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Review

Angiogenesis in ischaemic and hypertrophic hearts induced by long-term bradycardia

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Abstract

Angiogenesis and improved left ventricular function as a consequence of long-term bradycardia were first demonstrated in normal hearts, either electrically paced (rabbits, pigs) or treated with a selective sinus blocking drug alinidine (rats). Here we review the evidence that chronic heart rate reduction can have similar effects in the heart with compromised vascular supply, due to either hypertensive or haemodynamic overload hypertrophy (rats, rabbits) or ischaemic damage (rats, rabbits, pigs). Bradycardia induced over several weeks increased capillarity in all hypertrophied hearts, and in border and remote left ventricular myocardium of infarcted hearts. In some, but not all cases, coronary blood flow was improved by heart rate reduction, suggesting enlargement of the resistance vasculature in some circumstances. Cardiac or left ventricular function indices, which were depressed by hypertrophy or ischaemic damage, were preserved or even enhanced by chronic heart rate reduction. The expansion of the capillary bed in the vascularly compromised heart induced by bradycardia may be stimulated by mechanical stretch of the endothelium and/or VEGF activated by chamber dilation and myocyte stretch. The increased number of capillaries and more homogeneous distribution of capillary perfusion would support the better pump function, even in the absence of higher coronary flow. The beneficial impact of chronic heart rate reduction on myocardial angiogenesis and function in cardiac hypertrophy and infarction may be major factor in the success of beta-blockers in treatment of human heart failure.

Abbreviations: CD – capillary density; C:F ratio – capillary-to-fibre ratio; FGF – fibroblast growth factor; LV – left ventricle; VEGF – vascular endothelial growth factor

Introduction

Bradycardia and the coronary circulation

It is well known that bradycardia improves the oxygen balance of the heart by reducing demand and increasing supply, particularly in the sub-endocardial layers, through better perfusion during a prolonged diastolic period [1]. There is also an acknowledged inverse relationship between heart rate and capillary supply in that 'athletic' animals such as the hare or wild rat have a lower heart rate and higher capillary density (CD) when compared with related sedentary species such as the rabbit and laboratory rat [2, 3]. Conversely, capillary density is lower in animals with a higher heart rate [4]. Our laboratory has pioneered experimental models in which heart rate was manipulated to be lower on a chronic basis to determine whether myocardial capillary supply would be changed accordingly. A reduction in resting heart rate of approximately 40% was achieved in rabbits [5] and of 30% in pigs [6, 7] by cardiac electrical pacing. When maintained for 3–4 weeks this procedure increased capillary density by 30% and 25% respectively in the absence of heart hypertrophy. In rats, a smaller chronic decrease in heart rate (28%) was achieved pharmacologically by the sinus node inhibitor alinidine, leading to a 20% increase in CD [8]. This

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intervention did result in heart hypertrophy which is a common feature of exercise training-induced bradycardia. Experimental heart rate reduction in normal animal hearts also enhanced left ventricular work capacity [9, 10] whereas coronary blood flow, either at rest or during maximal work challenge, was in general not different from control normal values [8, 9]. This could be explained by a more homogeneous distribution of coronary flow, estimated from the relationship between blood flow and measurements of capillary diffusion capacity [11].

There is a significant body of evidence showing the clinical benefits of treatment with beta-blockers in reducing mortality in post-myocardial infarction [12] or heart failure patients [13, 14]. Aside from the protective effects of beta-blockade against catecholamine damage or beta-receptor downregulation in the myocardium, the associated bradycardia has been considered to be a major factor in its ability to preserve cardiac function. For example, ST segment elevation after gradual occlusion of the coronary artery was eliminated by atenolol, but cardiac pacing that returned heart rate to its original level prevented this effect [15]. Pacing also abolished the improvement induced by beta blockade in myocardial contraction in dogs with left ventricular (LV) dysfunction due to chronic mitral regurgitation [16]. Experiments in animal models of infarction have shown that prolonged administration of atenolol led to left ventricular remodelling and improved function [17], while acute administration of beta-blockers increased the contractile force in ischaemic canine myocardium [18, 19]. With regard to possible effects of beta-block on coronary vasculature, Grover et al. [20] found a higher proportion of perfused capillaries and greater volume fraction of capillaries and arterioles after acute use of practolol in normal rabbit hearts. In ischaemic hearts, acute beta blockade improved perfusion distal to a coronary stenosis [21] and normalised the sub-endo/sub-epicardial flow ratio [19], which could be related to improved capillary perfusion. Chronic administration of propranolol in normal hearts led to increased capillary density [22], but so far there are no data to show if beta-blockers induce angiogenesis in vascularly compromised hearts, nor if their beneficial effects in patients are due solely to decreased heart rate.

There is, however, evidence that pharmacological bradycardia can diminish the area of myocardium at risk after acute coronary artery ligation in rats [23] and pigs [24]. Furthermore, clinical observations of better coronary collateral development in coronary artery disease patients who have a low (< 50 beats min^{-1}) compared to a higher (> 60 beats min^{-1}, [25]) heart rate suggest that bradycardia could have a lasting effect on the coronary vascular bed. In this article, we would like to review the work which demonstrates that bradycardia, induced either by drugs or by electrical pacing, can result in capillary growth and improved left ventricular function in hearts that have a compromised coronary circulation due to chronic hypertension or infarction, and to discuss the factors which could explain cardiac angiogenesis under these conditions.

Effects of long-term bradycardia on coronary angiogenesis

Cardiac hypertrophy

Pathological cardiac enlargement can be modelled experimentally either by volume or pressure overload [26] and in both situations, cardiac remodelling *via* myocyte hypertrophy and interstitial fibrosis are accompanied by a reduction in the capillary supply [27–29], increased minimum coronary resistance [30] and decreased coronary vasodilator reserve [31]. Here we report on the effects of long-term bradycardia on myocardial capillarity in both types of hypertrophy.

Volume overload hypertrophy

Volume overload hypertrophy was induced in adult rabbits by surgical damage to the aortic valve. One month later, heart rate was decreased by electrical cardiac pacing [5] and maintained at approximately 55% of normal rate for a further 4 weeks. Myocardial capillary density was estimated in left ventricular tissue by histochemical staining for alkaline phosphatase, and regression analyses of heart weight on body weight, and capillary density on heart weight in a large sample of control animals were used to predict changes in heart weights and capillary densities for comparison with observed values. In animals with no pacing but valve lesion of 8 weeks duration, hearts were 16% heavier than expected for body weight and capillary density was 10% lower than expected for heart weight. Pacing resulted in coronary capillary growth, and even though heart weights were 25% heavier than expected, capillary density was actually 43% higher for this size of heart. This was an even greater increase than induced in normal hearts by pacing alone (35% increase in CD) (Figure 1).

Pressure overload hypertrophy

Moderate heart hypertrophy was produced in rats by induction of one-clip two kidney Goldblatt renal hypertension. Mean arterial blood pressure after 7 weeks was 162 ± 15 mmHg compared with 87 ± 14 mmHg in normotensive control rats ($P < 0.001$), and heart-to-body weight ratios were increased by 19%. Bradycardia was induced in groups of normo- and hypertensive rats by repeated intraperitoneal injections over 5 weeks of alinidine, a K_{ATP} channel antagonist that increases the A-V node refractive period [32], at a dose that acutely reduced heart rate from 380 ± 20 beats min^{-1} to 288 ± 10 beats min^{-1} ($P < 0.001$). Final heart rates after alinidine treatment were 28% lower in

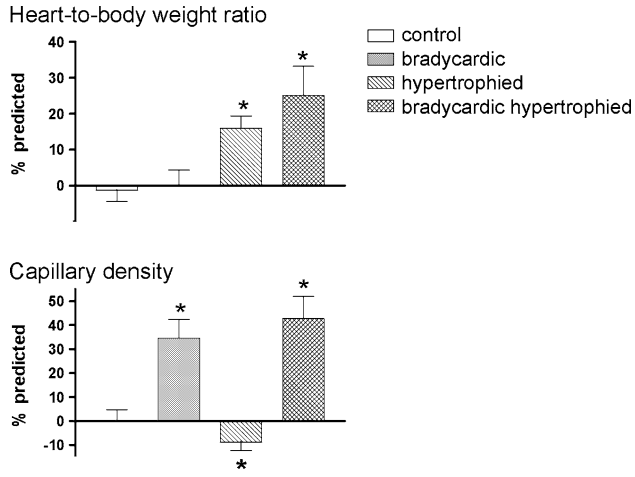

Figure 1. Top – Mean ± S.E.M. percentage deviation of heart-to body weight ratio for control and experimental groups of rabbits (control $n = 10$, pacing-induced bradycardia for 4 weeks $n = 8$, volume overload hypertrophy by aortic valve lesion for 8 weeks $n = 6$, hypertrophy and 4 weeks pacing $n = 8$) from that predicted by the regression of heart weight on body weight in a total sample of 26 normal adult New Zealand Red rabbits. Bottom – Mean ± S.E.M. percentage deviation of capillary density (estimated from alkaline phosphatase stained cryosections) for the same groups as above from that predicted by the regression of capillary density on heart weight for the same sample of normal rabbits. $^{*}P < 0.05$ vs. predicted.

normotensive rats and 16% lower in hypertensives. Chronic heart rate reduction did not affect the rise in blood pressure in hypertensive rats but exacerbated cardiac hypertrophy (heart-to-body weight was 43% greater than in normotensives). Capillary-to-fibre ratios were significantly increased by alinidine in both groups, by 20% in normo- and 15% in hypertensives (both $P < 0.05$) (Figure 2).

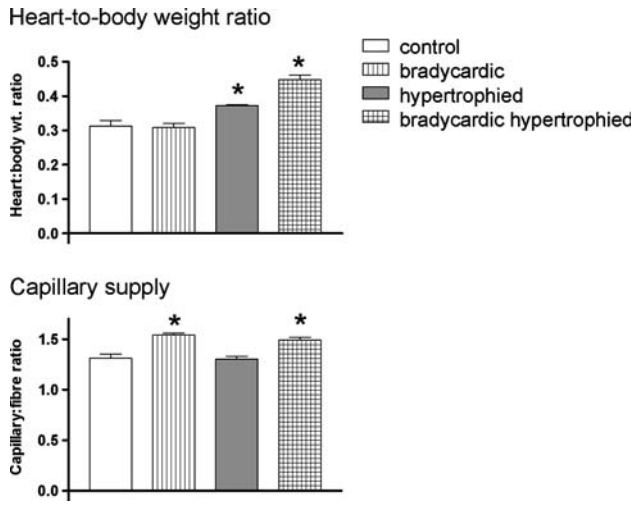

Figure 2. Top – Heart-to-body weight ratios in control rats ($n = 4$), rats rendered bradycardic by alinidine treatment for 5 weeks ($n = 7$), rats with pressure overload hypertrophy ($n = 6$) and hypertensive rats treated with alinidine ($n = 6$). Bottom – capillary:fibre ratio in the left ventricle of hearts from the same groups as above. $^{*}P < 0.05$ vs. controls.

Myocardial damage

The pathogenesis of myocardial damage after episodes of ischaemia involves cell necrosis and apoptosis that can be confined to a specific area of the myocardium when flow to a large coronary artery is interrupted, or diffuse and scattered when temporary occlusion of a large artery is followed by reperfusion and microvascular dysfunction or focal ischaemia accompanies small vessel disease and inhomogeneity of perfusion. In either case, ventricular function is impaired by irreversible loss of myocyte viability, and by sublethal myoctye damage known as myocardial 'stunning'. In the longer term, salvage of ischaemic-reperfused myocardium and restoration of cardiac function are critically dependent upon reversing the deficit in blood flow, to which end induction of coronary angiogenesis in areas at risk is crucial.

Diffuse ischaemic myocyte damage

The fact that high doses of catecholamines produce myocardial damage was established long ago in both rats [33] and patients [34]. Increased sympathetic activity and catecholamine release contribute to the occurrence of malignant arrhythmias in patients with coronary heart disease [35]. In rabbits, intravenous infusion of noradrenaline (4 μg kg^{-1} min^{-1}) for 1 h increased blood pressure and caused cardiac arrhythmias with occasional ST segment elevation, indicative of myocardial ischaemia. Within 2 days, it resulted in diffuse myocardial necrosis that was identified histologically in the left ventricle and quantified in a total area of 2.6 mm^2 per heart to occupy between 0 and 32% in individual fields, predominantly in the sub-endocardial region [36]. Of all fields, 21% showed damage of greater than 5% in area and 11% were without damage. In unaffected viable myocardium of the left ventricle, catecholamine treatment also decreased capillary density in the sub-endocardium versus the sub-epicardium (ratio of 0.86 vs. 1.03 in controls), resulting in an unfavourable transmural capillary distribution.

Prior induction of bradycardia in rabbits by electrical pacing for 3–4 weeks conferred protection against catecholamine damage. On histological analysis, fewer areas showed evidence of cellular necrosis (15%) and more (22%) were without damage. In undamaged myocardium, a normal transmural distribution of capillaries was maintained (sub-endo- to sub-epicardial capillary density ratio of 0.98, NS vs. controls). Whereas noradrenaline treatment led to a decrease of 10% in heart-to-body weight ratio after 2 days, the decrement was smaller (6%) in heart of animals that had been paced.

Ischaemic damage by coronary artery ligation

Lei et al. [37] studied the length density of capillaries in rat hearts 3 weeks after ligation of the left coronary artery that resulted in infarcts ranging in size from 50–83% of the left ventricular free wall. The bradycardic

drug alinidine, administered by an osmotic minipump during the three weeks post-ligation, lowered heart rates in conscious animals by 20–30%. Capillary length density was 40% higher in the infarct border zone and 14% higher in remote regions of the septum. Moreover, the length density of small pre-capillary terminal arterioles was increased by 62% in the septum after alinidine. The treatment did not affect infarct size, but resulted in a greater degree of compensatory hypertrophy in comparison with that observed in hearts with infarct alone.

In another recent study of rats with myocardial infarction, 3 weeks treatment with agents that normalised post-infarction tachycardia (aspirin, methylprednisolone) led to increases in capillary-to-fibre ratio in the remaining viable left ventricle despite myocyte hypertrophy [38], and in the same model, even modest heart rate reductions of 2–18% induced over 3 months by a sinoatrial node modulator ivabradine almost doubled CD in viable tissue compared to untreated infarcted hearts [39].

Ischaemic damage by coronary artery thrombosis

A more gradual occlusion of the coronary artery was produced in Yorkshire farm pigs in our laboratory by inserting a copper wire coil (1 mm diameter, length 7–8 mm) into the left anterior descending coronary artery and allowing a thrombus to form around it. The coil was introduced via the carotid artery on the end of a catheter guide wire and deposited approximately two thirds of the way down the artery from its origin (Figure 3). During the same operation all pigs were instrumented with telemetric equipment to record ECG for heart rate monitoring, and some received Medtronic® dual chamber pacemakers in order to decrease heart rate by a technique of atrial–atrial pacing [6]. Heart rate pacing was commenced a few days after surgery and in freely moving conscious animals, rates were maintained in the range of 60–80 beats min^{-1} compared with 120 beats min^{-1} in non-paced animals for periods of 4–6 weeks.

This procedure resulted in anteroseptal infarcts comprising around 15% of the left ventricle (Figure 3). In pigs with infarcts that were paced, heart-to-body weight and left ventricle-to-heart weight ratios (0.45 ± 0.03 and 0.43 ± 0.01 respectively) were no different from those in unpaced animals (0.45 ± 0.03 and 0.42 ± 0.01, NS). Pacing-induced bradycardia significantly increased the capillary density, evaluated in lectin-stained cryosections (Figure 3), in both sub-endo- and sub-epicardial layers of the left ventricle. In remaining viable normal LV myocardium of the infarcted hearts, the increase in capillary density after pacing (14%) was smaller than in non-infarcted hearts (25%), but in the infarct border zone, it was comparable (28%) (Figure 4). Pacing also increased the number of proliferating capillaries in normal hearts (3.3% vs. 0.8% capillaries positive for proliferating cell nuclear antigen in unpaced, $P < 0.05$). In the infarct border zone, proliferating capillaries were increased similarly in paced (3.7%) and unpaced (4.6%) hearts, but it was only in the former that growth of new capillaries actually ensued.

All these results show clearly that long-lasting bradycardia, whether induced by pacing or by drugs, stimulates capillary growth in both normal and ischaemic

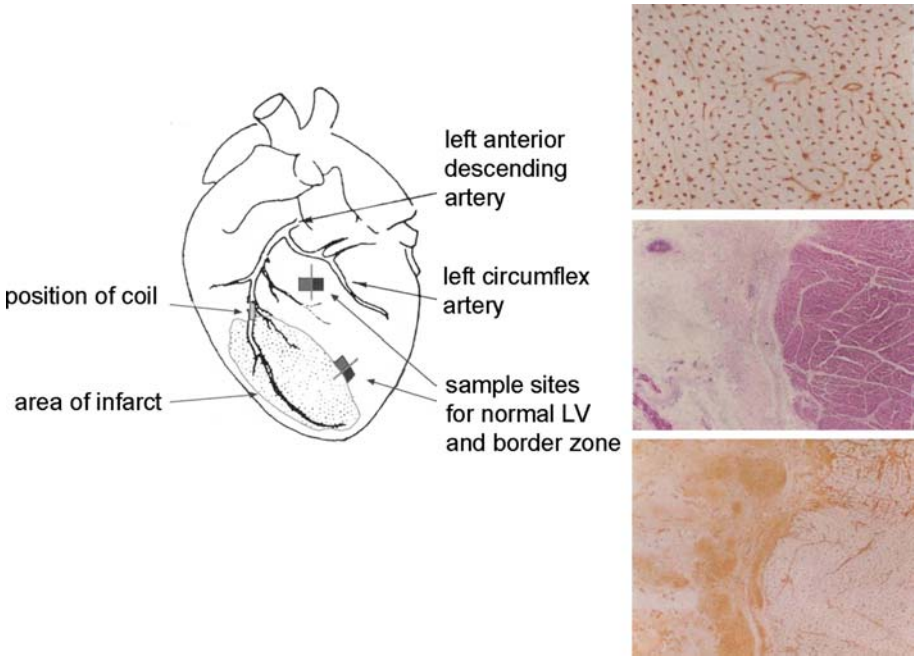

Figure 3. Left – Scheme to show location of copper coil placement to induce thrombus and the resultant ventricular infarct in pig hearts. Also shown are the sites of tissue sampling for estimation of capillary supply in viable myocardium and border zone. Right – Top panel shows GSI-1 lectin staining of capillaries in normal pig myocardium. Middle and lower panels show serial sections stained for H & E and lectin respectively illustrating the border zone adjacent to an area of infarct.

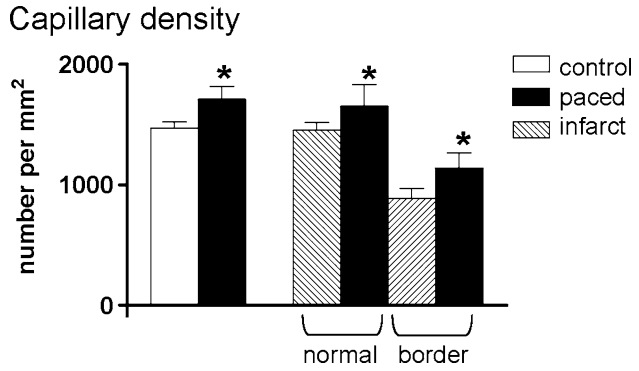

Figure 4. Capillary density in left ventricle of control normal and bradycardic paced pig hearts, and in normal and border regions of infarcted hearts and paced infarcted hearts. $^{*}P < 0.05$ vs. control.

damaged myocardium of vascularly compromised hearts, irrespective of myocyte hypertrophy. In normal hearts, the magnitude of angiogenic response over time was related to the degree to which heart rate could be lowered. Two important questions, then, are whether the expanded capillary bed is related to improvements in coronary perfusion and, ultimately, to better cardiac function.

Effects of long-term bradycardia on coronary blood flow and cardiac function

Cardiac hypertrophy

In groups of rabbits and rats with volume overload or pressure-induced hypertrophy respectively, coronary blood flow was measured using radiolabelled microspheres and maximal cardiac minute work (product of cardiac output and arterial pressure) estimated under conditions of rest and peak work elicited by inotropic challenge (noradrenaline infused at incremental doses to maximal effect, [40]).

Volume overload hypertrophy decreased peak coronary blood flow but despite the increased capillarity resulting from prolonged bradycardia in both normal and hypertrophic hearts, there was no improvement overall in coronary flow (Figure 5). Cardiac minute work was reduced by 15% at rest and by 35% during inotropic challenge in valve-lesioned hypertrophied hearts. Pacing-induced bradycardia enhanced both values by 19% and 46% respectively in normal hearts, and in hypertrophied hearts, cardiac work was 46% greater than in unpaced hypertrophied hearts under both conditions (Figure 5). Maximal work capacity in hearts hypertrophied by volume overload was therefore normalised by bradycardia.

Likewise, pressure-induced hypertrophy in rat hearts resulted in lower coronary blood flow during cardiac work, but treatment with alinidine did not alter this [8] (Figure 6). Cardiac work performance during inotropic challenge was also no different between treated and untreated hearts, either normal or hypertrophied (Figure 6).

Coronary blood flow was therefore unchanged by either pacing- or drug-induced bradycardia, implying that there was no expansion of the resistance vasculature. Cardiac function, however, was enhanced in volume overload hypertrophic hearts, although not when hypertrophy was pressure-induced.

Myocardial damage

Diffuse ischaemic myocyte damage

Maximal cardiac minute work during inotropic challenge was depressed by 30% in rabbit hearts with diffuse catecholamine ischaemic damage compared with normal hearts. In hearts that had been previously paced to bradycardia for 3–4 weeks, maximal work was normalised (101% of control values). Myocardial contractility, assessed as left ventricular dP/dt, was also reduced in catecholamine-damaged hearts but preserved at normal levels in damaged hearts that had been paced [36]. There is no accompanying data on coronary blood flow in these hearts.

Ischaemic damage by coronary artery ligation

In infarcted rat hearts, the study by Lei et al. [37] showed similar values for coronary conductance in the border zone and the septum at rest and during maximal dilation with dipyridamole. Concurrent treatment with alinidine, which reduced heart rate by up to 30%, did not alter conductance at rest but it was increased in both border zone and septum at maximal vasodilation, and coronary reserve – the ratio of maximal to resting conductance – was increased by 23%. This was associated with the observation of significant arteriolar growth which exceeded the increase in left ventricular mass observed with alinidine treatment, similar to capillary growth described above in hypertensive hypertrophied rat hearts in our own experiments. The regions which showed akinesis or dyskinesis, imaged by echocardiography, were the same size in infarcted and alinidine-treated infarcted rat hearts. Ejection fractions were also similar immediately post-ligation of the coronary artery, but whereas they fell by 31% over the following 3 weeks in untreated hearts they decreased by only 9% with concurrent alinidine treatment [37]. Mulder et al. [39] also reported preservation of cardiac output in infarcted rat hearts through enhancement of stroke volume and improved left ventricular wall shortening function after treatment with doses of ivabradine that reduced heart rate chronically by 18%.

Ischaemic damage by coronary artery thrombosis

Coronary sinus blood flow was measured by thermodilution during inotropic challenge (dobutamine infused to maximal effect) in normal and infarcted pig hearts with and without pacing to bradycardia for 4–6 weeks. There was no difference in peak values of flow between normal control and paced hearts [7], nor between unpaced and paced infarcted hearts (Figure 7).

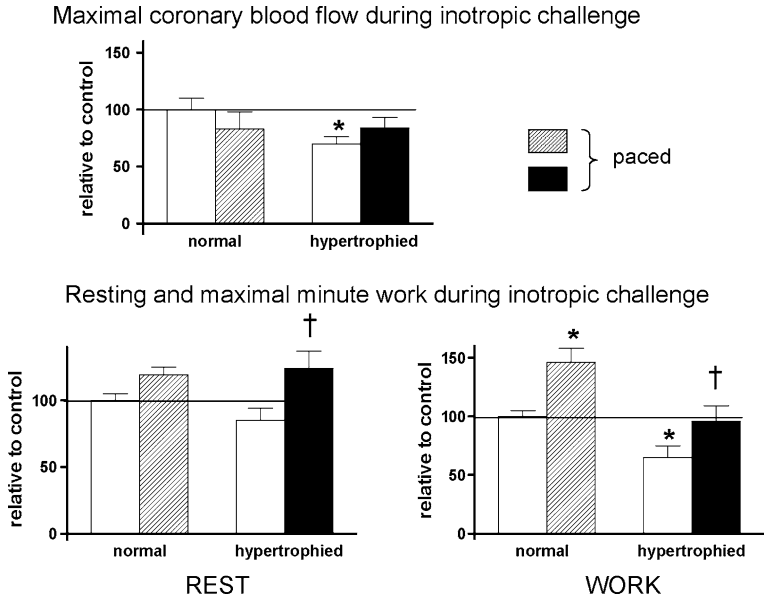

Figure 5. Top – Maximal coronary blood flow during inotropic challenge with noradrenaline. Bottom – Resting and maximal cardiac minute work in normal (N) and volume overload hypertrophied (H) rabbit hearts without and with pacing-induced bradycardia (control normal $n = 10$, paced normal $n = 6$, volume overload hypertrophied $n = 6$, paced hypertrophied $n = 6$). In all cases, data for experimental groups are shown relative to values in control normal animals (=100%). $^{*}P < 0.05$ vs. control, †$P < 0.05$ vs. unpaced hypertrophied.

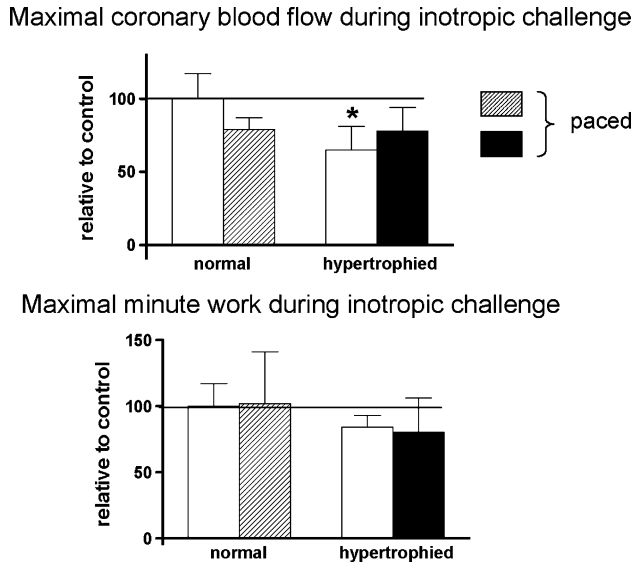

Figure 6. Coronary blood flow (top) and cardiac minute work (bottom) during maximal inotropic challenge with noradrenaline in rats without and with pressure-induced cardiac hypertrophy, without and with concurrent alinidine treatment. $^{*}P < 0.05$ vs. control.

In normal pigs, chronic bradycardia did not significantly alter cardiac performance measured either as stroke work index or left ventricular dP/dt at rest but maximal stroke work capacity during inotropic challenge was 47% greater [7]. In infarcted hearts, pacing also led to a significant improvement in stroke work capacity (Figure 7).

Bradycardia was therefore capable of bringing about improvements in cardiac function in most cases of hypertrophic and ischaemically damaged hearts, but this was not necessarily associated with enhancement of overall coronary blood flow, despite the enlarged capacity of the capillary bed. This may depend on whether there is growth of the resistance vasculature as well as growth of the capillary network during chronic heart rate reduction. To date, the coordinated growth of arterial and capillary vessels in the heart has seldom been studied during bradycardic interventions, with the exception of the study by Lei et al. [37] described above. One critical study in this area is that by

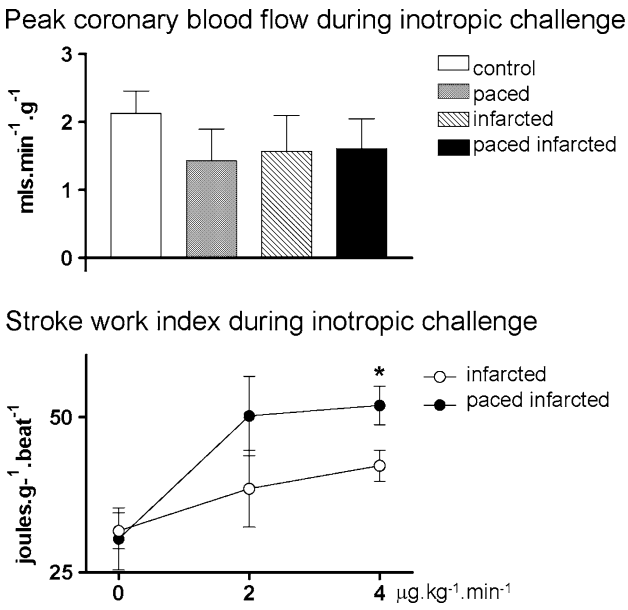

Figure 7. Top – Peak coronary sinus blood flow during inotropic challenge with dobutamine (8 μg kg^{-1} min^{-1} i.v.) in pig hearts (control $n = 7$, paced $n = 7$, infarcted $n = 5$ and paced infarcted $n = 3$). All NS Bottom – cardiac stroke work index during dobutamine challenge in infarcted (○) and paced infarcted ● pig hearts. $^{*}P < 0.05$ paced infarcted vs. infarcted.

White et al. [41] who performed morphometric analysis of capillary and arteriolar density in the hearts of healthy pigs during the course of a 16 week exercise training programme that induced resting bradycardia [42]. They demonstrated that capillary growth occurred within 3 weeks, followed by an increase in the density of small arterioles that coincided with higher maximal coronary blood flow. The implication is that expansion of the resistance vasculature occurs through transformation of capillaries at their proximal ends into small arterioles which eventually widen and contribute to the greater capacity for coronary flow [43]. In view of the disparate findings on whether coronary flow is increased by chronic heart rate reduction, this type of vascular growth and remodelling does not appear to be an inevitable corollary of bradycardia-induced angiogenesis in the vascularly compromised heart, but the duration of bradycardia may be an important factor because of the sequential pattern of capillary and arteriolar growth.

Even without accompanying resistance vessel growth, increased capillary density would shorten the diffusion distances for oxygen and improve oxygen delivery to mitochondria. Bradycardic pacing did result in a greater extraction of oxygen in isolated rabbit hearts [44] and in normal pig hearts ($45 \pm 11\%$ in paced versus $15 \pm 2\%$ in control hearts, $n = 7$ for both, $P < 0.05$), and it changed the relationship between coronary blood flow and oxygen consumption in pig hearts (Figure 8) so that oxygen consumption was consistently met by a lower coronary flow. This effect was less obvious in infarcted hearts (Figure 8).

A greater capillary density may also permit increased capillary diffusion capacity. This was estimated as permeability-surface area product (PS) in isolated rabbit hearts using the technique described by Mann and Yudilevich [45]. The tracers ^{22}Na and ^{51}Cr-EDTA were used, their trans-capillary transport being respectively flow-limited or partly diffusion-limited. Paced hearts had a higher PS and extraction for both tracers. The ratio of coronary flow/PS, which is an indicator of homogeneity of flow distribution, a value of 1.0 representing all capillaries with equal perfusion and transport capacity, was significantly lower in paced than in control hearts (Figure 9) [11]. The increased capillary bed as a result of lasting bradycardia was accompanied by more homogeneous distribution of capillary perfusion and transport. Although similar data are not available for either hypertrophic or damaged hearts, a similar redistribution of perfusion might help to explain the improved heart performance in hearts with enlarged capillary bed in the absence of changes in coronary blood flow.

Factors involved in bradycardia-induced angiogenesis

In view of the potency of bradycardia as a means for induction of capillary growth, attention has focussed on the likely mechanisms. Two growth factors, basic fibroblast growth factor (FGF-2) and vascular growth

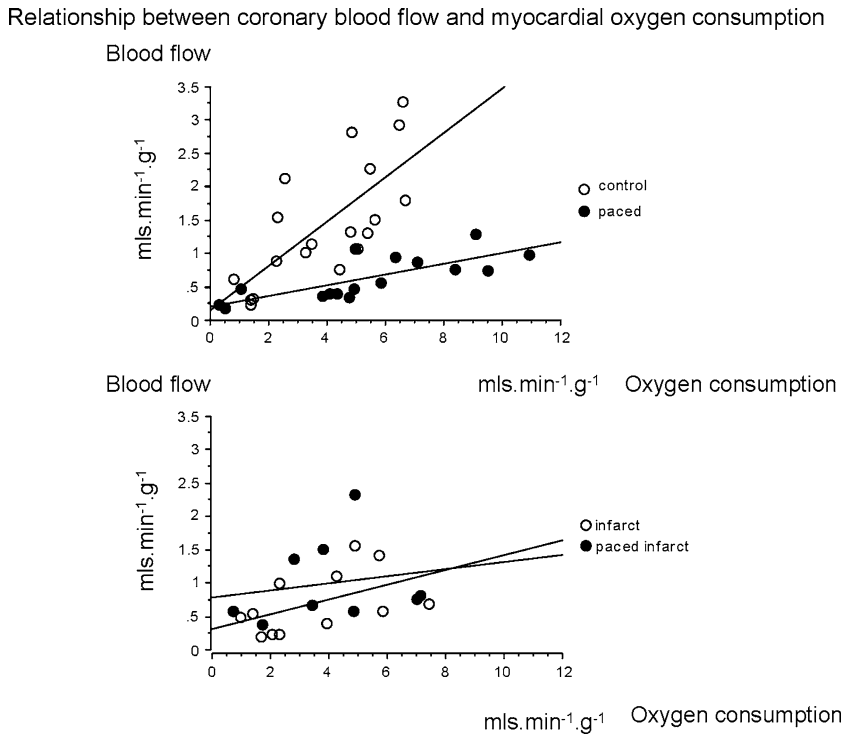

Figure 8. Relationship between coronary blood flow and myocardial oxygen consumption in control and bradicardially-paced pig hearts (top), infarcted and bradycardially-paced infarcted pig hearts (bottom). Blood flow was measured by thermodilution and oxygen consumption calculated using arterio-venous oxygen difference from oxygen content measured in femoral artery and coronary sinus blood samples by Tucker chamber.

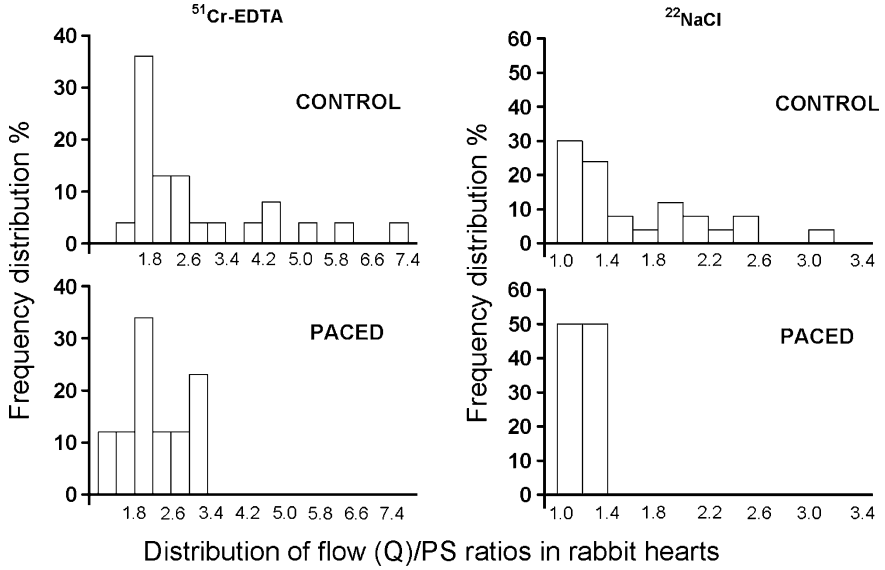

Figure 9. Relationship between coronary flow (Q) and permeability-surface product (PS) to tracers ^{51}Cr-EDTA (left) and ^{22}NaCl (right) in control (normal) and bradycardic (paced 4 weeks) rabbit hearts. Mean values for ^{51}Cr-EDTA were 3.264 ± 0.426 in controls and 2.227 ± 0.233 in paced (P<0.025) and for ^{22}NaCl, 1.588 ± 0.111 in controls and 1.194 ± 0.030 in paced (P < 0.005).

endothelial factor (VEGF) have been extensively studied in connection with angiogenesis in the heart. Whereas FGF-2 has been linked facilitation of arteriolar growth, both VEGF and FGF-2 modulate capillary growth during embryogenesis [46]. FGFs have also been identified in the ischaemic heart [47, 48], after coronary spasm produced by administration of vasopressin [49] and in ischaemia/reperfusion injury [50]. Both FGF-2 and VEGF were expressed on capillaries in rat ventricle in ischaemia/reperfusion [51]. There are many reports on their effects, both positive and negative, in the treatment of coronary vascular disease and this will be reviewed in the paper by Tirziu and Simons in this volume.

In bradycardiac normal rabbit hearts, however, there was no evidence of increased expression of FGF-2 mRNA [52] and since our data on coronary flow do not support the notion that chronic bradycardia induced arteriolar growth, it was not studied further. On the other hand, Zheng et al. [10] have demonstrated a more than twofold increase in VEGF mRNA with up-regulation of VEGF protein after 2 weeks and increased capillary length density in rats rendered bradycardic by alinidine treatment, and similar increases in VEGF expression and stimulation of both angiogenesis and arteriolar growth were observed by the same group in infarcted hearts [37]. Bradycardia may activate VEGF by mechanical stretch since it prolongs the duration of diastole so that the length of time for which cardiac myocytes are stretched by ventricular filling is greater. Stretch of the ventricular chamber activated vascular endothelial growth factor in the isolated heart [53] while in isolated cardiac myocytes, stretch increased VEGF and TGFβ1 but not FGF-2 [54]. Moreover, endothelial cells in capillaries are also exposed to stretch due to the fact that capillary diameters are wider during diastole than systole [55] and consequently stretched [56]. A combination of mechanical and growth factors may therefore be effective in the stimulation of capillary growth in hearts with a significantly reduced heart rate. This leads to the possibility that growth factors such as VEGF could be activated endogenously by chronic heart rate reduction in order to stimulate therapeutic angiogenesis and improve cardiac function without the need to introduce them exogenously.

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