Reoxygenated effluent of Tyrode-perfused heart affects papillary muscle contraction independent of cardiac perfusion

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Abstract

Objective: We determined, via a bioassay, if inotropic factors are released in the coronary circulation of the rat heart and if changes in cardiac perfusion change papillary muscle inotropy. Methods: An isolated isometrically contracting rat papillary muscle (n = 5, acceptor) was superfused with Tyrode or with reoxygenated coronary venous effluent from an isolated isovolumically beating rat heart (donor) at 27°C, which was perfused with Tyrode according to Langendorff. The superfusion solution in the muscle bath was exchanged completely in 90 s. During coronary venous effluent superfusion, the flow of the heart (donor) was changed in steps. Results: The peak force of the papillary muscle (acceptor) was unaffected by a change from Tyrode to coronary venous effluent superfusion, but time to half relaxation (RT1/2) significantly increased by 23.0 ± 9.0% (mean ± s.d.) and positive dF/dt max significantly decreased by 14.6 ± 4.7%. These twitch characteristics were unaffected by changes in coronary perfusion while in the heart isovolumic developed left ventricular pressure did increase with perfusion (the Gregg phenomenon). Conclusions: Factors that affect papillary muscle contractility are released into the coronary circulation, but their effect is independent of the magnitude of coronary perfusion.

Keywords: Bio-assay; Gregg’s phenomenon; Contractile function; Rat, ventricle

1. Introduction

In 1988 Brutsaert et al. [1] showed that removal of the endocardial endothelial cells of the isolated papillary muscle decreased peak tension and relaxation time of the muscle while positive dF/dt max was unaffected. Li et al. [2] found that the force of contraction of papillary muscles from hearts in which the vascular endothelium was removed was also lowered and the negative inotropic effects of removal of endocardial and vascular endothelium were found to be additive. The coronary microvessels are in close contact with the cardiomyocytes [3] and produce both up- and down-regulating factors that influence the contractile proteins in the myocardial cells [4] in proportion to local PO2 and possibly in relation to shear force on the vascular endothelium [5,6]. In a bio-assay study it has been shown recently that inotropic factors are released into the coronary circulation and that their effect on the accepting papillary muscle depends on the metabolic state of the donating heart muscle and the presence of vascular and endocardial endothelium [7]. However, these authors did not study the relation between heart and papillary muscle contractility during changes in coronary perfusion.

In 1957 Gregg reported that increased coronary perfusion leads to increased oxygen uptake [8]. A related finding is that the strength of cardiac contraction is increased by increased coronary perfusion. These combined effects are called the ‘Gregg phenomenon’ [9,10]. Originally, the search for the mechanism responsible for this phenomenon...
was focused on two hypotheses. Firstly, flow changes were assumed to alleviate relative or local ischaemia [11]. Secondly, changes in the volume of the coronary vessels were thought to stretch the surrounding cardiac myocytes ('garden-hose' effect [12]) which, via the ‘Frank-Starling’ mechanism could result in a higher force of contraction. However, in the isolated perfused papillary muscle of the rat in which perfusion can be freely changed without limiting the O$_2$ supply, it was found that neither ischaemia nor changes in muscle length can explain the relation between perfusion and force of contraction [13]. It was therefore concluded that the Gregg phenomenon represents a change in contractility of the cardiac muscle per se [13]. This conclusion is in line with the finding of Kitakaze and Marban [14] who found an increase in intracellular Ca$^{2+}$ with increased perfusion in the isolated ferret heart.

The aim of our study was twofold. The first aim was to investigate the effects of factors released into the coronary circulation on cardiac twitch characteristics. The second aim was to determine if changes in cardiac perfusion bring about changes in the effect of these factors. For this purpose an isolated, isometrically contracting rat papillary muscle (acceptor) was superfused either with Tyrode or with coronary venous effluent from an isolated Langendorff perfused rat heart (donor) after this effluent had been reoxygenated and rapidly transported to the muscle bath.

2. Methods

2.1. Preparation procedures

All animals were treated according to the guidelines of the DEC (Animal Experimental Committee) of the Free University of Amsterdam, the Netherlands. In each experiment ($n = 5$) two male Wistar rats weighing between 350 and 450 g were used. Anaesthesia was induced by placing the rat inside a box containing ether vapour mixed with air. Subsequently the thorax was opened and the heart was rapidly excised and submerged in cold HEPES buffer of the following composition (mM): NaCl, 140; KCl, 5; Na$_2$HPO$_4$, 2; CaCl$_2$, 1.36; MgSO$_4$, 1.2; glucose, 10; HEPES, 5. The pH was adjusted with NaOH to 7.3–7.4. The heart was used either as a donor (i.e., the isolated perfused rat heart setup, according to Langendorff) or acceptor (the isolated superfused papillary muscle). In Fig. 1 a schematic diagram of the bio-assay is shown.

2.2. Acceptor: The isolated superfused papillary muscle

The papillary muscle was isolated first because it needs a longer equilibration period (60–90 min) than the heart but is then stable over several hours. The aorta of the isolated heart was cannulated and immediately connected...
to a modified Langendorff perfusion setup at 27°C and perfused with the HEPES buffer. The HEPES buffer was equilibrated with 100% O₂, not recirculated and 15 mM KCl was added to stop the mechanical activity. The heart was placed under a dissection microscope. During cardioplegia the right ventricle was opened and a thin papillary muscle attached to a piece of adjacent septum was carefully taken out without touching its surface. The muscle was placed in an organ bath (volume 2.5 ml) and superfused, with a Tyrode solution of the following composition (in mM) NaCl, 128.3; KCl, 4.7; CaCl₂, 1.36; MgCl₂, 1.05; NaHCO₃, 20.2; NaH₂PO₄, 0.42; glucose, 10 equilibrated with 95% O₂ and 5% CO₂. A superfusion flow rate of 5.4 ml·min⁻¹ was induced by an Ismatec mini-S 620 pump. An isometric force transducer (type AES01, Mikro-Elektronikk, Horten, Norway) was connected via a thread to the tendinous end of the muscle with a small hook while the septum was clamped between a Perspex plate and a stainless-steel ring (diameter 3–4 mm). The cross-section of the papillary muscle was oval and the minor and major diameters were measured half-way between its base and the tendon with a calibrated grid in the dissection microscope. The papillary muscle was stimulated supramaximally with a frequency of 0.2 Hz. At regular intervals superfusion solution samples were withdrawn from the muscle bath and analyzed for PO₂, PCO₂ and pH (ABL 330 Laboratory, Radiometer, Copenhagen, Denmark). The temperature of the bath solution was continuously monitored. Before the protocol was started a force–length relationship was made and diastolic length was chosen such that the passive force was about 5% of developed force. Maximum developed force at the chosen length was determined by post-extrasystolic potentiation and/or a high calcium concentration in the superfusion solution (2.9 mM [13]).

2.3. Donor: The isolated perfused rat heart

After the isolated heart was excised and submerged in cold HEPES buffer, the aorta was cannulated and connected to a Langendorff perfusion setup. The isolated heart was perfused with a Tyrode solution (composition, see above, 27°C) which was not recirculated. An apex cannula was placed through the left ventricular wall to drain the Thebesian flow. A latex balloon, mounted on a catheter, was introduced into the left ventricle via the left atrium through the mitral valve and tied to the left atrium to allow isovolumic contractions. The balloon was filled with water and the end-diastolic pressure was set at 3 mmHg while developed left ventricular pressure was at least 60 mmHg at control flow (9.4 ml·min⁻¹). The catheter was connected to a pressure transducer (Statham 23 Db) to measure left ventricular pressure. The superior and inferior vena cava were ligated and the pulmonary artery was cannulated with a polyethylene tube to collect the coronary venous effluent. During the experiments the heart was paced with electrodes placed on the right atrium. Pace frequency (156 ± 13 beats per minute) was just above the heart’s own frequency. The flow through the coronary bed during the preparation and stabilization period was set at 9.4 ml·min⁻¹. The studied range of coronary flows (from 7.5 to 11.3 ml·min⁻¹) was generated by a roller pump (Gilson minipuls 3). The coronary perfusion pressure was continuously measured by a Statham (23 Db) pressure transducer just above the aorta. Arterial and venous samples were collected in glass capillaries and analyzed for PO₂, PCO₂ and pH. Arterial PO₂ was in all experiments above 700 mmHg and PCO₂ was approximately 32 mmHg (measured at 37°C). Myocardial oxygen consumption (VO₂, μmol O₂·min⁻¹·g⁻¹) at 27°C was calculated from the arteriovenous oxygen content difference times the coronary flow. The lactate concentration was measured in the venous effluent. The samples for lactate determination were frozen and stored at −20°C until analyzed. Lactate concentration was determined with the sensitive enzymatic cycling method, as described by Lowry and Passoneau [15]. At the end of the experiment the heart was removed and weighed. Dry weight was measured after the heart was placed in an oven (60°C) for minimal 48 h. Dry/wet ratio was used as an index of edema.

2.4. Transportation of the reoxygenated coronary venous effluent

A special hollow glass oxygenator was placed between the Langendorff heart and the isolated papillary muscle (Fig. 1) in which the Tyrode solution or venous effluent was exposed to 95% O₂ and 5% CO₂. The exposure surface (fluid flowed along the glass wall) and time (5 s) were sufficient to increase venous effluent PO₂ to values higher than 500 mmHg and to let pH and PCO₂ re-adjust to control values. From the lowest superfusion flow rate of 5.4 ml·min⁻¹ and the organ bath volume of 2.5 ml we calculated a time constant of 28 s for the fluid exchange. Complete exchange of fluid takes 3 time constants and therefore the superfusion solution of the muscle bath was fully replaced within 90 s. Larger coronary perfusion flows increased the amount of venous effluent and thus superfusion flow, thereby reducing the time constant.

Changes in coronary venous effluent flow rate had small effect on muscle bath temperature. We measured a maximal change in the muscle bath temperature of 0.2°C. Transition of superfusion fluid from Tyrode to coronary effluent and back also affected the temperature of the papillary muscle bath: from Tyrode to reoxygenated coronary venous effluent temperature changed (−0.4 ± 0.5°C) and upon return to Tyrode (0.2 ± 0.2°C). Therefore in separate isolated papillary muscle experiments muscle bath temperature was changed by 0.3°C through superfusion flow changes. The developed muscle force and twitch characteristics did not change.
The protocol was started after the Langendorff heart had stabilized during a 20 min period at control perfusion flow (9.4 ml·min⁻¹). At that moment the papillary muscle had stabilized for more than 60 min. The perfusion pressure (P_{perf}), left ventricular isovolumic pressure (P_{lv}), heart rate of the isolated rat heart, force (F), and muscle bath temperature (T) of the isolated papillary muscle were continuously monitored on a chart recorder (Graphitec thermal arraycorder WR3600) and also digitized and stored on disk using an Olivetti M290 personal computer (sample rate 300 Hz).

The protocol is shown in Fig. 2. Before the protocol was started, perfusion flow of the isolated heart was lowered from 9.4 ml·min⁻¹ during control to 7.5 ml·min⁻¹. First, the papillary muscle was superfused with the Tyrode solution for a period of 15 min. Then superfusion with coronary venous effluent was started. Subsequently, stepwise changes in coronary flow were applied. Five minutes after each change in coronary flow papillary muscle and heart reached new steady states. The symbols in Fig. 2 indicate when measurements were performed. Developed left ventricular pressure was averaged for the two measurements (closed circle and triangle, see Fig. 2). From a pilot experiment we obtained the twitch to twitch variation in the steady state of the isolated papillary muscle: the s.d. was less than 2% in all twitch characteristics. Therefore only one twitch was analyzed per condition. At the end of the protocol the superfusion solution of the papillary muscle was returned to the Tyrode solution to check for papillary muscle stability. The twitch characteristics of the papillary muscle were analyzed after the experiment on the digitized data. Developed peak force (dev F) was determined and the development of force of the twitch was described by positive dF/dt_{max} and the relaxing part of the twitch was described by the time between peak force and fall to 50% of peak force (RT_{1/2}).

To compare experiments, the twitch characteristics were normalized with respect to the values during the first superfusion with Tyrode at the start of the protocol. At each steady-state level, coronary venous flow was measured by weighing the fluid collected during 30 s. In the steady state we collected samples to determine lactate and PO_{2} in the coronary venous effluent during all perfusion flows and this was also done in the Tyrode superfusion fluid of the papillary muscle.

We performed three additional isolated papillary muscle experiments superfused with Tyrode in which we increased lactate concentration in the superfusion fluid (up to 100 μmol·L⁻¹) or increased lactate concentration (100 μmol·L⁻¹) in combination with lowered PO_{2} (down to 500 mmHg). We analyzed these changes on papillary muscle twitch characteristics.

2.6. Statistical analysis

In each experiment we used linear regression analysis to characterize the relationship between perfusion flow and developed left ventricular pressure and between perfusion flow and oxygen consumption (Gregg phenomenon). Experimental data were averaged after normalization by setting developed left ventricular pressure and oxygen consumption at 100% for the lowest flow studied (7.5 ml·min⁻¹). The relation between developed isovolumic left ventricular pressure and developed peak force of the isometric contraction of the papillary muscle was also examined in each experiment by linear regression analysis. The papillary muscle twitch characteristics, RT_{1/2} and positive dF/dt_{max} during all superfusion situations were averaged after normalization by setting developed force, RT_{1/2} and positive dF/dt_{max} at 100% during Tyrode superfusion at the start of the experiment and were analyzed by repeated measures of analysis of variance followed by a Tukey-Kramer multiple comparison test if appropriate. The effects of changing the superfusion solution from the Tyrode solution to coronary venous effluent solution on lactate content of the bath solutions were tested by a paired Student t-test. The significance level was set at 0.05.

3. Results

We performed 5 bioassay experiments. Fig. 3 shows a typical example of the force of contraction of the isolated papillary muscle and left ventricular isovolumic pressure of the isolated heart during a change in coronary perfusion flow from 7.5 to 11.3 ml·min⁻¹. There was no change in
peak force of contraction of the papillary muscle whereas there was a prominent change in left ventricular pressure. First the results of the donor hearts will be presented and then the twitch characteristics of the muscle including the effect of changes in coronary perfusion on papillary muscle twitch characteristics.

3.1. Donor

At control flow (9.4 ml·min⁻¹) the perfusion pressure was 50.5 ± 18.7 mmHg (mean ± s.d.), developed left ventricular pressure at a diastolic pressure of 3 mmHg was 96.6 ± 26.5 mmHg and oxygen consumption was 2.71 ± 0.73 μmol·min⁻¹·g⁻¹. Arterial coronary flow at control (9.4 ml·min⁻¹) corresponded to 6.3 ± 1.3 ml·min⁻¹·g⁻¹ (see Table 1). About 80% of coronary arterial flow was retrieved from the pulmonary artery cannula at control flow and this percentage was somewhat lower at higher flows. This means that the superfusion flow of the papillary muscle with the coronary effluent was between 6 and 9 ml·min⁻¹. In all isolated hearts a linear relationship was observed between perfusion pressure and perfusion flow, indicating that there was no coronary autoregulation present. The arteriovenous lactate difference of the coronary perfusion fluid was 15.8 ± 5.9 μmol·l⁻¹ at control flow. The lactate production did not differ significantly between perfusion levels. The dry/wet ratio of the hearts was 0.18 ± 0.02. Fig. 4 shows the increase in averaged developed left ventricular pressure and averaged oxygen consumption with perfusion flow (Gregg phenomenon) for all experiments. The reference value (mean ± s.d.) at 100% for developed left ventricular pressure and oxygen consumption was 90.2 ± 28.7 mmHg and 2.6 ± 0.6 μmol·min⁻¹·g⁻¹ at an initial flow rate of 7.5 ml·min⁻¹. The mean slopes of both relationships differed significantly from zero (mean ± s.d.: 4.6 ± 2.6 mmHg·ml⁻¹·min⁻¹ and 0.19 ± 0.10 μmol·g⁻¹·ml⁻¹).

![Fig. 3. Recordings of isometric force of the papillary muscle (F), left ventricular pressure (P_LV) and perfusion pressure (P_perf) after a change in perfusion flow (indicated by the arrow).](image-url)

![Fig. 4. The averaged results (mean ± s.d.) of the relations between normalized developed left ventricular pressure (dev P_LV) and coronary flow (Q_perf, panel A) and normalized oxygen consumption, (VO₂, panel B) and coronary flow (Q_perf).](image-url)
3.2. Acceptor

Mean cross-sectional area of the papillary muscles was 0.21 ± 0.06 mm². The mean passive force (2.6 ± 1.6 mN · mm⁻²) was about 4.9% of developed force (55.3 ± 31 mN · mm⁻²). All papillary muscles were able to change their contractile state at this length when calcium concentration was increased from 1.36 to 2.9 mM in the muscle bath or post-extrasystolic potentiation was applied (average 81.9 ± 29.9 mN · mm⁻²). All these initial values of the isolated papillary muscle during Tyrode perfusion (5.4 ml · min⁻¹) are presented in Table 2. The lactate content of the papillary muscle bath increased significantly by 15.8 ± 5.9 µmol · l⁻¹ when the superfusion fluid was changed from Tyrode to reoxygenated coronary venous effluent. The pH of the coronary effluent after reoxygenation differed only 0.05 pH units from the original superfusion medium and the PCO₂ differed only 3 mmHg.

In the three additional experiments we increased lactate concentration (100 µmol · l⁻¹) of the superfusion solution or increased lactate concentration (100 µmol · l⁻¹) in combination with lowered PO₂. Twitch characteristics of the isolated papillary muscle we found to change less than 2%. The developed peak force of the papillary muscle did not change with developed left ventricular pressure, mean slope (mean ± s.d.; 0.013 ± 0.009 mN · mm⁻² · mmHg⁻¹). The developed peak forces of the papillary muscle during Tyrode superfusion at the beginning and at the end of the protocol were not different, indicating that during the experimental period the preparation was stable.

Fig. 5 shows a typical example of the twitch of the isolated papillary muscle during superfusion with Tyrode and reoxygenated coronary venous effluent. The positive dF/dt_max decreases and time to half relaxation increases. Fig. 6 summarizes the effect of switching from Tyrode to coronary venous effluent superfusion and the effect of changing the cardiac coronary perfusion on the twitch characteristics of the papillary muscle. Significant effects were found when switching from Tyrode to reoxygenated coronary venous effluent superfusion and back. The time to half relaxation increased significantly (P < 0.05) from 169 ± 39 to 208 ± 50 ms and positive dF/dt_max decreased (P < 0.05) from 108.0 ± 27.9 to 84.7 ± 22.4 mN · s⁻¹.

Table 2

<table>
<thead>
<tr>
<th>Muscle</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Mean ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F_pass</td>
<td>0.7</td>
<td>3.1</td>
<td>5.0</td>
<td>1.5</td>
<td>2.5</td>
<td>2.6 ± 1.6</td>
</tr>
<tr>
<td>dev F</td>
<td>26.7</td>
<td>29.5</td>
<td>103.7</td>
<td>56.5</td>
<td>60.2</td>
<td>55.3 ± 31</td>
</tr>
<tr>
<td>dev Fmax</td>
<td>65.8</td>
<td>38.1</td>
<td>112.2</td>
<td>91.2</td>
<td>102.0</td>
<td>81.9 ± 29.9</td>
</tr>
<tr>
<td>dF/dt</td>
<td>72.6</td>
<td>99.9</td>
<td>150.3</td>
<td>107.7</td>
<td>109.6</td>
<td>169.0 ± 27.9</td>
</tr>
<tr>
<td>RT₁/₂</td>
<td>146.7</td>
<td>205.3</td>
<td>183.3</td>
<td>113.3</td>
<td>200.0</td>
<td>169.3 ± 38.5</td>
</tr>
<tr>
<td>CSA</td>
<td>0.26</td>
<td>0.29</td>
<td>0.15</td>
<td>0.15</td>
<td>0.19</td>
<td>0.21 ± 0.06</td>
</tr>
</tbody>
</table>

F_pass, dev F, dev Fmax = passive, developed and maximal developed force (all in mN · mm⁻²); dF/dt in mN · mm⁻² / s⁻¹; RT₁/₂ = time to half relaxation (ms); CSA = cross-sectional area (mm²).

Fig. 5. A typical registration of the twitch of the isolated papillary muscle during superfusion with Tyrode and reoxygenated coronary venous effluent.

Fig. 6. A summary of the normalized changes with respect to the initial Tyrode superfusion in the twitch characteristics (individual experiments, open symbols, mean ± s.d., closed symbols) of the papillary muscle during Tyrode or reoxygenated coronary venous effluent superfusion at different coronary flow levels (ml · min⁻¹). * Significantly different from Tyrode superfusion at start and end of the experiment.
The developed peak force did not change from Tyrode to coronary venous effluent superfusion over the period of measurement; it did, however, significantly decrease after 1 h of coronary effluent superfusion. All twitch characteristics (developed peak force, RT1/2, and positive dF/dt max) did not change with changes in the coronary perfusion of the isolated heart during coronary venous effluent superfusion of the papillary muscle (Fig. 6).

4. Discussion

We found a significant effect on the twitch characteristics of the superfused papillary muscle following the change from Tyrode to reoxygenated coronary venous effluent: the time to half relaxation increased and positive dF/dt max decreased. Return to Tyrode reversed these changes and showed that the preparation was stable. Initially developed peak force of the papillary muscle did not change, but after 1 h of coronary venous effluent superfusion, peak force decreased significantly. The increase in contractility seen with an increase in coronary perfusion in the isolated rat heart (donor) was not communicated through the coronary venous effluent to the isolated superfused papillary muscle (acceptor) since twitch characteristics did not vary with changes in coronary flow (Fig. 6).

4.1. Temperature and oxygenation of the heart

Both preparations, the isolated perfused heart and the isolated papillary muscle were kept at 27°C. This temperature was chosen to keep metabolism low so that limitations of oxygen supply do not play a role [16]. This is important because it has long been thought that the increase in contractility seen with an increase in perfusion may result from limitations of oxygen supply especially at low perfusion flows [11]. There were no signs of limited oxygen supply in the isolated heart because the veno-arterial lactate concentration difference was 15.8 ± 5.9 µmol·l⁻¹. In the literature a lactate concentration of 33 ± 5 µmol·l⁻¹ is supposed to be indicative for a normoxic heart at 37°C [17,18] and a concentration of 45.6 µmol·l⁻¹ for a rat heart at 25°C [19]. (The latter concentration was calculated from 1.52 µmol·min⁻¹·g dw⁻¹ as given by Kreutzler et al. [19] assuming a heart weight of 1.5 g, a dry weight wet/weight ratio of 0.2 and coronary flow of 10 ml·min⁻¹.) Also, the lactate production did not change with changes in coronary flow, and finally venous PO₂ was high (between 446 and 522 mmHg). From the literature it is known that lactate, in the millimolar range, at normal pH influences the contractile properties of papillary muscles [20]. In additional experiments (n = 3), we found that increasing lactate concentration in the superfusion fluid (up to 10 times the concentration found in the coronary venous effluent in our experiments) or increasing lactate concentration in combination with lowered PO₂ (down to 500 mmHg) had no effect on twitch characteristics. Thus, we can exclude that the lactate concentration and some what lowered PO₂ in the reoxygenated coronary venous effluent contribute to the changes in twitch characteristics.

The temperature chosen implies a lowered activity of the enzymes and which could affect endothelial function. However, in other experiments performed at the same temperature we found endothelial function to be present since an endothelium-dependent dilator (bradykinin) produced a vasodilation of the coronary bed (unpublished observations). The chosen temperature (27°C) had no influence on the aim of this study since the increase in contractility seen after an increase in perfusion still exists in the isolated heart. This indicates that if this increase in contractility originates from released inotropic substances, the amounts produced are effective.

4.2. Coronary venous PO₂ and superfusion flow

The coronary venous effluent with lowered PO₂ and increased PCO₂ and pH was transported in approximately 5 s to the papillary muscle bath. In this short period coronary venous effluent was exposed to 95% O₂ and 5% CO₂ which was sufficient to increase the PO₂ by 100–150 mmHg to at least 500 mmHg while PCO₂ and pH returned to control values. Although it was already known that a oxygen tension of 500 mmHg had no effect on the peak force of the papillary muscle [13], we performed three additional experiments in which it was found that neither the peak force nor other twitch characteristics were affected by lowering the PO₂ from approximately 730 to 500 mmHg in the muscle bath.

Two aspects of our experiments will be discussed: the effect of changing the superfusion solution of the papillary muscle from Tyrode to reoxygenated coronary venous effluent and the effect of changing the coronary perfusion of the isolated heart (the Gregg phenomenon) on the twitch characteristics of the papillary muscle.

4.3. Effect of changing the superfusion from Tyrode to coronary venous effluent

The coronary venous effluent significantly affects the twitch characteristics of the accepting papillary muscle. We found these changes to be consistent in all hearts and muscles studied, independent of the differences in reference conditions. The contractile state of the muscle was only affected after long-lasting exposure (1 h) to the coronary venous effluent.

In experiments comparable to ours by Ramaciotti et al. [7] an inconsistent effect of the reoxygenated coronary venous effluent on twitch characteristics was found. In-
creases, no effect, and decreases in developed peak force and time to half relaxation were observed, but positive $\frac{dF}{dt_{\text{max}}}$ did not change. They found a relation between coronary venous effluent $\text{PO}_2$ before reoxygenation and change in developed peak force [7]. Our unchanged developed peak force after changing the superfusion fluid from Tyrode to reoxygenated coronary venous effluent is in agreement with their data, considering the narrow range of venous effluent $\text{PO}_2$'s before reoxygenation found in our study (ranging from 446 to 522 mmHg). The changes in twitch characteristics (i.e., an increase in time to half relaxation and a decrease in positive $\frac{dF}{dt_{\text{max}}}$) without changes in peak force were not found by them. This could be due to some important differences between their and our studies. Ramaciotti et al. [7] used a preparation in which the perfusate was recirculated, possibly leading to accumulation of metabolites (lactate), mixed with inotropic factors and leading to a decrease in the concentration of nutrients. We did not recirculate the perfusion fluid to avoid complexity in the interpretation of the results. A second important improvement of our study was the short transportation time of the reoxygenated coronary venous effluent (within 90 s) while in Ramaciotti's experiments [7] it was much longer (4–5 min).

Changing the superfusion fluid from Tyrode to coronary venous effluent and back introduced small temperature changes of $-0.4 \pm 0.5^\circ\text{C}$ from Tyrode to effluent and $+0.2 \pm 0.2^\circ\text{C}$ for return to Tyrode. These temperature changes could at most explain a change in twitch characteristics of a few percent. We found a much larger change in $\frac{dF}{dt}$ of 14.7 $\pm$ 4.7%. In one of the experiments we found a rather large change in superfusion fluid temperature, namely $-1.3^\circ\text{C}$, when switching from Tyrode to coronary venous effluent and 0.6$^\circ\text{C}$ when switching back to Tyrode at the end of the experiment. This, however, did not correspond with a larger directional change than in the other experiments. Thus temperature changes cannot explain the findings. Also a change of 0.3$^\circ\text{C}$ in muscle bath temperature in the three separate experiments did not result in changes in muscle twitch characteristics.

Although the nature of the factors released into the coronary circulation is unknown we suggest they could originate from the endothelium on the basis of the following arguments. After removal of the endocardial and/or vascular endothelial cells, twitch characteristics of the papillary muscle change: peak force decreases, time to half relaxation (measured from the start of the contraction) shortens and velocity of tension development decreases in most cases [1,2]. The strength of these effects depends on experimental temperature and extracellular calcium concentration (smaller effects at lowered temperature and lowered calcium concentration). In the present investigation the Tyrode perfusing the donor heart was exposed to the coronary vascular endothelial layer before it superfused the papillary muscle. This can be viewed as the inverse of the removal of endothelial cells: time to half relaxation should increase and no effect on peak force is to be expected, the latter because of the relatively low temperature [1]. This prediction is in agreement with our results: we found no effect on peak force while positive $\frac{dF}{dt_{\text{max}}}$ was decreased, indicating that time to peak force had increased. Time to half relaxation measured from peak force was increased also.

4.4. Effect of changing the coronary perfusion of the isolated heart and its effect on the twitch of the papillary muscle

Although there was a wide variation in base-line hemodynamic parameters between the isolated hearts, they all showed an increase in developed left ventricular pressure and oxygen consumption after changes in coronary flow. This increase in contractile performance of the heart with increased coronary flow is not transmitted to the isolated papillary muscle by the coronary venous effluent because developed force as well as the other measured twitch characteristics were unaltered. An increase in coronary flow can influence the production of endothelium-derived inotropic factors by changes in shear forces [5,6]. The concentrations of inotropic factors that are present in the coronary circulation depend on their production and on coronary flow. With changes in coronary perfusion we found no effect on twitch characteristics and this can be explained when production increases in proportion to flow or by assuming that the factors produced in relation to flow diffuse mainly to the adjacent cardiomyocytes and/or have short half-lives with respect to the transportation time in the present experiment.

4.5. Conclusion

The isolated perfused heart releases factors into the coronary circulation that affect the twitch characteristics of an accepting isolated papillary muscle (i.e., positive $\frac{dF}{dt_{\text{max}}}$ decreased and time to half relaxation of the twitch increased). Developed left ventricular pressure and oxygen consumption of the isolated heart increase with flow, but this increase was not transmitted by the reoxygenated coronary venous effluent to the papillary muscle because developed peak force and other twitch characteristics remained the same. This implies that the factors released in the coronary circulation that affect the papillary twitch characteristics are independent of perfusion.

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References


