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Haemodynamic factors influencing myocardial ischaemia in a canine model of coronary artery stenosis: the effects of nitroglycerine

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1 At a critical degree of coronary stenosis (allowing a just adequate blood supply to the poststenotic area only at the expense of maximal hypoxic coronary vasodilatation), an additional loading of the heart induced marked local myocardial ischaemia, as indicated by appropriate biochemical, electrophysiological and haemodynamic changes.

2 In this model myocardial oxygen demand was increased in three different ways: (i) increasing heart rate by atrial pacing; (ii) increasing afterload by aortic occlusion and (iii) increasing preload by blood infusion. These procedures were compared in their ability to produce local myocardial ischaemia and characterized by ST-segment elevation recorded from the endocardium and epicardium.

3 Increasing afterload evoked the mildest degree of ischaemia since the resulting increase in coronary perfusion pressure and coronary flow almost met the augmented myocardial oxygen demand evoked by the elevated peripheral resistance and by the simultaneously increased preload. A rather more pronounced ischaemia was produced by increasing the preload. The most serious ischaemia of all was induced by atrial pacing. This reduced coronary flow and perfusion pressure and increased left ventricular end diastolic pressure (LVEDP).

4 Nitroglycerine transiently reduced blood pressure and coronary blood flow and increased epicardial and endocardial ST-segment elevation; the changes had disappeared 10 min after terminating the infusion. However, at this time a prolonged protective action against pacing-induced ST-segment elevation was observed. This protection was also seen after intracoronary injections of nitroglycerine. This indicated that part of the beneficial effect of nitroglycerine in ischaemia is due to direct coronary and/or myocardial actions.

Introduction

In earlier studies we described an experimental canine model for evaluating potential anti-anginal drugs (Szekeres, Csik & Udvary 1976) in which an analysis of the metabolic and electrophysiological consequences of coronary insufficiency could be determined in acute experiments. These studies were performed in anaesthetized, thoracotomized dogs with partial constriction of the left anterior descending coronary artery. A critical degree of coronary stenosis resulted in ischaemic changes, as assessed from epicardial electrograms, when an additional work load was imposed on the heart by atrial pacing. However, in clinical practice other kinds of cardiac loading elicit anginal attacks, for example increases in preload and afterload. We have therefore examined in this experimental model the effects of different kinds of loading on the development of severe ischaemia in the presence of a fixed stenosis of a major coronary artery. Elucidation of the major factors determining the severity of myocardial ischaemia in such an animal model might be of therapeutic importance since therapy, for example with new potential antianginal agents, could then be directed towards suppression, or prevention, of the most critical haemodynamic contributions to the severity of myocardial ischaemia.

In the present experiments three different types of loading stress have been used, namely increasing heart rate, augmented venous inflow (preload) and elevated peripheral vascular resistance (afterload). Because of the complexity of these different types of loading *in vivo*, no attempt has been made to produce equivalent loading in physical terms. The load applied to the heart was thus regulated to represent the

maximal possible burden which could be imposed without major impairment of cardiac function. Studies have also been made in this model of the effects of a standard anti-anginal agent nitroglycerine, administered both systemically and by local injection into the stenosed coronary artery.

Methods

The experiments were performed on a total of 32 mongrel dogs of either sex and weighing between 15 and 20 kg. The dogs were anaesthetized with sodium pentobarbitone (35 mg/kg by intravenous injection), intubated and ventilated by a Harvard respirator delivering room air; the tidal volume was 15 ml/kg and the rate 12/min. Arterial blood pH, P_{CO_2} and oxygen saturation was examined at regular intervals (Astrup blood gas analyser, and Kipp CC oxymeter type MD3). The control values were: pH, 7.408 ± 0.04, $P_{CO_2} = 30 \pm 5$ mmHg and oxygen saturation $92 \pm 4\%$ (mean $\pm$ s.e. mean).

After a left thoracotomy the pericardium was incised and the left anterior descending (LAD) coronary artery cannulated and autoperfused from the left femoral artery. The experimental arrangement is shown in Figure 1. Epicardial electrodes, similar to those described by Szekeres *et al.* (1976) were sutured to the left venticular wall supplied by this artery; this allowed simultaneous recording from three separate locations. Endocardial ST-segment changes were recorded from the tip of a 0.2 gauge copper wire, insulated except from the tip, and introduced through the myocardial wall into the ventricular cavity by means of a 0.55 gauge needle (see Figure 1.). The potentials from the endocardial and the epicardial unipolar leads were recorded on a Hellige recorder (Multiscriptor 9400/6/) with the sensitivity set to 2.5 mV corresponding to a 1 mm deflection.

Blood pressures were recorded by means of Statham Pb 23 transducers and Hellige electromanometers. Aortic blood pressure was measured from a polyethylene catheter introduced via a femoral artery and right atrial pressure from a catheter introduced via a femoral vein. A catheter was introduced through the left carotid artery into the left ventricular cavity and was used for the measurement of intraventricular pressure; this pressure signal was differentiated to allow for the assessment of myocardial contractility from the left ventricular (LV) *dP/dt max.* Left ventricular end-diastolic pressure (LVEDP) was recorded from the LV pressure trace using a higher amplifier gain. Cardiac output was measured by means of an electromagnetic flowmeter (Nycotron Mod 376 Oslo, Norway) on the ascending aorta. Coronary perfusion pressure was measured in a glass T-piece side branch of the polyethylene tube

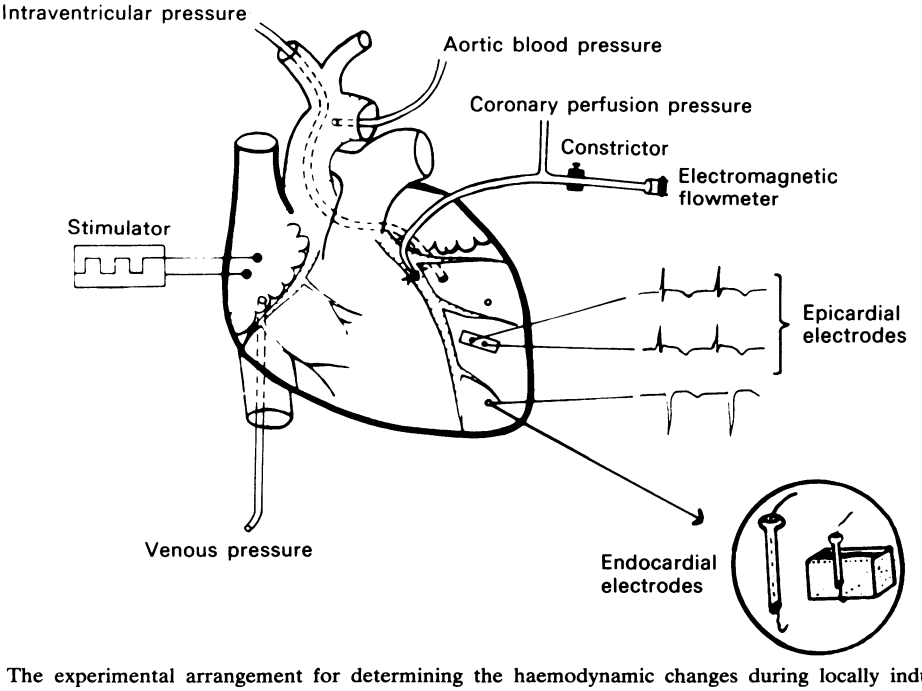

Figure 1 The experimental arrangement for determining the haemodynamic changes during locally induced myocardial ischaemia. For details see text.

of 2.5 mm internal diameter, tapered at the coronary end to 1.5 mm connecting the left femoral artery to the left descending coronary artery (see Figure 1) and subendocardial driving pressure was calculated from the difference between LVEDP and the coronary (diastolic) perfusion pressure (Marshall & Parratt, 1974). Inflow through the autoperfused LAD coronary artery was measured using a Nycotron 376 flowmeter. The animals were heparinized by giving 1000 iu/kg (i.v.), followed after 2 h by 250 iu/kg if necessary.

After a short rest period the following basic haemodynamic and electrophysiological parameters were determined: blood pressure, epicardial and endocardial electrograms, heart rate, coronary blood flow, coronary perfusion pressure, subendocardial driving pressure, LVEDP, LV$dP/dt\ max$ and cardiac output.

Coronary blood flow in the LAD artery was then reduced by means of a finely adjustable micrometer screw such that a short complete occlusion of the cannulated artery for 20 s was no longer followed by a hyperaemic response. This represents the so called *'critical stenosis'* (Gould, Lipscomb & Hamilton, 1974). Under these conditions no ischaemic changes could be detected from epicardial ECG recordings in the area perfused by the constricted artery unless the myocardium was subjected to the additional stress of pacing or volume loading (see below). This means that at rest the blood supply to this area is just sufficient to cope with the metabolic needs of the myocardium but only at the expense of a maximal hypoxic dilatation of the vascular bed. After establishing this critical degree of stenosis, different types of additional load were imposed on the heart and were maintained for periods of 5 min. The haemodynamic and electrocardiographic changes occurring during this experimental period were measured every minute. Additional stress was placed on the myocardium subjected to a critical coronary stenosis by: (1) augmenting the heart rate by 70 beats/min over the basic rate (which was 150 ± 15 beats/min) by pacing from electrodes sutured to the right atrium. This procedure results in reproducible and reversible changes (ST-segment elevation) in epicardial electrograms (Szekeres *et al.*, 1976). (2) Occluding the descending aorta, below the origin of the left subclavian artery, for 5 min (increased afterload). (3) Increasing venous inflow by transfusing blood (140 ml for 5 min) from a donor dog into a jugular vein (increased preload).

The effects in this model of administering nitroglycerine were investigated in a total of 20 dogs. Nitroglycerine was given either as an intravenous infusion (in a dose at 2 $\mu g\,kg^{-1}\,min^{-1}$ for 10 min into the femoral vein) or by intracoronary (LAD) administration in a dose of 1.5 μg/kg which was calculated to produce concentrations in coronary blood equivalent to that following the intravenous infusion. Pacing was induced 10 and 40 min after nitroglycerine administration. In all experiments elevation of the ST-segment in the epicardial and endocardial electrograms was used as an indicator of the myocardial oxygen supply: demand ratio. Student's *t* test was used for statistical analysis of the data.

Results

Effects of creating a critical coronary stenosis

Critical stenosis of the left anterior descending coronary artery evoked no substantial changes in blood pressure, LV $dP/dt\ max.$, cardiac output, right atrial

Table 1 Haemodynamic and electrocardiological effects of atrial pacing and of increasing afterload and preload in dogs with a critically stenosed LAD coronary artery

	Control	*Atrial pacing*	*Control*	*Aortic occlusion*	*Control*	*Blood transfusion*
Arterial blood pressure (mmHg)	107 ± 3	94 ± 3**	113 ± 6	144 ± 8*	110 ± 8	142 ± 12**
LVEDP (mmHg)	3.0 ± 0.1	7.0 ± 0.4**	5.0 ± 1.0	11.0 ± 1.7**	6.0 ± 1.4	43 ± 10.4**
Coronary blood flow (ml $100g^{-1}\,min^{-1}$)	79 ± 6	63 ± 6**	79 ± 8	99 ± 10**	74 ± 7	140 ± 25**
Coronary perfusion pressure (mean, mmHg)	59 ± 2	49 ± 3**	56 ± 3	75 ± 5**	57 ± 3	79 ± 7**
Subendocardial driving pressure (mmHg)	49 ± 5	36 ± 4**	47 ± 6	58 ± 7**	46 ± 6	33 ± 9
ST-elevation (epicardial; mV)	4.7 ± 0.5	11.4 ± 0.8**	5.4 ± 0.9	6.6 ± 1.6	7.0 ± 1.1	11.0 ± 0.8**
ST-elevation	1.4 ± 0.3	11.0 ± 1.0**	1.5 ± 0.7	1.5 ± 0.7	2.5 ± 0.7	9.0 ± 0.8**

Values are means ± s.e.mean.

*Significantly different from control at level $P < 0.02$; **Significantly different from control at level $P < 0.01$.

pressure or LVEDP. However there was a significant (32%) decrease in flow through the stenosed artery (from 117 ± 7 to 80 ± 5 ml 100 g^{-1} min^{-1}); coronary perfusion pressure decreased by 37% (from 102 ± 4 to 64 ± 3 mmHg) and subendocardial driving pressure by 39% (from 80 ± 3 to 49 ± 2 mmHg). Only slight changes occurred in the epicardial and endocardial electrocardiograms (less than 2 mV ST-segment elevation). The endocardium proved to be more sensitive to this disturbance in local myocardial blood supply as indicated by a rather more marked elevation of the ST-segment.

Effects of frequency load

Increasing the atrial rate by 70 beats/min evoked small, but significant, reductions in blood pressure (Table 1), significantly reduced cardiac output (from 1.48 ± 0.12 l/min to 1.13 ± 0.13 l/min; $P < 0.02$) and increased right atrial pressure (from 2.0 ± 0.3 mmHg to 2.5 ± 0.4 mmHg; $P < 0.02$). Coronary flow was further reduced by 20% and coronary perfusion pressure by 17% (Table 1). This reduction, together with a marked elevation in LVEDP was responsible for the marked (27%) decrease in subendocardial driving pressure. This indicates a considerable deterioration in the endocardial microcirculation as indicated by a marked elevation of the ST-segment recorded from this region (Table 1). A similarly significant elevation of the ST-segment in epicardial electrograms indicates that the ischaemia extended over the whole thickness of the left ventricular wall.

It was important for the validity of this model, particularly for the assessment of potential antianginal activity of drugs, to establish the reproducibility of several consecutive atrial pacing periods. That this is indeed so is illustrated in Figure 2, which shows the effects on epicardial and endocardial ST-segment changes of five 5 min periods of atrial pacing over 2–3 h.

Effects of increasing afterload

Afterload was increased by occluding the descending aorta for 5 min periods. The results are presented in Table 1 and the time course of the most significant changes is illustrated in Figure 3. In spite of the substantially increased afterload (increase in blood pressure from 113 ± 6 to 144 ± 8 mmHg) no substantial changes occurred in ST-segment elevation recorded from either epicardial or endocardial electrograms (Table 1), presumably because alterations in myocardial oxygen demand were more than adequately met by the increased coronary perfusion pressure and blood flow. Unfortunately, aortic occlusion also resulted in a substantial increase in preload (LVEDP; Table 1) and a slight increase in LV d*P/dt max* (from 5470 ± 825 mmHg^{-1} to 5940 ± 700 mmHg^{-1}). These parameters can only be adequately controlled in either isolated hearts or in

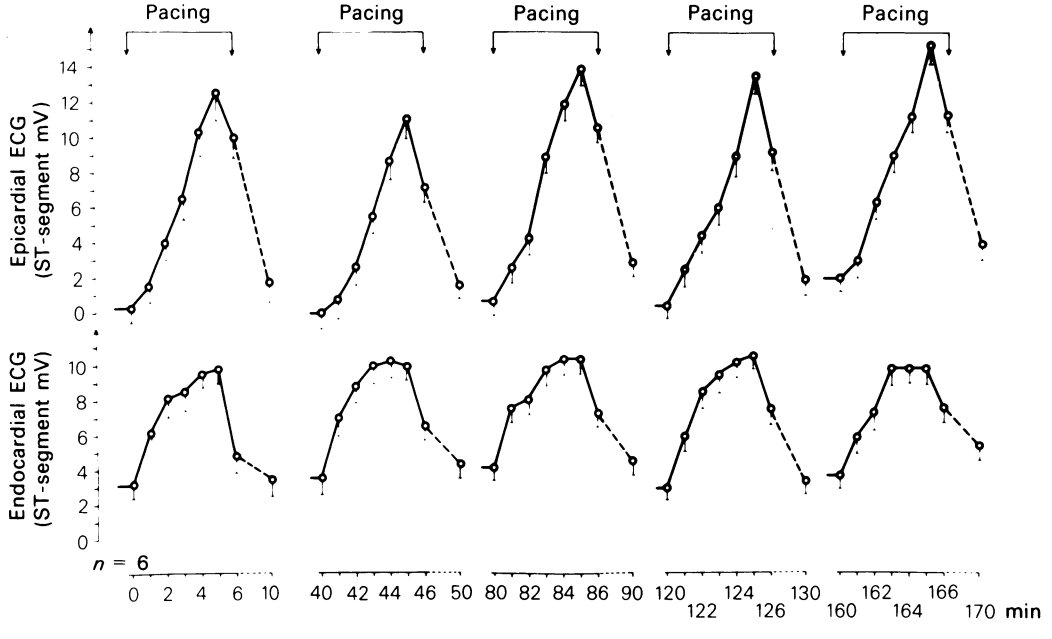

Figure 2 Effect of frequency loading on the endocardial and epicardial ST-segment elevation.

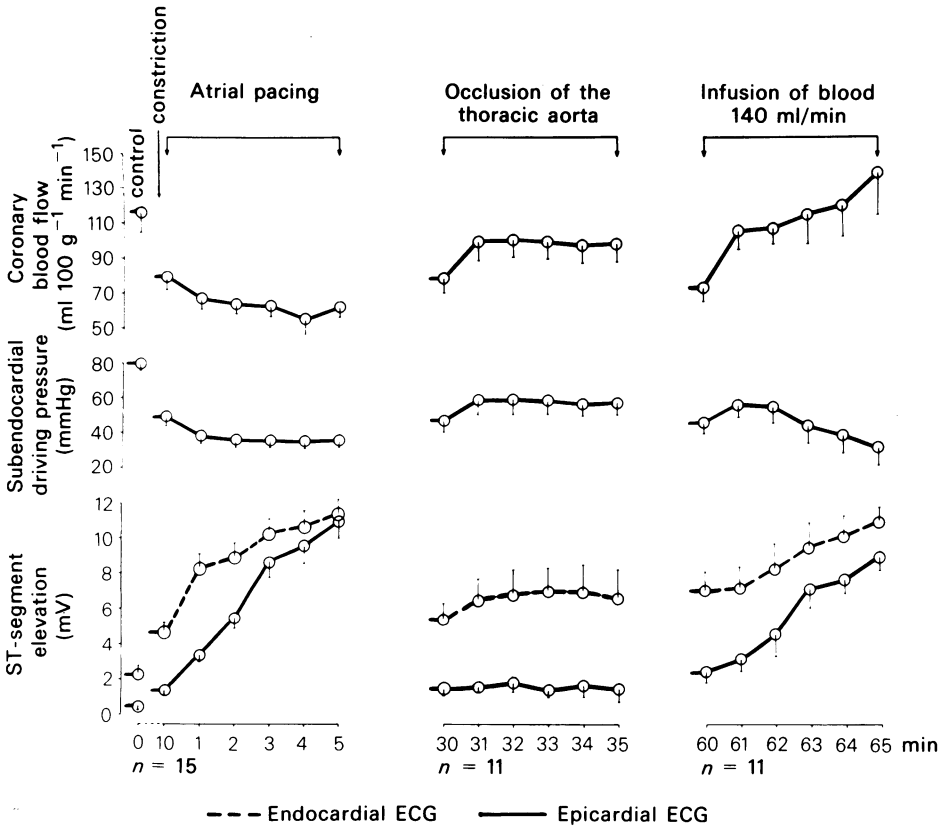

Figure 3 Haemodynamic effects of three different kinds of cardiac loading in dogs with a critically stenosed left coronary artery.

heart-lung preparations. However, it is clear from the present studies that substantial increases in both preload and afterload do not markedly intensify myocardial ischaemia in this model.

Effects of increasing preload

Very marked increases in preload were induced by transfusing a total of 700 ml of blood from a donor dog over a 5 min period. This resulted in increases in artrial pressure (Table 1), left ventricular filling pressure (values in excess of 40 mmHg; Table 1), right atrial pressure (from 1.8 ± 0.5 mmHg to 11.0 ± 1.6 mmHg; $P < 0.01$), and in cardiac output (from 1.4 ± 0.2 to 2.9 ± 0.3 l/min; $P < 0.01$). There was a slight increase in LV dP/dt *max* (from 5200 ± 780 to 5910 ± 860 mmHg/s) which, taken together with the marked increases in both preload and afterload, probably indicates a reduction, rather than an increase, in myocardial contractility. Blood transfusion clearly resulted in a marked intensification of myocardial ischaemia in the region supplied by the stenosed coronary artery. There were marked increases in ST-segment elevation recorded from both epicardial and endocardial electrodes (Table 1) with the endocardial changes being especially severe.

Effects of intravenously administered nitroglycerine

The effects of nitroglycerine were examined in dogs with a critically stenosed coronary artery subjected to the additional stress of atrial pacing. The intravenous infusion of 20 μg/kg of nitroglycerine administered over a 10 min period resulted in substantial haemodynamic changes which had mainly returned to control pre-drug values 10 min after the end of infusion period (Table 2). However, it is clear that at this time ST-segment elevation was significantly reduced particularly in the endocardium (i.e. from 5.7 ± 1.1 mV to 4.5 ± 1.0 mV; Table 2). This probably reflects, at least in part, the reduced left ventricular filling pressure and the slight, but significant, increase in coronary blood flow (Table 2).

When tachycardia was induced by atrial pacing 10 min after nitroglycerine administration, the marked increases that occurred in the control period

Table 2 Direct haemodynamic and electrocardiologic effects of nitroglycerine (NTG) infused intravenously (2 μg kg^{-1} min^{-1} for 10 min) and locally into the constricted LAD coronary artery

	Control	*Intravenous NTG*		*Intracoronary NTG*	
		post NTG	*10 min post NTG*	*Control*	*post NTG*
Arterial blood pressure (mmHg)	92±6	76±4**	95±7	97±3	94±4
LV *dP/dt max* (mmHg/s)	4340±120	2750±140**	3360±120	5070±125	4720±145
LVEDP (mmHg)	6.5±1.3	4.6±0.9*	5.8±1.1**	5.5±1.0	5.4±1.0
Coronary blood flow (ml $100g^{-1}$ min^{-1})	54±4	48±3	60±3**	47±6	49±6
Coronary perfusion pressure mean; mmHg)	52±3	44±3	56±2**	52±3	48±3*
Subendocardial driving pressure (mmHg)	47±3	41±1*	51±9	48±4	44±3*
ST-segment elevation (endocardial; mV)	5.7±1.1	6.2±1.2	4.5±1.0**	5.6±0.3	5.5±0.6
ST-segment elevation (epicardial; mV)	2.5±0.6	3.2±1.1*	2.1±0.6	3.4±0.5	2.2±0.5**

Values are means ± s.e.mean

*Significantly different from control at level $P<0.02$; **Significantly different from control at level $P<0.01$.

during pacing, in LVEDP and the ST-segment elevation was significantly attenuated (Figure 4). This protection is especially clear if values obtained at the end of the 5 min pacing period are compared (Figure 4). For example, at this time, the increase in LVEDP induced by pacing was reduced by 27% after nitroglycerine administration and the increase in epicardial ST-segment elevation was reduced by 44%. Similar results were obtained even 40 min after nitroglycerine administration.

Effects of locally administered nitroglycerine

The haemodynamic effects of the intracoronary administration of nitroglycerine are summarized in Table 2. The only pronounced direct effect was a reduction in ST-segment elevation recorded from epicardial electrodes (from a mean of 3.4 ± 0.5 mV to a mean of 2.2 ± 0.5 mV, a reduction of 36%). The local intracoronary administration of nitroglycerine beneficially modified the effects of atrial pacing (Figure 5), as indicated by the less pronounced increases in ST-segment elevation. This protection was rather less than that resulting from systemic administration (Figure 4) but, since it occurred in the absence of changes in systemic arterial pressure or LVEDP, this might indicate that some at least of the beneficial effects of nitroglycerine in ischaemia are due to direct coronary and/or myocardial actions.

Discussion

A comparison of three different means of loading the *in situ* heart with fixed 'critical' stenosis of the LAD has shown that the most severe ischaemia is produced by frequency loading, that less severe changes appear after volume-loading and particularly after pressure-loading. No attempt was made in these studies to apply equal degrees of loading; this is difficult because of the lack of easily measurable and comparable physical equivalents of load. Exploratory calculations suggest that the external work performance of the heart does not reflect the ability of the different types of loading to promote ischaemia. A better approach, and one we used here, is to compare coronary flow, coronary perfusion pressure, subendocardial driving pressure and LVEDP under all three types of loading.

It is clear that in the case of frequency loading changes in all three factors are involved in determining the degree of ischaemia. Besides the unfavourable pressure changes evoked by frequency loading (a substantial increase in LVEDP and a decrease in coronary perfusion pressure) a reduction in the coronary flow to the stenosed area also plays an important role in the development of ischaemia. This is probably at least partly due to a frequency induced shortening of diastole (and an augmented time of systolic compression of the coronary vessels). Less time is thus available for blood to flow to the deeper myocar-

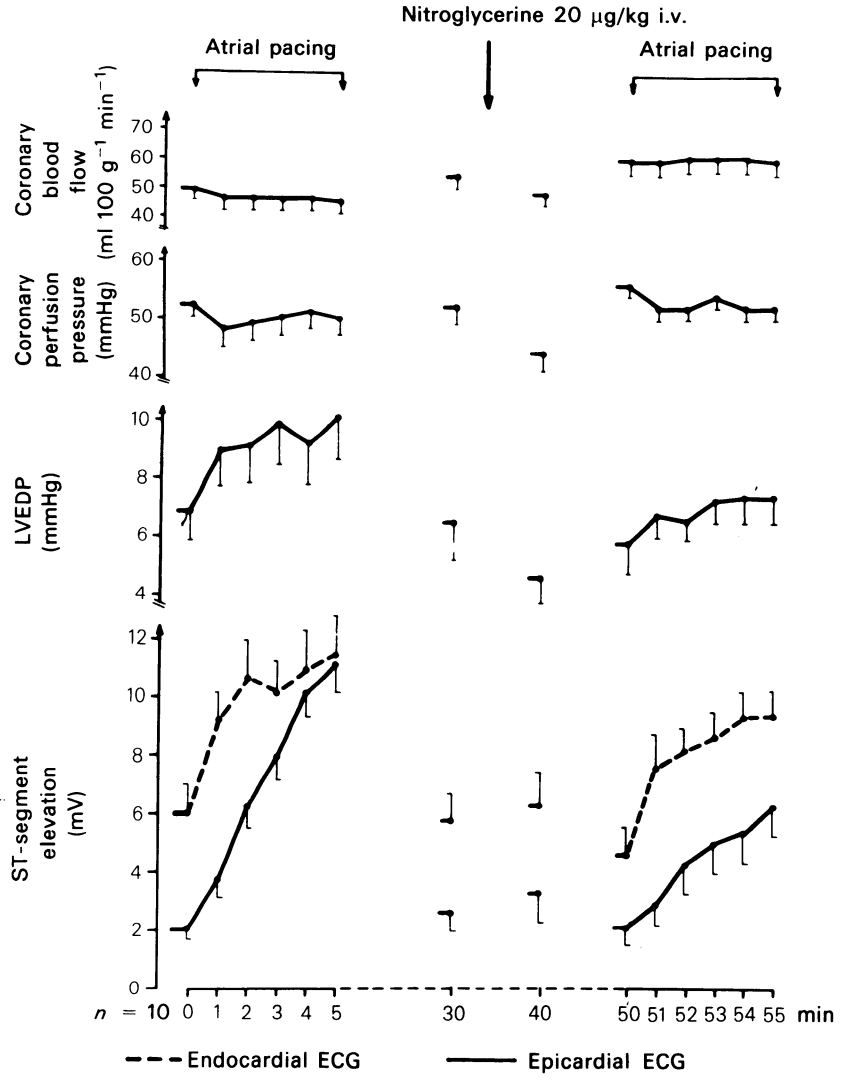

Figure 4 The time of the action of intravenously administered nitroglycerine on various haemodynamic and electrophysiological parameters during atrial pacing in dogs with a critically stenosed coronary artery.

dial layers. The above hypothesis is in accordance with the findings of Neil, Phels, Oxendine, Mahler & Sim (1973) who found in tranquilized dogs without coronary constriction that atrial pacing-induced tachycardia diminished the subendocardial/subepicardial flow ratio, as estimated by radioactive microspheres. This shift of coronary blood flow from the subendocardial to the subepicardial layers is also reflected by a similar shift of endo/epicardial carbon dioxide tension (Gerry, Schaff, Kallman & Flaherty 1981). Coronary stenosis alone increased the endocardial/epicardial P_{CO_2} ratio and this was further aggravated by pacing. Thus it can be expected (Neill *et al.*, 1973) that in patients with coronary stenosis the shift of the blood flow away from the subendocardial layers is accentuated by tachycardia, resulting in a more severe subendocardial ischaemia.

In contrast to frequency loading, volume and pressure-induced loading increased blood flow to the area supplied by the constricted coronary artery. This was due to the considerable increase in coronary perfusion pressure resulting in an augmented subendocardial pressure. However, both types of loading also elevated LVEDP and this counteracted the beneficial effect of increased coronary blood flow. If we

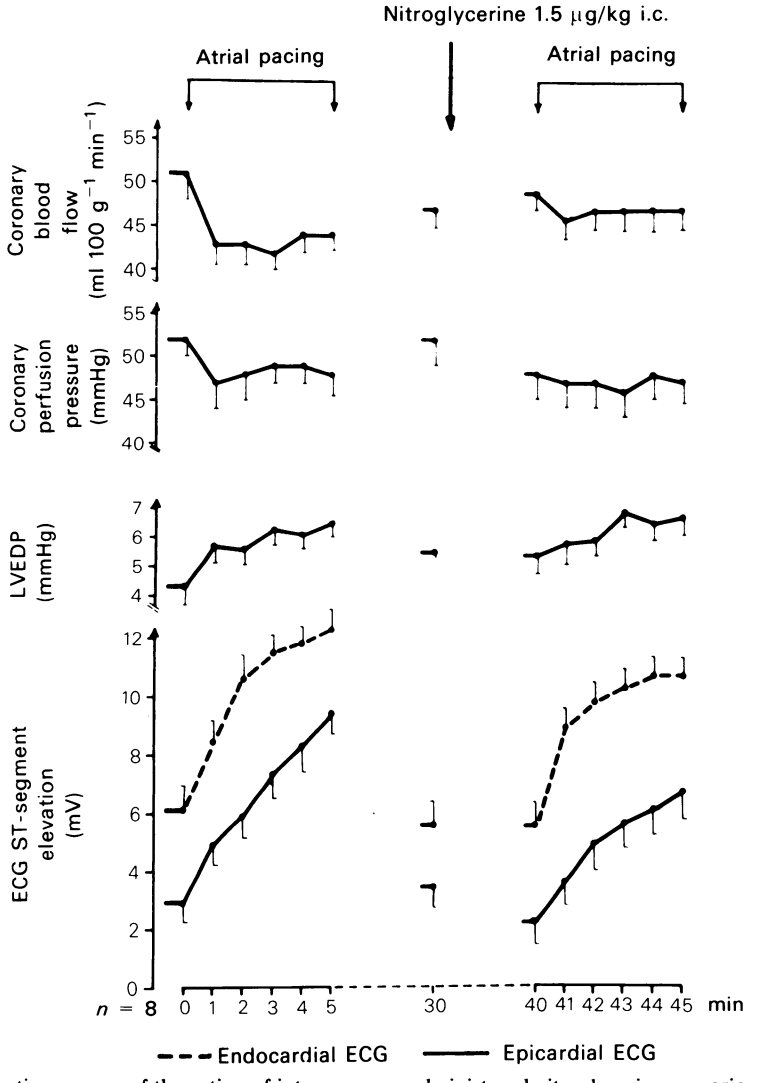

Figure 5 The time course of the action of intracoronary administered nitroglycerine on various haemodymamic and electrophysiological parameters during atrial pacing in dogs with a critically stenosed coronary artery.

take into consideration that LVEDP is not only a determinant of subendocardial driving pressure but also reflects changes in cardiac volume, then marked ventricular dilatation may be present with increased tension of myocardial fibres at rest. This increases the myocardial oxygen demand and would contribute to the severity of ischaemia. This is supported by the observation of Wyatt, Da Luz, Waters, Swan & Forrester (1977) that elevating LVEDP by infusing dextran increases cardiac output and regional systolic shortening. However, regional lactate production also increased, indicating an aggravation of ischaemia despite an augmentation in coronary flow.

In our present studies nitroglycerine reduced ST-segment elevation in the endocardium more than it did in the epicardium. This accords with the finding that nitroglycerine increases oxygen tension in the endocardium without affecting that in the epicardium (Winbury, 1971) and with the observation of Gerry *et al.*, (1981) that nitroglycerine reduces the elevated subendocardial P_{CO_2} tension in the ischaemic area. This speaks in favour either of a selective arteriolar dilatation by nitroglycerine in the subendocardium or of a preferential effect on the transmural arteries (Winbury, 1971; Weiss, Howe & Winbury, 1973).

An intramyocardial redistribution of flow between

outer (subepicardial) and inner (subendocardial) layers of the myocardium (Bache, 1978) or between normal and ischaemic areas (Udvary, Csik & Szekeres, 1976) would explain the beneficial action of nitroglycerine.

Besides a more favourable redistribution of flow, nitroglycerine-induced haemodynamic changes may also contribute to the anti-ischaemic effect of the drug. The most important of these actions seems to be the marked reduction in LVEDP which may considerably increase the subendocardial driving pressure (a major determinant of subendocardial blood flow) despite a diminished coronary perfusion pressure secondary to systemic hypotension. Our finding that the beneficial action of the drug is not as marked when it is given directly into the coronary artery supports this idea.

On the basis of these results some conclusions may perhaps be drawn regarding the clinical situation of angina. Thus therapeutic interventions aimed at reducing local myocardial ischaemia should tend to reduce first any existing tachycardia. This also involves the use of drugs that are able to reduce heart rate (e.g. β-adrenoceptor blocking agents) rather than those that reduce peripheral resistance (vasodilators).

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Reprint requests to L.S. please.

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