Perfusion-induced changes in cardiac contractility and oxygen consumption are not endothelium-dependent

Marieke A. Dijkman *, Johannes W. Heslinga, Pieter Sipkema, Nico Westerhof

Laboratory for Physiology, Institute for Cardiovascular Research, ICaR-VU, Free University of Amsterdam, Van der Boechorststraat 7, 1081 HT Amsterdam, Netherlands

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Abstract

Objective: Are substances released from rat coronary endothelial cells responsible for the increase in contractility and oxygen consumption (Gregg phenomenon) seen with an increase in cardiac perfusion? Methods: In an isovolumically contracting, Langendorff, crystalloid perfused rat heart (n = 6) at 27°C, coronary flow was changed (from 4.4 to 15.4 ml. min⁻¹ . g⁻¹) before and after the endothelium was made dysfunctional by Triton X-100. Vascular endothelium and smooth muscle function were tested with bradykinin (BK, 1 μM, an endothelium dependent dilator) and papaverine (PAP, 1 μM, an endothelium independent dilator) in a preconstricted vascular bed (vasopressin, VP, 3 nM). Results: Before Triton X-100, coronary resistance (at constant flow) decreased significantly in response to BK and to PAP. After Triton X-100 treatment the dilatory response to BK was abolished while the PAP response was still present, suggesting endothelial dysfunction with intact smooth muscle function. Due to Triton X-100 treatment, coronary resistance increased significantly. Therefore coronary flow changes were also applied during a similar increase in coronary resistance induced by VP infusion (3 nM) before Triton X-100 treatment. During control, developed left ventricular pressure (dev P,) increased with 68 ± 21% and oxygen consumption (VO₂) increased with 122 ± 25% in response to the maximal increase in coronary flow. During increased coronary resistance with and without functional endothelium, dev P, increased by 57 ± 16 and 64 ± 22%, respectively, and VO₂ increased by 126 ± 21 and 103 ± 20%, respectively, in response to the maximal increase in flow. These changes were not significantly different from control. Conclusion: The results suggest that the arterial endothelium is not involved in the Gregg phenomenon.

Keywords: Gregg's phenomenon; Coronary resistance; Triton X-100; Bradykinin; Papaverine; Vasopressin; Rat, heart

1. Introduction

Recently it has become established that the endothelium is not only involved in the regulation of vascular smooth muscle tone [1] but also influences myocardial contractile performance [2,3]: removal of the endocardial endothelial cells results in changes in the contraction of isolated papillary muscle [2] and more recently the vascular endothelium was shown to affect myocardial contractile performance [3,4]. In cell-culture experiments, it has been proven that the vascular endothelial cells produce a flow-induced release of inotropic substances that influence the cardiomyocytes [5]. In the isolated unperfused papillary muscle both the endocardial and vascular endothelial cells were found to modulate the contractile characteristics of the myocardium and their effects were additive [6].

In 1963 Gregg reported that increased coronary perfusion leads to increased oxygen uptake and increased cardiac strength [7]. The mechanism of this so-called 'Gregg phenomenon' is still not clear [8]. Previous work has focused on two possibilities: firstly, the increased cardiac performance with perfusion is attributable to alleviation of limited O₂ supply (ischaemia effect), particularly in the subendocardium [9] and secondly, distension of the intramyocardial coronary vasculature with perfusion stretches the myocardial cells surrounding the vessels, resulting in an increase in contractile force via the Frank-Starling mechanism—the so-called 'garden-hose effect' [10]. In the
isolated perfused papillary muscle it was shown that neither ischaemia nor muscle length changes were the cause of the Gregg phenomenon but that it is a change in contractility per se [11].

The aim of the present study was to determine whether the vascular endothelial cells are involved in the perfusion-induced change in contractility and oxygen consumption of the myocardium. We therefore applied in the isolated rat heart a range of perfusion flows before and after the endothelium was made dysfunctional. Our results indicate that although the vascular endothelial cells lie in close proximity to the cardiomyocytes, they are not involved in the perfusion-induced change in myocardial contractility and oxygen consumption (Gregg phenomenon).

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. Hearts of male Wistar rats weighing 350–450 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 HEPES buffer (for composition, see below) which was placed on ice. The aorta was cannulated and connected to a Langendorff perfusion setup (27°C). A Tyrode’s solution (for composition, see below), equilibrated with 95% O₂ and 5% CO₂ was used as perfusion solution and was not recirculated. The inferior and superior caval veins were ligated. A latex balloon, mounted on a catheter, was introduced into the left ventricle through the left atrium to allow isovolumic contractions and ventricular pressure measurements. The balloon was filled with water and end-diastolic pressure was set at approximately 8 mmHg. The catheter was connected to a pressure transducer (Statham 23 Db). At the apex a cannula through the left ventricular wall was used to drain the Thebesian flow. The pulmonary artery was cannulated with a polyethylene tube to collect the coronary venous effluent. During the experiments the hearts were paced at a rate just above the heart’s own frequency (mean ± s.d.: 2.6 ± 0.2 Hz) at 27°C using electrodes placed on the right atrium. The flow through the coronary bed during the preparation and stabilization period was set at 9.4 ml · min⁻¹ (6.6 ± 0.4 ml · min⁻¹ · g⁻¹) called ‘control coronary flow’) using a calibrated roller pump (Gilson minipuls 3). The arterial pressure was continuously measured by a Statham pressure transducer (23 Db) just above the aorta. Arterial inflow and venous outflow samples were collected in glass capillaries and analysed for PO₂ and pH (ABL 330 Laboratory Radiometer, Copenhagen, Denmark). Myocardial oxygen consumption (VO₂: μmol O₂ · min⁻¹ · g⁻¹) was calculated from the product of arterio-venous oxygen content differences and coronary flow. The isolated perfused hearts were allowed to stabilize for 20 min. In all experiments, the left ventricular systolic pressure after stabilization was 70 mmHg or more at control coronary flow. The coronary resistance was taken as arterial pressure over coronary flow.

2.2. Experimental protocol

A schematic drawing of the protocol is given in Fig. 1. After the equilibration period a control series of 4–6 coronary flows within the range of 4.4–15.4 ml · min⁻¹ · g⁻¹ were given. The effects of these changes in coronary flow were continuously monitored on a chart recorder (Graphtec thermal arraycorder WR3600), and digitized and stored on disk using an Olivetti M290 personal computer (sample rate 300 Hz). Four minutes after each change in coronary flow steady-state conditions were observed and all variables—left ventricular peak systolic and diastolic pressure (P(vp), arterial pressure (Pvp) and oxygen consumption (VO₂)—were measured. The effect of Triton X-100 treatment on the characteristics of the left ventricular pressure curve were analysed using the digitized data. The positive rate of pressure development by the left ventricle was described by positive dP/dt max and the relaxation of the left ventricle was described by time between peak left ventricular pressure and fall to 50% of left ventricular pressure (time to half-relaxation, RT1/2). To compare experiments the data were normalized with respect to the values during control at the lowest perfusion flow.

The endothelium was made dysfunctional by a bolus of Triton X-100 in a dilution ratio of 1:200. The amount injected was 1% of the minute volume: i.e., with a perfusion flow of 6.6 ± 0.4 ml · min⁻¹ · g⁻¹ an amount of 0.07

![Fig. 1. A schematic drawing of the protocol. The 4 min perfusion period of bradykinin (BK, 1 μM) and papaverine (PAP, 1 μM) is indicated by vertical arrows. Vasopressin (VP, 3 nM) perfusion period is indicated by the horizontal arrow. The grey blocks indicate flow step periods and the time scale is indicated by the arrow.](image-url)
ml diluted Triton X-100 solution was injected. The solution was injected within 1 s into the aorta cannula so that the vascular endothelial cells and the right ventricular endocardial endothelial cells were exposed to the substance. Triton X-100 produced vasoconstriction in the coronary bed. Therefore, before Triton X-100 was used, we preconstricted the coronary bed with vasopressin (VP, 3 nM, an endothelium-independent vasoconstrictor) to the same degree. During this condition coronary resistance was increased while endothelial function was present. The concentration of vasopressin (3 nM) used does not have any effects on contractile strength of the cardiac muscle [15]. During VP perfusion a second series of coronary flow changes (same range as above) was performed.

During 'control coronary flow', endothelial and vascular smooth muscle function were tested before and after Triton X-100 treatment using bradykinin (BK; 1 µM), an endothelium-dependent vasodilator, and papaverine (PAP: 1 µM), an endothelium-independent vasodilator in a preconstricted coronary bed (VP, 3 nM). Both vasodilators were infused for 4 min. Fifteen minutes after application of Triton X-100, the coronary resistance had increased and BK and PAP were infused again for 4 min without preconstricting the coronary bed. If the dilatory response of bradykinin still existed, a second amount of Triton X-100 was injected and endothelial function was tested again. Therefore bradykinin was given before papaverine. When the endothelial response (BK) was abolished and muscle responsiveness (PAP) was unaffected, a third series of coronary flow changes (same range as above) was performed. At the end of the protocol all hearts were removed and weighed (wet weight). Of 4 out of 6 hearts, dry weight was measured after the hearts were placed in an oven (60°C) for at least 48 h. Dry/wet ratio was calculated.

2.3. Histology

In two experiments, the heart was fixed by perfusion with 4% formaldehyde. The hearts were cut into 3 pieces, left and right ventricle and septum, and dehydrated in ethanol. The pieces were embedded in methacrylate (JB embedding kit, Polysciences Inc., Warrington, UK). Microscopic sections (4 µm) were coloured using haematoxylin and subjected to light microscopy for examination of the effect of Triton X-100 on the vascular endothelium.

2.4. Chemicals

All solutions except for Triton X-100 were made fresh on the day of the experiment. The HEPES solution during the isolation procedure of the heart had the following composition (in mM): NaCl, 140; KCl, 5; CaCl2, 1; NaH2PO4, 2; MgSO4, 1.2; glucose, 10; and HEPES, 5. The pH was adjusted with NaOH to 7.3–7.4 at room temperature and the fluid was equilibrated with 100% O2.

The perfusion solution (Tyrode) contained (in mM): NaCl, 128.3; KCl, 4.7; CaCl2, 1.36; MgCl2, 1.05; NaHCO3, 20.2; NaH2PO4, 0.42; glucose, 11.1; and was equilibrated with 95% O2 and 5% CO2. Bradykinin was first dissolved in 70% ethanol as stock solution and the final ethanol concentration in the Tyrode solution was 7 × 10−4%. Papaverine and vasopressin were dissolved in the Tyrode solution as used for perfusion. Triton X-100 was diluted (1:200) in Tyrode. All drugs were obtained from Sigma Chemicals Co. (St Louis, MO, USA).

2.5. Statistical analysis

We characterized the Gregg phenomenon during control, during VP perfusion (increased coronary resistance with functional endothelium) and after Triton X-100 treatment (increased coronary resistance without functional endothelium) as the slope of the relationships between peak developed left ventricular (isovolumic) pressure and oxygen consumption, as a function of coronary flow, by linear regression analysis. The slope, r2 and P-value were calculated. Within each experiment the slopes of both relations in control, during VP perfusion and after Triton X-100 treatment, were compared using the Friedman non-parametric repeated measures test. The effects of BK, PAP, Triton X-100 and VP on coronary resistance in each experiment were given in mean ± s.d. and were compared using Wilcoxon's signed-rank test. P-levels of < 0.05 were considered to be significant.

3. Results

We performed 6 experiments in which the coronary flow was changed in control, during VP perfusion (in-

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± s.d. 0.4 | 2.6 | 1.0 | 2.9 | 2.7 | 1.4 | 0.9 | 2.8 | 1.2

Coronary resistance at constant flow (6.6 ± 0.4 ml·min−1·g−1) in mmHg/(ml·min−1·g−1) during control perfusion (C), vasopressin perfusion (CVP) and after Triton X-100 treatment (CVP). The effect of bradykinin (BK, 1 µM) and papaverine (PAP, 1 µM) perfusion on coronary resistance in a preconstricted coronary bed (VP, 3nM) and after Triton X-100 treatment in each experiment.

Significantly different (P < 0.05) from control. Significantly different from CVP. Significantly different from CVP.
creased coronary resistance with functional endothelium) and after Triton X-100 treatment (increased coronary resistance with dysfunctional endothelium). At the start of the experiment, left ventricular systolic pressure at the chosen diastolic pressure of approximately 8 mmHg was 88.2 ± 14 mmHg and mean arterial pressure was 48.3 ± 3.1 mmHg at control coronary flow (6.6 ± 0.4 ml · min⁻¹ · g⁻¹). In pilot experiments, the addition of adenosine or papaverine to the perfusate did not affect perfusion pressure, indicating that the coronary bed of our isolated hearts was maximally dilated. At control flow, vasopressin perfusion increased arterial pressure significantly from 48.3 ± 3.1 to 83.7 ± 22.9 mmHg and thus coronary resistance increased from 7.3 ± 0.4 to 12.6 ± 2.6 mmHg/ml · min⁻¹ · g⁻¹. Triton X-100 treatment increased arterial pressure to 80.8 ± 25.6 mmHg (coronary resistance 12.1 ± 2.9 mmHg/ml · min⁻¹ · g⁻¹; Table 1). In all experiments arterial pressure increased linearly with coronary flow, suggesting lack of autoregulation capacity. The dry/wet weight ratio (n = 4) was 0.16 ± 0.005 (mean ± s.d.).

3.1. Perfusion-induced changes in cardiac contractility and oxygen consumption

Fig. 2 shows a typical example of the increase in left ventricular pressure after an increase in coronary flow from 11.3 to 15 ml · min⁻¹ before and after Triton X-100 treatment. In both conditions Pcv increased with increased coronary flow and perfusion pressure. Both aspects of the Gregg phenomenon, developed left ventricular pressure and oxygen consumption, plotted as a function of coronary flow, are shown in Fig. 3A,B (closed circles) for a typical experiment. All hearts showed during control perfusion both aspects of the Gregg phenomenon; i.e., developed left ventricular pressure and oxygen consumption depended on coronary flow (mean slope ± s.d.: 2.5 ± 0.6 mmHg/ml · min⁻¹ and 0.19 ± 0.02 μmol · ml⁻¹ · g⁻¹, n = 6). Fig. 3A,B (open squares) also shows the effect of changes in coronary flow during increased coronary resistance (VP perfusion). The slope of both relationships remained unchanged (mean ± SD: 2.9 ± 1.4 mmHg/ml · min⁻¹ and 0.25 ± 0.10 μmol · ml⁻¹ · g⁻¹, n = 6). The effect of an increase in coronary flow from 11.3 to 15 ml · min⁻¹ on left ventricular pressure after Triton X-100 treatment is shown in Fig. 2 (lower panel). Developed left ventricular pressure and oxygen consumption as a function of coronary flow are plotted in Fig. 3A,B (closed triangles). The slope of both relationships remained the same (mean ± s.d.: 2.5 ± 1.2 mmHg/ml · min⁻¹ and 0.22 ± 0.10 μmol · ml⁻¹ · g⁻¹, n = 6).
3.2. Endothelium and smooth muscle function

Endothelium-dependent and endothelium-independent vascular smooth muscle relaxation were tested with bradykinin (BK, 1 μM) and papaverine (PAP, 1 μM), respectively. Because the coronary arteries were maximally dilated during control perfusion the vascular bed was first preconstricted with vasopressin (VP, 3 nM). Fig. 4 shows a typical registration of the effects of bradykinin and papaverine before and after Triton X-100 application on left ventricular and arterial pressure during control coronary flow (6.6 ± 0.4 ml·min⁻¹·g⁻¹). Coronary resistance decreased significantly from 12.6 ± 2.6 to 8.8 ± 1.0 mmHg/ml·min⁻¹·g⁻¹ due to BK and from 12.7 ± 1.4 to 7.9 ± 0.9 mmHg/ml·min⁻¹·g⁻¹ as a result of PAP, respectively. After Triton X-100 treatment, a transient effect of BK was often seen, but it was abolished after 4 min and coronary resistance in the steady state did not change significantly (from 12.1 ± 2.9 to 11.9 ± 2.7 mmHg/ml·min⁻¹·g⁻¹, Table 1). Coronary resistance during PAP decreased significantly from 13.9 ± 2.8 to 10.2 ± 1.2 mmHg/ml·min⁻¹·g⁻¹ after Triton X-100.

On the light-microscopic sections of the Triton-X-100-treated hearts, the nuclei of the vascular endothelial cells of capillaries and larger vessels as well as of endocardial endothelial cells were still present (Fig. 5). The results of the functional tests led us to conclude that endothelial function was abolished but that vascular smooth muscle...
function was unaffected after Triton X-100 treatment. Except for papaverine none of the drugs had a significant effect on the mechanical performance of the isolated heart indicated by left ventricular isovolumic pressure (using the Wilcoxon signed-rank test). The left ventricular systolic pressure increased significantly from 99.5 ± 16.7 to 116.3 ± 20.0 mmHg during papaverine (1 µM) and this positive inotropic effect remained after Triton X-100 treatment (from 93.7 ± 14.9 to 106.6 ± 17.5 mmHg).

There was no effect of Triton X-100 treatment on the mechanical performance of the heart since systolic left ventricular pressure did not change significantly (from 84.6 ± 26.9 to 93.1 ± 25.6 mmHg). Time to half-relaxation at a perfusion flow of 4.8 ml·min⁻¹ was 124 ± 11 ms before and 122 ± 13 ms after Triton X-100 and positive dPw/dtmax was 1360 ± 393 and 1768 ± 404 mmHg·s⁻². The relation between time to half-relaxation and positive dPw/dtmax and perfusion flow before and after Triton X-100 treatment are shown in Fig. 6.

4. Discussion

The aim of our study was to determine whether the perfusion-induced increases in developed left ventricular pressure and oxygen consumption in the isolated Langendorff-perfused rat heart were related to vascular arterial endothelium function. In the present experiments an increase in coronary flow induced an increase in developed left ventricular pressure and oxygen consumption with functional as well as with dysfunctional endothelium. This suggests that the endothelial cells are not involved in the Gregg phenomenon. The finding that the endothelium does not play a role in the Gregg phenomenon makes more detailed studies like NO blockade superfluous. Dysfunctional endothelium increases coronary resistance, but an increase in coronary resistance did not alter the Gregg phenomenon [12] (Fig. 3).

4.1. Critique of methods; temperature

The Gregg phenomenon is present at 37°C and at 27°C. Our experiments were performed at 27°C. This temperature was chosen to avoid myocardial ischaemia as much as possible. This is important because it has long been thought that the Gregg phenomenon may result from local or diffuse ischaemia [9] at lower perfusion. In similar experiments it was found that lactate production (23 ± 4.3 µmol/l) did not increase and was in the normoxic range [13] for the flows studied here, indicating that ischaemia did not exist in the present experiments [14]. A disadvantage of this low temperature is the low activity state of several enzymes. However, damage to the endothelial cells still leads to an increase in peripheral resistance and bradykinin was able to dilate the coronary bed, indicating proper function of several enzymatic pathways. Despite this low temperature, developed left ventricular pressure and oxygen consumption still increased after an increase in perfusion.

4.2. Triton X-100 and coronary resistance

Coronary resistance increased significantly by 17.5 ± 8.5% after Triton X-100 treatment. This increase in coronary resistance is a result of the fact that vasoactive substances (e.g., NO) produced and released by the vascular endothelium during control were no longer released after Triton X-100, because smooth muscle function was unaffected by Triton X-100 (Fig. 2, Table 1). The constricting effect of dysfunctional endothelial cells on coronary vascular tone has also been observed by other investigators in isolated heart preparations [4] as well as in isolated vessel studies [1]. This increase in coronary resistance is not likely to be due to a compressive effect of oedema. An increase in permeability of the endothelial cells after Triton X-100 treatment can be excluded because the dry/wet ratios (n = 4) were found to be 0.16 ± 0.005. Ratios of 0.16 are comparable to those found by others in isolated Langendorff-perfused heart experiments [16] (0.15 ± 0.02), suggesting that the permeability of the endothelial cells is probably not changed after Triton X-100 treatment.

We studied an increase in coronary resistance per se by vasopressin (3 nM) infusion before Triton X-100 treatment. Changes in coronary flow during increased coronary resistance (due to vasopressin perfusion or Triton X-100 treatment) led to an increase in developed left ventricular pressure and oxygen consumption similar to that during control conditions, indicating that increased coronary tone did not affect the Gregg phenomenon [12] (Fig. 3). It has been reported that vasopressin has a negative inotropic effect on the heart [17]. However, in the isolated papillary muscle with intact endocardial endothelium, it was shown by Schoemaker et al. [18] that vasopressin in a concentration range between 1 pM and 1 nM had no effect on the contractile state of cardiac muscle. In another study performed by us we showed that the same dose range of vasopressin had no effect on contractile state of the myocardium [12]. We can thus assume that in the present experiments vasopressin had no significant direct effect on left ventricular pressure development.

4.3. Triton X-100 and cardiac contractility

It is well known that the twitch characteristics of the papillary muscle are affected by exposure to Triton X-100. Peak isometric tension is often decreased, contraction time and time to half-relaxation are shortened [2]. These changes are explained by the finding that both endocardial and vascular endothelium modulate cardiac contraction [4]. In our isolated heart experiment left ventricular isovolumic pressure, time to half-relaxation and maximal positive dPw/dt were not changed after Triton X-100 treatment.
The endocardial endothelial cells of the left ventricle are most likely damaged from the start of the experiment by the ventricular balloon and the right endocardial endothelium probably only affects the contractility of the right ventricle which we did not measure. The lack of effect of dysfunctional vascular endothelium on the shape of the left ventricular pressure curve is probably due to the complex structure of the heart, which might mask the modulation of the myocardial contraction by the vascular endothelium.

4.4. Triton X-100 and endothelium and smooth muscle function

The effect of Triton X-100 was analysed in two ways—histologically and functionally. Histological analysis of the Triton-X-100-treated hearts did not show loss of vascular endothelial cells. Others have also reported that the endothelial cells were still present after Triton X-100 inside the vascular tree using light microscopy, scanning electron microscopy and cell viability factors [4]. The vascular endothelium and smooth muscle were functionally tested with two vasodilators, bradykinin (BK, 1 µM) an endothelium-dependent dilator, and papaverine (PAP, 1 µM), an endothelium-dependent dilator, in a preconstricted (VP, 3 nM) coronary bed and after Triton X-100 treatment while perfusion flow was held constant. In the preconstricted bed bradykinin induced a long-lasting dilatory effect (20 min) while after Triton X-100 treatment the bradykinin response was abolished or the response was short-lasting (less than 4 min) and during the steady state was abolished. Bradykinin is a potent vasoactive agent which relaxes different vascular smooth muscle preparations via an endothelium-dependent mechanism. The bradykinin response in the isolated perfused rat heart exists due to an EDRF-mediated response [19]. The BK-induced release of PGI₂ in vascular endothelial cells is coupled to that of NO and is mediated by the activation of kinin B₁ receptors. Inhibitors of the NO-mediated response in isolated perfused rat hearts reduce the duration of the bradykinin response but not the amplitude [19]. This is similar to the response of bradykinin found after Triton X-100 treatment and supports our conclusion that if the bradykinin response was abolished within 4 min after the start of bradykinin infusion, the endothelium is dysfunctional.

By definition, the Gregg phenomenon is related to the perfusion changes in the coronary bed. In the present study a relation between the Gregg phenomenon and vascular endothelial cells could not be demonstrated. However, we could only show that the endothelium of the arterial and arteriolar part of the coronary vasculature was made dysfunctional. Also other parts of the coronary vessel wall may be involved in the Gregg phenomenon because it was recently shown by Li et al. that a vascular-derived contractile factor (VDCF) may play a role in cardiac muscle function [20].

4.5. Conclusion

We conclude that the Gregg phenomenon is independent of the level of coronary resistance and was present in the isolated hearts with both functional and dysfunctional endothelium, suggesting that the arterial and arteriolar endothelial cells are not involved in the Gregg phenomenon.

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References


