Chronic cardiac denervation affects the speed of coronary vascular regulation


Published in:
Cardiovascular research

DOI:
10.1016/S0008-6363(99)00257-6

Citation for published version (APA):
Chronic cardiac denervation affects the speed of coronary vascular regulation

Isabelle Vergroesen\textsuperscript{a,*,} Daphne Merkus\textsuperscript{a}, Jurgen W.G.E. van Teeffelen\textsuperscript{a}, Jenny Dankelman\textsuperscript{a}, Jos A.E. Spaan\textsuperscript{a}, Harry B. van Wezel\textsuperscript{b}, Mark I.M. Noble\textsuperscript{c}, Angela J. Drake-Holland\textsuperscript{c}

\textsuperscript{a}Department of Medical Physics, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands
\textsuperscript{b}Department of Anaesthesiology, University of Amsterdam, Amsterdam, The Netherlands
\textsuperscript{c}National Heart and Lung Institute, Imperial College of Science Technology and Medicine, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK

Received 2 June 1999; accepted 20 July 1999

Abstract

 Objective: We tested the hypothesis that the rate of adaptation of coronary metabolic vasodilatation and autoregulation is modulated by the cardiac nerves. Methods: Anaesthetised dogs (seven innervated (control) and seven with denervated hearts) were subjected to controlled pressure perfusion of the left main coronary artery. Heart rate was controlled by pacing. Results: The steady state autoregulation curves and metabolic regulation curves were similar in the two groups. A sudden increase or decrease in heart rate was associated with a faster response (22\% shorter half-times) in the innervated than the denervated dogs (\(P<0.001\)). A sudden increase or decrease in coronary arterial perfusion pressure was associated with a slower response (24\% longer half-times) in the innervated than the denervated hearts (\(P<0.005\)). Conclusions: We conclude that the speed of response to metabolic and perfusion pressure changes is partly mediated by cardio-cardiac reflexes. Reflex coronary vasodilatation appears to reinforce the metabolic vasodilatation of a heart rate increase and oppose the vasoconstriction in response to increased perfusion pressure. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Coronary circulation; Innervation; Regional blood flow; Vasoconstriction/dilation

1. Introduction

The response of the coronary vascular bed to changes in perfusion pressure minimises changes in coronary blood flow (autoregulation), whereas large changes in coronary blood flow accompany variations in metabolic rate (metabolic regulation). The contribution of various mechanisms to these two regulatory phenomena is still debated [1–6]. One of the purposes of the present study was to assess the contribution of the extrinsic nerve supply of the heart by studying these effects in globally chronically denervated hearts when both the parasympathetic and sympathetic neuronal influences have been removed.

We have previously characterised the speed of response to changes in perfusion pressure and metabolic rate by imposing step changes of these independent determinants, and studying the time course of the response in coronary blood flow [7–10]. A second purpose of the present study was to determine whether these time courses of response were altered in the denervated heart.

2. Methods

The local ethical committee on animal experimentation approved the procedures used in this study which were in accordance with European Commission Recommendations.
2.1. Preparation of cardiac denervated dogs

Adult mongrel dogs (24–30 kg) of either sex were premedicated with glomoxil 24 h before the cardiac denervation procedure. Anaesthesia was induced with midazolam/sufenta followed by ventilation with O₂/N₂O/ Isoflurane. The dogs were denervated under sterile conditions by the method of surgical regional neural ablation under 1.5% halothane in N₂O/O₂ (60:40) by the method of Drake et al. [11]; this procedure achieves preganglionic parasympathetic and postganglionic sympathetic denervation. In addition, a ligature was placed around the left main coronary artery. This ligature could be used for fixation of the perfusion cannula on the day of the measurements. Adequate post-operative pain-killing drugs were administered routinely. Following recovery from the cardiac denervation the coronary blood flow study was made after 3–4 weeks. Previous studies have shown that at least three weeks are required to allow endogenous tissue catecholamines to deplete [11–14]. The major nature of the surgical procedure with recovery caused us to consider it unethical to perform a greater number of experiments in the denervated group.

2.2. Surgery for physiological measurements

Seven innervated dogs (mongrel weighing 19–27 kg) and seven denervated dogs (mongrel 24–30 kg) were used for this study. The dogs were pre-medicated by an intramuscular injection of a mixture of 1.5 ml Rompun (20 mg/ml, Bayer, Leverkusen, Germany) and 1 ml methadone. Anaesthesia was induced by intravenous injection of 4 ml sodium pentobarbital (60 mg/ml). The dogs were intubated and ventilated by a Harvard respirator using a 2:1 N₂O/O₂ mixture. Anaesthesia was maintained by i.v. administration of 40 ml fentanyl in 30 min (0.05 mg/ml). After preparative surgery the anaesthesia for the experiments was continued by injection of 5 ml fentanyl and 2 ml sodium pentobarbital/hour. The use of barbiturate anaesthesia is not ideal, compared with chloralose which enhances cardiovascular reflex activity. However, deep surgical anaesthesia was required. The α-adrenoceptor blocking properties of Rompun would not have still been effective at the time of the measurements. Opioid receptors would have been affected by fentanyl, but we are not aware of these receptors being involved in the responses studied (see Discussion).

A left thoracotomy was performed. In the innervated dogs, the left main coronary artery was dissected proximal to its bifurcation and a ligature placed around it. To induce a low intrinsic heart rate the His bundle was destroyed and the right ventricle was paced with a fixed rate external pacemaker. Heart block was not induced in the denervated dogs because their intrinsic heart rate was already sufficiently low. Pacing was achieved via right atrial electrodes at frequencies above the spontaneous sinus rate. In all dogs (innervated and denervated) a catheter-tip manometer was inserted into the left ventricle through the apex. The dogs were given 3 ml, 5000 IU/ml heparin followed by a continuous infusion of 5000 IU heparin/h. The left carotid and subclavian arteries were cannulated and a stainless steel cannula was introduced through the latter into the aorta. An arterial controlled-pressure perfusion system, as described by Spaan [15], was connected to both cannulae and was used to pump blood from the carotid artery into the aorta under controlled pressure. The subclavian/aortic cannula was then ligated into the left main coronary artery giving controlled continuous perfusion pressure. Perfusion pressure was measured at the tip of the coronary cannula and coronary blood flow (CBF) was measured by an electromagnetic cannulating flow transducer in the perfusion line, just above the cannula. A catheter was introduced into the coronary sinus to obtain steady state oxygen consumption measurements from simultaneous arterio-venous blood samples (oxygen content by Hemoximeter, Radiometer, Copenhagen) and blood flow. A recording of reactive hyperaemia was made for the purposes of normalisation of variables; the cannula supplying blood to the left main coronary artery was occluded for 15 s, and the subsequent maximum blood flow-rate recorded. The zero flow pressure (wedge pressure) was recorded via the perfusion pressure cannula at the end of the 15-s occlusion.

2.3. Protocols

2.3.1. Protocol A

Steady state autoregulation curves were measured by making a series of measurements of final steady blood flow at different perfusion pressures.

2.3.2. Protocol B

Final steady myocardial oxygen consumption was measured and could be related to coronary blood flow at different heart rates all at the same constant perfusion pressure. This was not possible in 4 denervated dogs due to technical difficulties with coronary sinus catheterisation which is done by manipulation of a catheter inserted from a peripheral vein; positioning by manual manipulation (in the absence of fluoroscopy) and in the presence of adhesions from a previous thoracotomy is an uncertain technique.

2.3.3. Protocol C

The rates of coronary flow adaptation to heart rate steps (induced by pacing the heart at different rates) were studied, (this was not possible in 3 denervated dogs due to technical difficulties with pacing and, in one denervated dog, the presence of atrial fibrillation).

2.3.4. Protocol D

The rate of coronary flow adaptation to both step
increases and step decreases in perfusion pressure were studied. Pressure steps and autoregulation curves were performed before heart rate changes in half the experiments, and then vice versa.

2.4. Data analysis

In order to pool the results from individual experiments, it was necessary to normalise coronary blood flow to that obtained with maximum reactive hyperaemia. Myocardial mass was not used for normalisation as this has been shown to be poorly related to maximum flow [5]. Maximum reactive hyperaemic variables were constant for repeated determinations in each animal and were accepted as the maximum physiological flow capacity of the vascular bed. Coronary flow was normalised (CBF
sub
sup
) to a peak reactive hyperaemic flow of 10 ml/min at a perfusion pressure of 100 mmHg above zero flow pressure (wedge pressure).

2.4.1. Steady-state pressure–flow data

The steady-state behaviour of the coronary bed was quantified by pressure–flow relations at constant heart rate. Straight lines with the formula: CBF
sub
= a×P
sup
+b (where CBF
sub
= normalised coronary blood flow, P
sup
= perfusion pressure, and a and b are parameters), were fitted to the data of each dog for the autoregulation curve measured at a heart rate of 100 beats per min. The fitted curves were averaged per dog group and the curves were presented between P
sup
= 50 and 130 mm Hg above wedge pressure. When myocardial oxygen consumption (MVO
sub
) was measured, CBF was related to it according to the method previously described in great detail [5].

2.4.2. Dynamic flow responses

All pressure and flow data were digitized on line, 15 to 20 s before the step change in heart rate or perfusion pressure until 80 to 100 s after the step change. During heart rate steps, every effort was made to hold perfusion pressure constant, but it sometimes did change slightly (Fig. 1). We therefore chose to measure the time course of the effect from the resistance change rather than the flow change; this however does not affect the statistical analysis (see below). Coronary perfusion pressure, coronary venous pressure and coronary blood flow were averaged per beat. The response rate of the coronary bed was analysed in terms of half-times (t
50
values) as described by Dankelman [9].

Reproducibility of t
50
measurements was tested by analysing the same experiment by two persons; results were extremely close. Although absolute values for t
50
determined by this method or by calculation of the half time of flow change were not always identical, the order statistics for both methods are identical; these were therefore used for statistical analysis. This was done by non-parametric analysis of variance; statistical variables were calculating using INSTAT software (Graph Pad, San Diego). The between-group sum of squares and degrees of freedom were calculated by subtraction of the within-experiment sum of squares and degrees of freedom and within-group sum of squares and degrees of freedom from the total sum of squares and degrees of freedom. The variance ratio was calculated by dividing the between group variance by the within group variance.

These studies were adequately powered to give sufficient degrees of freedom, as given in the Results section. A comparable analysis can be applied to compare up-steps with down-steps. However, the protocol was not designed for these comparisons and only the pressure-step study was adequately powered. These and the HR-steps have been previously studied exhaustively in innervated animals [9].

2.4.3. Comparison of oxygen supply and demand

All the values for supply (supplied flow×arterial oxygen content) and demand (measured flow×arterial oxygen content difference) at the varying heart rates were compared by parametric analysis of covariance (software: PRISM, Graph Pad, San Diego). Supply and demand were also studied as functions of heart rate and compared between innervated and denervated hearts by the same analysis of covariance method.

3. Results

There was no significant difference in the range of heart rates or other haemodynamic variables studied (see Table 1).

3.1. Reactive hyperaemia

Fig. 2 illustrates the response to 15-s occlusion of the perfusion line in representative innervated and cardiac denervated dogs; the 15 s used for induction of reactive hyperaemia was kept constant throughout the experimental procedures. There were no observable differences between the two groups in these responses. It can be observed that the pressure in the perfusion line distal to the occlusion decays asymptotically to a value which we have defined as the zero flow pressure or wedge pressure. The peak reactive hyperaemic flow following release of occlusion was similar in both groups (P>0.05).

3.2. Protocol A

All the autoregulation curves of the denervated hearts are compared to those of the normally innervated hearts. Fig. 3 shows the linear regression lines for individual experiments all of which had correlation coefficients above 0.96. The dashed lines in Fig. 3 are the peak reactive hyperaemic relationships which determine the normalisa-
Fig. 1. Tracing of a step heart rate increase and decrease of 30 beats per min in each case. The instability in the perfusion pressure during these steps was minimal. Note delayed responses of coronary blood flow.

Equation (including zero flow intercept pressure set to the origin); these are therefore fixed. The linear regression equation for the innervated dogs was: $\text{CBF}_n = 0.525 + 0.02415(P_p)$ and for the denervated group: $\text{CBF}_n = 1.585 + 0.01372(P_p)$. Analysis of covariance yielded an $F$ variance ratio for comparison of slopes of 2.165 ($p=0.14$). The $F$ variance ratio for comparison of elevations was 0.454 ($p=0.50$), i.e., not significant.

3.3. Protocol B

There were no differences in the relationships between
coronary blood flow and myocardial oxygen consumption between denervated hearts and the controls (Fig. 4). The linear regression equation for the relationship for innervated dogs was: $\text{supply} = 5.485 + 1.193(\text{demand})$ and for denervated dogs was: $\text{supply} = 4.573 + 1.196(\text{demand})$ (where $\text{supply} = \text{CBF} \times \text{arterial oxygen content}$, demand = myocardial oxygen consumption [16]). Analysis of covariance yielded an F variance ratio for comparison of slopes of 0.0004 ($P = 0.98$). The F variance ratio for comparison of elevations was 0.7484 ($P = 0.39$).

Myocardial Oxygen Consumption: Higher values for supply and demand were recorded in denervated hearts than the range in innervated hearts (Fig. 4). Analysis of covariance showed that the myocardial oxygen demand was higher (slopes N.S., for elevations $F = 29.6; P < 0.0001$) in the cardiac denervated dogs at any given heart rate. This was also the case for myocardial oxygen supply (slopes NS, for elevations $F = 22.6; P < 0.0001$).

### 3.4. Protocol C

The average values for the haemodynamic variables during Protocols C & D are shown in Table 1. The perfusion pressure was set to 70, 100, and 130 mmHg in each individual dog (hence the large SD in Table 1). The denervated dogs show a statistically significant slower response after heart rate steps compared to the innervated dogs (Table 2).

### 3.5. Protocol D

The range of heart rates at which these experiments were performed were 83–164 beats per min innervated and 92–164 beats per min cardiac denervated (N.S.) After perfusion pressure steps, the innervated dogs showed slower coronary flow adaptation compared to the denervated dogs. This is the case for both pressure steps up and down (Table 2).

### 4. Discussion

This study shows that the cardiac nerves alter the speed of response of the coronary vascular bed to heart rate and perfusion pressure changes. The cardiac nerves are not required for steady state coronary flow autoregulation and metabolic regulation as would be expected from the primary role of chemical mediation of these phenomena by tissue oxygen and/or metabolites [1,3,5].

Denervation slows the response of coronary flow and resistance to a step heart rate change but speeds up the response to a perfusion pressure change. What cardiac nervous reflexes might subserve these effects? A wealth of different cardio-cardiac reflexes and cardiac afferent fibres have been described for many years since the first description by von Bezold [17]. Early studies using intracoronary veratridine stimulation [18] could not distinguish between coronary and ventricular receptors, but these were later separated into ventricular mechanoreceptors [19,20], coronary mechanoreceptors [21], and chemoreceptors [22]. The reflex effects of stimulation of these receptors have also been studied and discussed extensively [23]. The present results may indicate importance for those potentially involved in the transient changes in coronary blood flow described here.

Heart rate changes in the range used do not materially affect cardiac output or arterial pressure [24]; arterial pressure and peak left ventricular pressure are constant. In the heart rate step experiments, coronary perfusion pressure is constant whereas it obviously changes in the perfusion pressure step experiments. It is therefore almost certain that different sets of receptors are responsible for the two effects observed. Recordings have been made from the possibly corresponding two types of receptors in the ventricle and coronary artery [25].

The main reflex effect of stimulation of coronary and ventricular mechanoreceptors is ‘depressor’, i.e. decreased sympathetic and increased vagal tone [23] with dilatation being the net effect in vascular beds [26]. The vascular bed usually studied is that of the perfused hind limb, or lower body [26], but the responses of the coronary vascular bed are similar [27].

One might postulate that ventricular mechanoreceptors would increase their rate of firing with an increase in heart rate, since the mean left ventricular pressure is increased, although not peak ventricular pressure. The receptors are stimulated more often per minute. In this case we would expect them to produce a reflex coronary vasodilatation [26]. Since the main effect of a step-up in heart rate is also a vasodilatation (metabolically-induced), the reflex vasodilatation would be expected to speed up this vasodilatation. Ventricular mechanoreceptors could conceivably be stimu-
lated also by an increase in perfusion pressure, which would increase myocardial blood volume [28], and thus increase the turgor of the ventricular wall. This might produce a reflex coronary vasodilatation with coronary pressure increase. The main effect of the perfusion pressure increase is a vasoconstriction, i.e. the opposite effect. One would therefore expect a slowing down of the response when the reflex is present, as found in this study.

A more attractive alternative explanation would be to postulate stimulation of coronary arterial baroreceptors, which would also produce reflex vasodilatation [29]. Such vasodilatation, in the face of simultaneous autoregulatory
Fig. 3. Summary of autoregulation curves in all dogs. Continuous lines indicate regression relationships for individual experiments; all regression lines were significantly positive with $r^2$ values >0.92. Dashed lines are the reference flow/pressure relationship at peak reactive hyperaemia which set the normalization. Left: innervated dogs; right: dogs with denervated hearts.

coronary vasoconstriction, would again slow down the speed of the response when the reflex is present as in our innervated animals. The presence of these reflex responses to mechanoreceptor stimulation in innervated hearts can be postulated as having evolved because of a protective role, e.g., protection against coronary arterial wall damage consequent upon a pressure rise. The absence of such a mechanism in transplanted hearts could conceivably play a role in the accelerated coronary artery disease of these hearts.

There is evidence that an increase in aortic and coronary pressure accompanied by increase in left ventricular systolic pressure causes a release of tissue catecholamines, mainly noradrenaline, in the innervated heart. However, this appears to be solely due to the left ventricular pressure increase since it occurs when the aortic and coronary pressures are held constant [30]. This effect is therefore unlikely to apply to our experiments. Catecholamine release would cause vasoconstriction not vasodilatation. This would tend to speed up the vasoconstriction of autoregulation occurring at the same time. These considerations would appear to rule out an interpretation based on a consequence of myocardial tissue catecholamine depletion rather than absence of cardiac reflexes. There can be no tissue release of catecholamines in the globally chronically denervated hearts as used in this study as the tissue catecholamines, particularly noradrenaline, concentrations are severely depleted [14].

Demand, and therefore supply were increased by

Table 2

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Test</th>
<th>Mean±SD</th>
<th>F variance</th>
<th>Degrees of freedom</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Innervated</td>
<td>Denervated</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ratio</td>
<td></td>
<td>Between group</td>
<td>Within group</td>
</tr>
<tr>
<td>Constant pressure perfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_{HRUP}$</td>
<td>Anova</td>
<td>3.81±1.19</td>
<td>4.96±1.34</td>
<td>39.82</td>
<td>1</td>
</tr>
<tr>
<td>$t_{HRDOWN}$</td>
<td>Anova</td>
<td>4.76±1.46</td>
<td>5.06±1.28</td>
<td>26.97</td>
<td>1</td>
</tr>
<tr>
<td>$p$ (UP versus DOWN)</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_{PUP}$</td>
<td>Anova</td>
<td>4.59±1.24</td>
<td>3.72±0.69</td>
<td>52.27</td>
<td>1</td>
</tr>
<tr>
<td>$t_{PDOWN}$</td>
<td>Anova</td>
<td>3.93±0.78</td>
<td>3.34±0.78</td>
<td>40.50</td>
<td>1</td>
</tr>
<tr>
<td>$p$ (UP versus DOWN)</td>
<td>&lt;0.025</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The study was adequately powered for innervated versus denervation, and for P steps up versus down, but not for HR steps up versus down.
chronic denervation for any given heart rate. This supports our earlier finding [31] measured by a completely different technique (radioactive microspheres for myocardial blood flow and correction for external work) that the myocardial oxygen consumption is greater in the denervated heart. This is the underlying observation for metabolic inefficiency in transplanted hearts.

A negative aspect of the study is the failure to find any difference in the slope of the supply/demand curves (Fig. 4). If there is a tonic sympathetic vasoconstriction in innervated hearts, denervation will lead to an increase in the slope of the supply demand curve; this is clearly not found (Fig. 4). The conclusion is that under the circumstances of acute surgical thoracotomy under anaesthesia, tonic sympathetic coronary vasoconstriction is negligible. This is in agreement with findings from regionally denervated humans in the basal state and with adenosine vasodilatation [32]; sympathetic stimulation of course, does induce sympathetically mediated vasoconstriction [27,32] as does exercise [33].

Acknowledgements

The authors are grateful to Aart Boekee, Michiel van der Meer and Cor Brandsma for their assistance and technical support during the experiments described in this study. We also thank all the animal care workers for their excellent care for the denervated dogs after the denervation procedure. MIMN was supported by the Garfield Weston Trust, AJDH by the British Heart Foundation and Daphne Merkus by The Netherlands Heart Foundation.

References