesian flow a cannula was introduced through the apex of the left ventricle.

The atrio-ventricular node was destroyed by crushing the nodal tissue. The heart was paced by two electrodes, one attached to the apex cannula and the other to the right ventricle. Pace frequency was set at approximately 0.7 Hz, just above the ventricular rhythm. A latex balloon, filled with distilled water was placed in the left ventricle via the left auricle and through the mitral valve. The heart was beating isovolumically. Left ventricular balloon pressure (LVP) was measured using a pressure transducer (Gould P23ID, USA).

2.2. The modified Langendorff set up (Fig. 1)

The Langendorff set up consisted of two separate perfusion lines to change the flow to the left heart specifically. The two perfusion lines were connected to two Tyrode-filled reservoirs (a and b in Fig. 1) to obtain different perfusion pressures (see protocol). A rapid pneumatic switch was used to alternate perfusion of the heart from either reservoir (a) or (b). Both the function generator and the pneumatic switch were controlled by a digitimer. The right perfusion line, containing a thermistor and an electromagnetic flow probe (EM, Skalar Instruments, The Netherlands), terminated in the aortic cannula. The left coronary perfusion line contained a transit-time flow probe (TT, Transonic Systems, Ithaca, New York, U.S.A.) and was connected to a small cannula (see inset Fig. 1), that was inserted through the aortic cannula into the left main stem, where it was wedged in position and secured with a suture. Left coronary perfusion pressure (P_c , Gould P23ID USA) was measured at the proximal side of the left main stem cannula.

To check whether the left main stem cannula was tight in place in the left main stem, two tests were performed. Ink was injected into the heart through the left or right perfusion line respectively. Fluid leakage was found to be unlikely when the section of the heart not perfused with ink did not color. Second, zero flow pressures were measured in the left main stem by occluding both perfusion lines and then occluding only the left perfusion line. If the difference between these two zero-flow pressures was within 5 mmHg we assumed that perfusion of the left coronary bed occurred solely through the left main stem cannula. When leakage was found, the cannula was repositioned and the three tests were repeated.

2.3. Protocol (Fig. 2)

The experiments were divided in four sections (see bottom section of Fig. 2). Each section is divided in three volume settings and at each volume a left coronary pressure flow relation is determined. In the first section the heart was perfused with Tyrode and beating. In the second

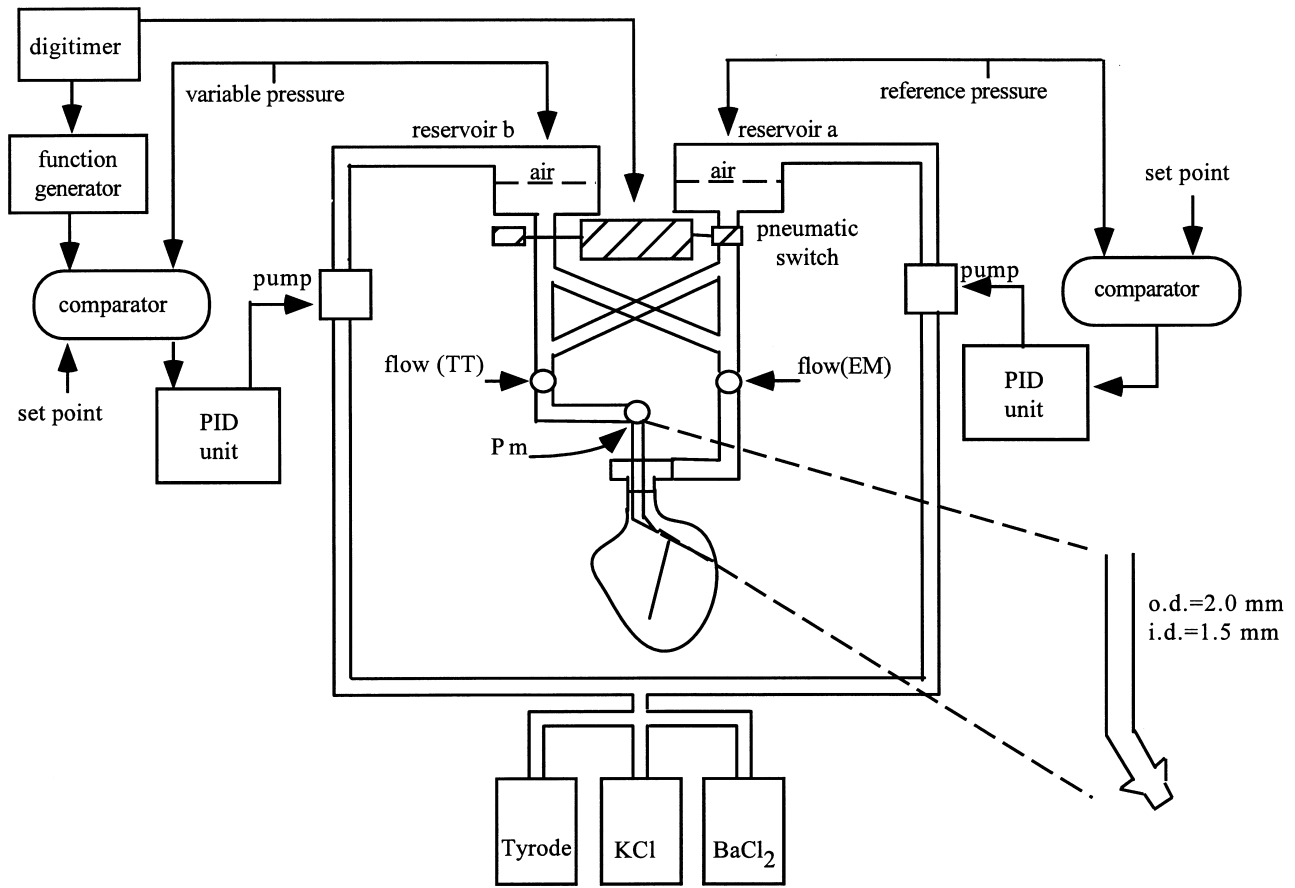


Fig. 1. Experimental set-up of the isolated heart. The left coronary artery cannula is shown in the inset. The pneumatic switch, controlled by the digitizer, connects both perfusion lines to either reservoir **a** (reference pressure) or reservoir **b** (variable pressure). PID unit=Proportional Integral Derivative control unit; flow (TT)=transit time flow meter; flow (EM)=electromagnetic flow meter; P_m=pressure transducer left coronary artery.

section the heart was arrested in diastole (static diastole) by changing the perfusion solution to a Tyrode solution containing 20.0 mM KCl (NaCl concentration was lowered to 113 mM). In the third section the heart was perfused with normal Tyrode and beating again to check the stability of the preparation. Pressure–flow relations had to be statistically not significantly different from the first section of the experiment, in order to accept the experiment. In the fourth and final section of the experiments, the heart was stopped in systole (static systole) by lowering the calcium concentration of the Tyrode (0.078mM CaCl₂·2H₂O) and adding 2.0–3.0 mM BaCl₂ (Barium contracture). Since BaCl₂ is known to cause vasoconstriction, adenosine (50 μM) and Isoproterenol (55 nM) (Sigma I-2760, St. Louis MO, USA) were also added. In pilot experiments we found that left ventricular pressure in static systole was approximately 80% of the peak systolic left ventricular pressure in the beating heart. Systolic left ventricular pressure in the beating heart (first and third section of the experiments) was therefore lowered to 80% of the original value by adding Verapamil (Sigma V-4629, St. Louis MO, USA).

In each section ventricular chamber volume was

changed (V_0 , V_1 , V_0). The first and last chamber volume settings were identical to test the stability of the heart.

At each volume setting a perfusion pressure protocol consisting of nine steps (lasting 6 s each, sufficient to reach a steady state, Fig. 2 top part) was generated for the left coronary bed to determine a pressure–flow relation. Perfusion pressure was after each step returned to the reference pressure (90 mmHg) to check the stability of the preparation by testing if the flow rate at the reference level was constant. When the reference flow rate was not stable ($\geq 10\%$ difference compared to the starting flow rate), the preceding and following steps of the protocol were not used for analysis.

At the end of the experiment the wet and dry (60°C, 48 h) were measured. By calculating the dry–wet weight ratio, formation of edema could be quantified.

2.4. Measurements and analysis of data

Left ventricular pressure (mmHg), left coronary pressure (P_c, mmHg), and flow rate (ml/min) were recorded on a Thermal Array recorder WR3600 (Graphtec Corporation,

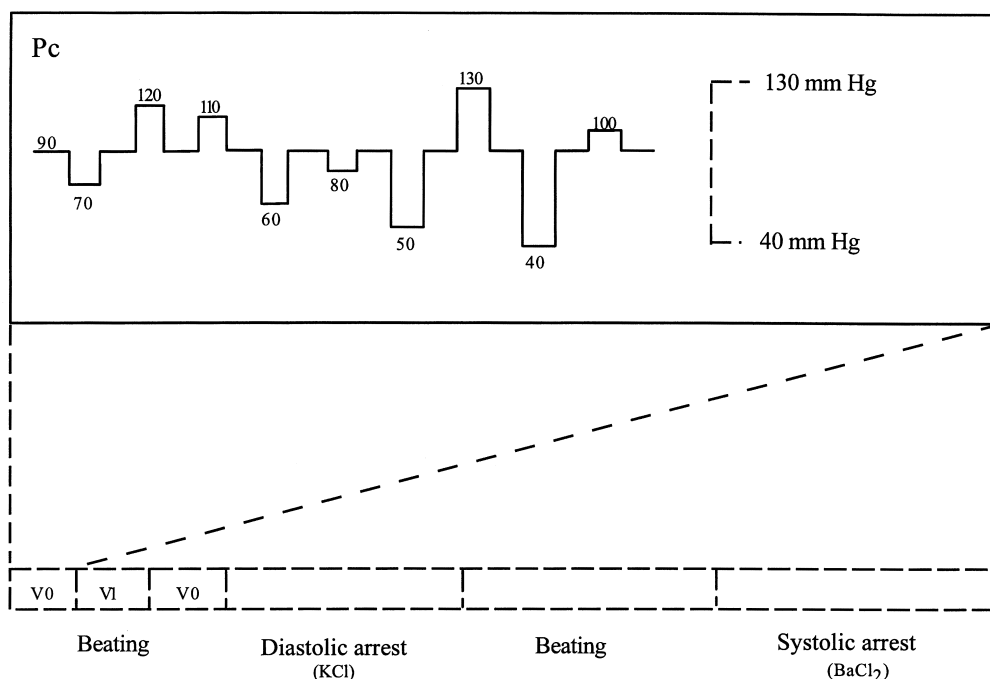


Fig. 2. Experimental protocol. The entire protocol is indicated at the bottom. Each condition is divided in three parts: V_0 , V_1 and V_0 . The dashed lines indicate a blow-up of the first section of the protocol line. A nine step pressure protocol was generated at different ventricular volumes in both the beating and the arrested (systolic and diastolic) heart. Steps in perfusion pressure are applied between 40 and 130 mm Hg. Between steps pressure is at its reference level of 90 mm Hg. The flow values measured at the reference level are used to check the stability of the preparation. The return to beating is also used for stability testing. From each step-protocol a pressure–flow relation is determined.

Tokyo, Japan). Left coronary pressure and flow rate values were also stored (sampling rate 200 Hz) on a personal computer (M290S, Olivetti). For each flow rate, the pressure drop across the left main stem cannula was calculated and subtracted from the measured left coronary perfusion pressure (P_m) in order to determine the true left coronary pressure (P_c) at the tip of the cannula.

Left coronary pressure (P_c)–flow relations were made by applying linear regressions (Left coronary pressure is the independent variable). The Akaike information criterion [18] was employed to investigate if relations could be fitted better by a quadratic equation than a linear one. The intercepts and slopes of the linear regression analysis were used in further statistical comparisons.

Left coronary pressure–flow relations in the beating and the arrested hearts were analyzed at selected left ventricular chamber volumes that resulted in end-diastolic pressures of 1–2 mmHg (V_0) and 20 mmHg (V_1) and in systolic left ventricular pressures of approximately 80 mmHg (V_0) and 115 mmHg (V_1).

The effects of cardiac contraction, ventricular chamber volume and type of contraction on slopes and intercepts of left coronary pressure–flow relations were investigated using analysis of variance with repeated measures, i.e., each heart is investigated under the three conditions. The analysis extracts the variance for the three main effects: (A) phase of contraction, (B) chamber volume and (C) type of contraction. (Systat software, release 4, Evanston

IL 60201). Mean values of slopes and intercepts are given with Standard Error. Significance was chosen at $p < 0.05$.

3. Results

Six successful experiments on isolated rabbit hearts were performed. The left main stem cannula provided the perfusion fluid to the left heart and did not leak. Ink injected in the left main stem cannula only showed up in the left heart. Ink injected in the perfusion line (right perfusion) did not become visible in the left heart. The mean measured zero flow intercept (occlusion of the left heart perfusion flow) in the beating heart was 0.33 ± 0.5 mmHg. We analyzed 24 step-protocols each consisting of nine steps in the six experiments. We left out 15 steps (7 steps in experiment 6) in the analysis because the control flow before and after the step was different.

Two left ventricular chamber volumes were studied: V_0 and V_1 . In the beating and diastolic arrested hearts V_0 resulted in left ventricular end-diastolic pressures of 1.0 ± 0.9 mmHg and 1.8 ± 0.5 mmHg respectively, while at V_1 these values were 19.8 ± 1.5 mmHg and 20.3 ± 1.3 mmHg, respectively. In the systolic arrested hearts we measured left ventricular pressures of 78.0 ± 5.2 mmHg at V_0 and 111.7 ± 4.9 mmHg at V_1 , pressures not significantly different from the systolic left ventricular pressures in the beating hearts (78.2 ± 3.2 mmHg and 119.7 ± 13.2 mmHg,

respectively). Wet weight of the hearts ($n=6$) was 14.5 ± 1.0 gram and dry weight was 2.63 ± 0.2 gram. The dry–wet weight ratio was 0.18 ± 0.01 .

Table 1 shows slopes and intercepts (individual and their mean values) of left coronary pressure–flow relations in diastole and systole at the two ventricular chamber volumes (diastolic pressure at V_0 was 1–2 mmHg and at V_1 20 mmHg) in the beating and arrested heart for all experiments. The p -values from analysis of variance are given at the bottom.

Fig. 3 and Fig. 4 show systolic and diastolic pressure–flow relations of the left main coronary artery in the six **beating** and **arrested** hearts, respectively, at two chamber volumes. Differences in pressure–flow relations between the two chamber volumes are small. The **intercept** of the pressure–flow relations was found to depend on the contraction phase (diastole/systole, $p=0.00032$) and slightly on the chamber volume of the ventricle ($p=0.037$). Post hoc tests on intercepts show that all individual comparisons between ‘diastole and systole’ are significant. Individual comparisons of intercepts between V_0 and V_1 are not significant in systole, but are significant in diastole.

The pressure–flow relations of beating and arrested hearts at the smallest chamber volume (V_0) are compared in Fig. 5. Diastolic flow (indicated with D) is smaller at the same perfusion pressure in the arrested hearts (open square) compared to the beating heart (filled square). Systolic flow (indicated with S) in the arrested hearts (open squares) and the beating hearts (filled squares) are about the same. Only the **slopes** of pressure–flow relations comparing the beating and arrested heart were slightly, but significantly different, a small clockwise rotation was found. The intercepts were not different ($p=0.43$).

Normalized pressure–flow relations at chamber volume V_0 of all six experiments are displayed in Fig. 6. It is evident that in both the beating and the arrested hearts

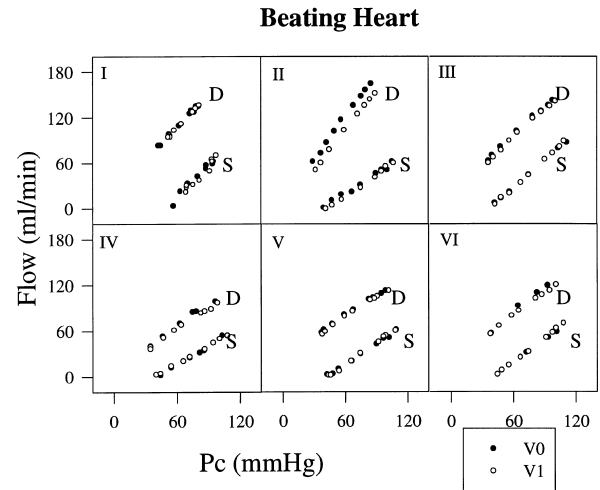


Fig. 3. Left coronary pressure–flow relations at left ventricular volumes V_0 and V_1 in the beating hearts ($n=6$). Each panel contains four relations. The filled symbols are relations at V_0 and the open symbols at V_1 . The two upper relations are diastolic and the two lower relations are systolic. Pc (mmHg)=left coronary artery pressure; Flow=left coronary artery flow rate. The roman numbers are the experiment numbers.

systolic flow is significantly impaired compared to diastolic flow, at all perfusion pressures studied.

4. Discussion

The results from this study show that left coronary pressure–flow relations in the maximally dilated left coronary bed of a Tyrode-perfused rabbit heart differ significantly between systole and diastole, i.e., contraction causes a parallel shift to lower flows. Increase in left ventricular chamber volume, corresponding with a diastolic ventricular pressure rise from ~ 1 to ~ 20 mmHg, did result in a small but significant decrease in the flow rate.

Table 1

(A) Slopes (ml/min/mmHg) and pressure axis intercepts (mm Hg) of left coronary pressure–flow relations ($\text{Flow}=(P_c-P_i) \times \text{slope}$) in beating and arrested hearts at two different volumes (V_0, V_1); (B) p -values of slopes and intercepts of left coronary pressure–flow relations

(A)	Beating (systole)				Beating (diastole)				Arrested (systole)				Arrested (diastole)			
	Slope		Intercept		Slope		Intercept		Slope		Intercept		Slope		Intercept	
	V_0	V_1	0	V_1	V_0	V_1	V_0	V_1	V_0	V_1	V_0	V_1	V_0	V_1	V_0	V_1
Experiment #																
I	1.42	1.46	49.6	51.7	1.44	1.43	-14.9	-14.2	1.10	1.10	35.6	40.4	1.42	1.33	-3.4	-5.3
II	0.87	0.94	35.6	43.1	1.86	1.75	-4.7	-1.3	0.94	0.95	38.0	41.1	1.50	1.52	3.6	6.4
III	1.19	1.25	36.9	38.6	1.24	1.26	-17.3	-13.4	0.98	0.91	32.7	37.3	1.16	1.16	-6.1	-3.7
IV	0.86	0.77	42.0	37.1	0.98	0.90	-8.3	-9.2	0.67	0.63	42.4	43.3	0.86	0.84	1.4	4.5
V	0.89	0.97	41.7	43.7	0.87	0.89	-32.6	-28.0	0.76	0.72	37.2	41.7	0.83	0.83	-15.3	-11.3
VI	0.94	1.04	39.1	41.6	0.92	1.00	-23.3	-19.7	0.75	0.76	46.5	44.5	0.98	0.97	-10.9	-4.7
Mean	1.02	1.07	40.8	42.7	1.21	1.21	-19.2	-14.3	0.87	0.85	38.7	41.4	1.13	1.11	-5.1	-2.4
SEM	0.09	0.10	2.0	2.1	0.16	0.14	5.3	3.7	0.07	0.07	2.1	1.0	0.12	0.11	2.9	2.7
(B)																
P -values					Diastole/systole				Volume V_0/V_1				Beating/arrested			
Slope					0.10				0.89				0.00021			
Intercept					0.00032				0.037				0.43			

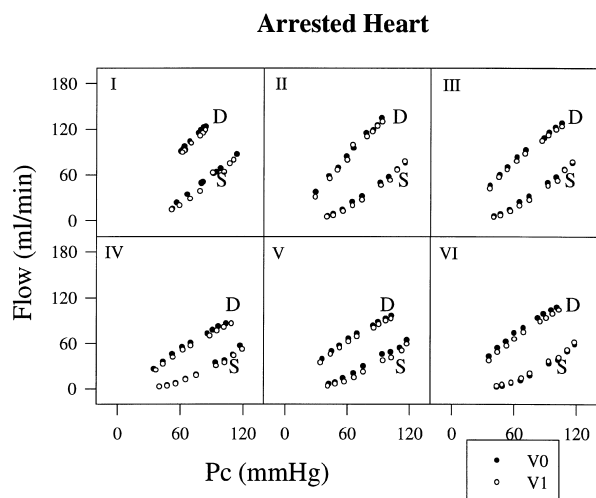


Fig. 4. Left coronary pressure–flow relations at V_0 and V_1 in the hearts ($n=6$) both arrested in systole and in diastole. Pc (mmHg)=left coronary artery pressure; Flow=left coronary artery flow.

Furthermore, comparing beating with arrested hearts, shows that the slopes of the left coronary pressure–flow relations in the arrested heart are smaller than in the beating heart (clockwise rotation).

4.1. Discussion of methods

The mean dry to wet ratio in this study was found to be 0.18 ± 0.01 . In other studies in our laboratory [1,19] a similar value (0.17 ± 0.05) was found. The steady conditions of the hearts were checked by measuring the flow throughout the experiment at the reference pressure. No significant change was found. Any increase in edema throughout the experiment would be reflected in a decrease in this reference flow [16].

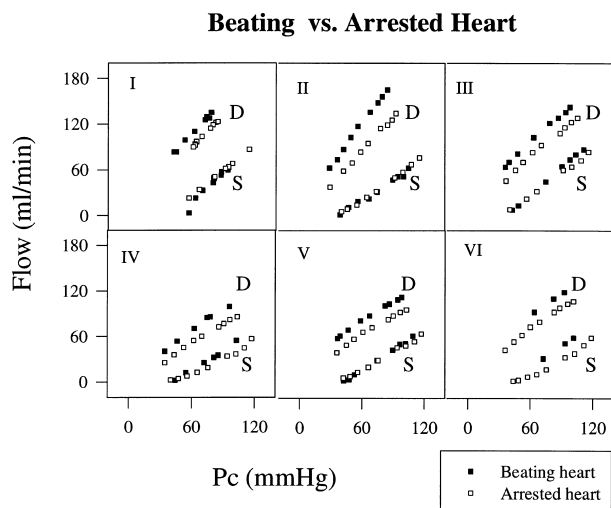


Fig. 5. Comparison of left coronary pressure–flow relations in the beating versus the arrested hearts ($n=6$) at the smallest left ventricular volume (V_0). Pc=left coronary artery pressure; Flow=left coronary artery flow rate.

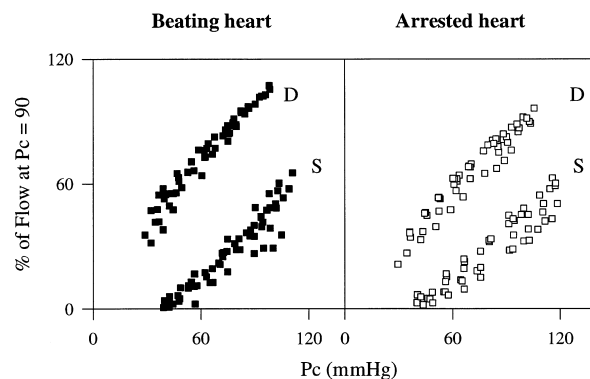


Fig. 6. Normalized pressure–flow relations of all experiments. On the left, results of measurements in the beating heart are displayed. On the right, results of measurements in the arrested heart are displayed. ‘% of Flow at $P_c=90$ ’ is defined as measured left coronary artery flow / reference diastolic left coronary artery flow at a left coronary artery pressure of 90 mmHg*100%; P_c =left coronary artery pressure.

In the beating hearts we added adenosine to the perfusion medium in order to maximally dilate the coronary bed, i.e., flow mediated dilation and myogenic effects could not influence the pressure–flow relationships. In the diastolic arrested heart there is some remaining tone due to the fact that we did not counteract the potassium constriction completely and a small contribution of the myogenic response or shear stress to the pressure–flow relation could be possible. This might explain the slightly smaller flows in the diastolic arrested hearts as compared to diastolic flows in the beating hearts (Fig. 5).

We used Barium contracture to study mechanical interaction in the systolic arrested heart. It could be reasoned that the tissue deteriorates during this intervention. However, the metabolic and functional consequences of the Barium induced contracture are studied by Shibata et al. [20]. They used Tyrode perfused rabbit hearts at 37°C and found that after Barium contracture the tissue is stable for approximately 30 min with a perfusion flow of 35 ml/min. We used a lower temperature (27°C) during our experiments. The Q_{10} for heart metabolism is 2.1 [21] and therefore a flow of ~ 17 ml/min (35 divided by 2.1) would have been sufficient. Our systolic arrest protocol lasted less than 30 min. and our perfusion flow during systolic arrest at reference pressure was minimally 31.9 ml/min. Thus, conditions were chosen to prevent cardiac deterioration. Temporarily, during our step protocol, we went down to lower flows, but the duration (six sec) was less than the actual delay time for the start of autoregulation. We also tested cardiac conditions in the step protocol (Fig. 2) and had stable pressure development. We conclude that deterioration of the tissue during the period of Barium arrest is unlikely.

We applied the Akaike criterion to all experiments to investigate whether the pressure–flow relations were best described by a linear or quadratic function. This was done because it seemed that the pressure–flow relations in Figs.

3 and 4, tended to be curvilinear, as suggested in the literature [15,22–24]. Curvilinearity is accentuated at lower pressures and may reflect changes in the number of perfused channels as well as the size of the individual channels. Hanley et al. [23] support the latter option. Curvilinear systolic coronary pressure–flow relations were predicted by a model for the effect of cardiac contraction on coronary blood vessels [9] developed in our institute. However, although in 70% of the cases a curvilinear relationship fitted the data somewhat better according to the Akaike criterion over the pressure range studied, it can be seen that the difference between a linear and a quadratic relation was very small. Therefore, and also for the sake of simplicity, we decided to use linear regression for the comparison of different left coronary pressure–flow relations. The intercept of the linearized pressure–flow relation is then not necessarily the zero flow pressure intercept.

4.2. Diastolic/systolic differences in beating and arrested hearts

We found that cardiac contraction causes a significant shift in left coronary pressure–flow relations to lower flows both in the beating and in the arrested heart. Livingston and colleagues [24] observed similar shifts of the coronary pressure–flow relations in the isolated maximally dilated perfused septum of the dog heart comparing the diastolic (not beating) and tetanized states. Using linear fits they found that the intercept between tetanized and diastolic relations was significantly different while the slope was not. In a more recent study in the same experimental model by Livingston et al. [25], a small portion of this rotation was found to be attributable to the Gregg effect (The increase of coronary perfusion causes an increase in contractility and myocardial oxygen consumption [26]). The pressure–flow relation was therefore not affected uniformly, because at higher perfusion pressure, and thus higher contractility, flow is relatively more affected so that the pressure–flow relation rotates clockwise. We analyzed the Gregg effect, as the relation between **systolic** left ventricular pressure and perfusion pressure (P_s/P_p), in the beating and arrested heart at V_0 . We found that the mean values of the slope of the relations to be 0.0737 ± 0.282 and 0.186 ± 0.114 for beating and arrested conditions resp. (NS, $p=0.313$). Thus, the Gregg phenomenon does not explain the relative rotation between the beating and arrested conditions in systole in our experiments.

The effect of capacitive flow on diastolic left coronary pressure–flow relations is expected to be negligible, because we used a very low heart rate and we measured diastolic flow at the end of diastole, and at this point in time most capacitive effects, if any, have already disappeared.

4.3. The arrested heart

Potassium chloride (used for diastolic arrest) causes vasoconstriction. In a separate study (not published) we investigated the effect of potassium and adenosine on the diameter of isolated rabbit left coronary arteries ($n=7$) at 28°C . The diameter was $86.8 \pm 3.0\%$ (from the maximal diameter) during perfusion with 20 mM potassium and after adding 10 μM adenosine $93.3 \pm 3.2\%$. Since we used these concentrations of potassium and adenosine in the present study, we cannot exclude some remaining vasoconstriction. The significant reduction of the intercept of the diastolic pressure–flow relation as observed in the arrested hearts compared to the beating hearts (reduction of diastolic flow by approximately 20% at reference flow) can thus be explained by the incomplete vasodilatation, since a diameter reduction of 6% will result, on basis of Poiseuille's law, in a flow reduction of approximately 22%.

Barium is a good model for systolic arrest [27,28], but known to constrict coronary arteries [29]. In pilot studies we studied Barium-induced contraction of rabbit coronary arteries, and found that effective dilatation was established with a mixture of adenosine (50 μM) and isoproterenol (55 nM) at 28°C [29]. We used that same mixture in the present study and therefore concluded that there was no barium-induced constrictive effect on the diameter of coronary vessels. This mixture of adenosine and isoproterenol was also used by Goto et al. [30], but their study was performed at 37°C . At that temperature we found in the same pilot data that this mixture was not sufficient to counteract the Barium effect on the vessels.

4.4. Effect of chamber volume

The effect of chamber volume did overall result in a small but significant decrease in flow rate (intercept $p=0.037$, slope NS). In the arrested hearts in diastole and systole the effect of chamber volume was not significant ($p=0.16$). We used a left ventricular pressure of 80 mmHg and 115 mmHg. Iwanaga et al. [30] discussed the role of the subepicardium in extramural coronary flow pulsatility. The subepicardium may shunt in systole most of the retrograde flow from the subendocardium. Thus the observation that capacitive effects in global flow are small, as observed in the present study, may be due to the shunting phenomenon as discussed by Iwanaga et al. [30].

In summary, in this experimental model left coronary pressure–flow relations are significantly different in systole and diastole, left ventricular chamber volume has little influence on these relations, and capacitive effects are small. Ideally one could think that the in vivo situation is best suited to study the sensitivity of coronary pressure–flow relations to interventions, but this is not always the case when one wants to study certain phenomena without

the confounding contributions of other factors. Therefore, the conditions were chosen to be able to test the hypothesis. The results of this study may, for instance, be applied to the stenotic coronary circulation where the bed distal to the stenosis is (maximally) dilated. Since we found that increased contractility has a stronger impeding effect on flow than an increase in ventricular volume, an increase in stroke volume should be obtained by a volume increase rather than by increased contractility. Future studies in this model will be directed to determine these effects on left coronary pressure–flow relations in different layers of the myocardium.

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References

- [1] Bouma P, Sipkema P, Westerhof N. Coronary arterial inflow impediment during systole is little affected by capacitive effects. *Am J Physiol* 1993;264:H715–H721.
- [2] Bouma P, Sipkema P, Westerhof N. Vasomotor tone affects diastolic coronary flow and flow impediment by cardiac contraction similarly. *Am J Physiol* 1994;266:H1944–H1950.
- [3] Krams R, van Haelst ACTA, Sipkema P, et al. Can coronary systolic–diastolic flow differences be related to left ventricular pressure? A study in the isolated rabbit heart. *Basic Res Cardiol* 1989;84:149–159.
- [4] Krams R, Sipkema P, Westerhof N. The varying elastance concept may explain coronary systolic flow impediment. *Am J Physiol* 1989;257:H1471–H1479.
- [5] Krams R, Sipkema P, Westerhof N. Contractility is the main determinant of coronary systolic flow impediment. *Am J Physiol* 1989;257:H1936–H1944.
- [6] Krams R, Sipkema P, Westerhof N. Coronary oscillatory flow amplitude is more affected by perfusion pressure than ventricular pressure. *Am J Physiol* 1990;258:H1889–H1898.
- [7] Suga H, Sagawa K, Shoukas AA. Load independence of the instantaneous pressure–volume ratio of the canine left ventricle, and effects of epinephrine and heart rate on the ratio. *Circ Res* 1973;49:314–322.
- [8] Suga H, Sagawa K. Instantaneous pressure–volume relationships and their ratio in the excised, supported canine left ventricle. *Circ Res* 1974;35:117–126.
- [9] Vis MA, Sipkema P, Westerhof N. Modelling pressure–flow relations of cardiac muscle in diastole and systole. *Am J Physiol* 1997;272:H1516–H1526.
- [10] Downey JM, Kirk ES. Inhibition of coronary flow by vascular waterfall mechanism. *Circ Res* 1975;36:753–763.
- [11] Arts MGJ, Reneman RS. Interaction between intra myocardial pressure (IMP) and myocardial circulation. *J Biomech Eng* 1985;107:51–56.
- [12] Spaan JAE, Breuls NPW, Laird JD. Forward coronary flow normally seen in systole is the result of both forward and concealed back flow. *Basic Res Cardiol* 1981;76:582–586.
- [13] Spaan JAE, Breuls NPW, Laird JD. Diastolic–systolic coronary flow differences are caused by the intra myocardial pump action in the anesthetized dog. *Circ Res* 1981;49:584–593.
- [14] Bellamy RF. Diastolic coronary artery pressure–flow relations in the dog. *Circ Res* 1978;43:92–101.
- [15] Aversano T, Klocke FJ, Mates RE, et al. Preload induced alterations in capacitance free diastolic pressure–flow relationship. *Am J Physiol* 1984;246:H410–H417.
- [16] Van Dijk LC, Krams R, Sipkema P, et al. Changes in coronary pressure–flow relation after transition from blood to Tyrode perfusion. *Am J Physiol* 1988;255:H476–H482.
- [17] Gregg DE, Green HD. Registration and interpretation of normal phasic inflow into a left coronary artery by an improved differential manometric method. *Am J Physiol* 1940;130:114–125.
- [18] Landaw EM, DiStefano JJ. Multiexponential, multicompartamental and noncompartmental modelling. II. Data analysis and statistical considerations. *Am J Physiol* 1984;246:R665–R677.
- [19] Hak JB, van Beek JHGM, van Wijhe MH, et al. Influence of temperature on the response time of mitochondrial oxygen consumption in the isolated rabbit heart. *J Physiol* 1992;447:17–31.
- [20] Shibata T, Berman MR, Hunter WC, et al. Metabolic and functional consequences of barium-induced contracture in rabbit myocardium. *Am J Physiol* 1990;259:H1566–H1574.
- [21] Hak JB, van Beek JH, van Wijhe MH, et al. Influence of temperature on the response time of mitochondrial oxygen consumption in isolated rabbit heart. *J Physiol* 1992;447:17–31.
- [22] Klocke FJ, Mates RE, Canty Jr. JM, et al. Coronary pressure–flow relationships. Controversial issues and probable implications. *Circ Res* 1985;56:310–323.
- [23] Hanley FL, Messina MR, Grattan MT, et al. The effect of coronary inflow pressure on coronary vascular resistance in isolated dog heart. *Circ Res* 1984;54:760–772.
- [24] Livingston JZ, Resar JR, Yin FCP. Effect of tetanic myocardial contraction on coronary pressure–flow relationships. *Am J Physiol* 1993;265:H1215–H1226.
- [25] Livingston JZ, Halperin HR, Yin FCP. Accounting for the Gregg effect in tetanized coronary arterial pressure–flow relationships. *Cardiovasc Res* 1994;28:228–234.
- [26] Feigl EO. Coronary physiology. *Phys Rev* 1983;63:13–18.
- [27] Judd RM, Levy BI. Effects of barium-induced cardiac contraction on large- and small-vessel intra myocardial blood volume. *Circ Res* 1991;68:217–225.
- [28] Munch DF, Comer HT, Downey JM. Barium contracture: a model for systole. *Am J Physiol* 1980;239:H438–H442.
- [29] Sipkema P, van der Linden PJW, Zeeuwe PEM, et al. Contraction and dilatation of the rabbit coronary artery due to BaCl₂ at different temperatures. *J Vasc Res* 1994;31(S1):47.
- [30] Iwanaga S, Ewing SG, Hussini WK, et al. Changes in contractility and afterload have only slight effects on subendocardial systolic flow impediment. *Am J Physiol* 1995;269:H1202–1212.