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Nicardipine in models of myocardial infarction

B. J. ALPS, C. CALDER. & A. WILSON
Syntex Research Centre, Heriot-Watt University, Edinburgh EH14 4AS, UK

1 In a dog model of partial myocardial ischaemia, superimposed ST segment elevations in epicardial ECGs were inhibited by nicardipine over a cumulative i.v. dose range of 1–20 $\mu g\ kg^{-1}$.

2 Over the cumulative i.v. dose range of 0.5–166.5 $\mu g\ kg^{-1}$, nicardipine had little overall effect on gross cardiac conduction, at spontaneous heart rate.

3 Dogs that received oral 1–2 $mg\ kg^{-1}$ nicardipine daily for 16 weeks and then survived 1 week occlusion of the left anterior descending coronary artery (LAD) developed a superior coronary collateral circulation compared with untreated animals.

4 Nicardipine given by three different dosing schedules to baboons markedly limited myocardial infarction over a 6 h period of LAD occlusion.

5 Compared with a group of completely untreated dogs, there was protection of the myocardium in the animals given nicardipine that survived 3 months occlusion of the LAD.

Keywords myocardial infarct animal models nicardipine pharmacology

Introduction

Nicardipine belongs to the group of drugs known as calcium entry blockers, which collectively have been shown to reduce cardiac work, modify cellular ion loss, reduce substrate utilisation, dilate systemic arteries (reduce afterload), dilate systemic veins (reduce preload) and dilate coronary arteries (improve myocardial blood flow). Whether or not one drug in this group can favourably influence all or any aspects of ischaemic heart disease will depend on how the various pharmacological properties in an individual agent's profile interact *in vivo*, with or without the intervening effect of metabolism.

Our interest in nicardipine initially centered on its potent vasodilator activity, particularly its effect on coronary arteries (Takenaka *et al.*, 1976) where coronary blood flow was increased by 8–130% with i.v. doses from 1 to 10 $\mu g\ kg^{-1}$ in normal dogs. It was reasoned that if this property was an important part of the drug's usefulness in treating ischaemic heart disease, it should be examined in appropriate animals over this dose range.

Early unpublished studies were conducted with nicardipine in a dog model of electrical stress pacing-induced ischaemia, based on the work of Szekeres *et al.* (1976), and a dog model of myocardial infarction where animals survived a 1 week occlusion of the left anterior descending coronary artery (LAD). Another study examined the effect of nicardipine on gross cardiac conduction to determine whether this feature would be compromised at i.v. doses affording protection in ischaemic myocardium.

Using information obtained from these various studies, recent experiments were carried out in baboons to examine the protective effect of nicardipine in limiting acute infarct size (Alps *et al.*, 1983*a*) and then in dogs to determine whether combined pre-infarct and post-infarct treatment could favourably influence the quality of infarct healing and limit disposition of scar tissue (Alps *et al.*, 1983*b*).

The purpose of this paper is to review the main findings from these studies.

Methods

General

All dogs were premedicated with 0.2 mg kg^{-1} acetylpromazine i.m. General anaesthesia was induced (30 mg kg^{-1} i.v.) and maintained with sodium pentobarbitone. In baboons the pentobarbitone dose was about 8 mg kg^{-1} i.v. since phencyclidine HCl at 1 mg kg^{-1} i.m. was used for sedation. General haemodynamic function and electrocardiograms (ECGs) were recorded on an 804 (Siemens Ltd) Mingograf inkspray oscillograph system. Animals with a thoracotomy were ventilated with room air by a Harvard respiration pump.

In all studies on myocardial infarction a prophylactic i.v. dose (baboons 1.0 ml, dogs 2.0 ml) of lignocaine HCl (Xylocaine, 2% (w/v) aqueous solution: 21.2 mg ml^{-1}, Astra Pharmaceuticals Ltd) was given to offset development of early ventricular dysrhythmia following LAD ligation. Further doses (baboons 0.5–1.0 ml, dogs 1.0–2.0 ml) were used as necessary during the course of any experiment.

Preparation of test drugs

In procedures (i), (ii), and (iv) below, drugs were made up in 1% hydroxy-propyl methyl cellulose in 0.9% (w/v) sodium chloride solution. In procedures (iii) and (v), 0.9% (w/v) saline alone was used as the vehicle. The doses of nicardipine HCl, verapamil HCl, and nifedipine were calculated in terms of base substance to present 10 $\mu g\ kg^{-1}$ of each drug as 2.09, 2.19 or 2.89×10^{-5} $\mu mol\ kg^{-1}$ respectively. Oral doses of nicardipine were administered by capsules containing 10 mg active constituent.

Histological examination of tissues

Myocardial tissue was embedded in paraffin, sectioned at 5–8 μm and stained with haematoxylin and eosin.

Statistical analysis

Results for group mean differences were evaluated for statistical significance using the Student's *t*-test for unpaired samples with $P < 0.05$ as the criterion for significance. Group absolute or percentage values are expressed as mean $\pm$ s.e. mean.

Procedure

(i) *Electrical stress pacing-induced ST segment changes in partially ischaemic dog heart* Three groups of three to six adult beagles of either sex (11.0–16.0 kg) were anaesthetised and ventilated artificially; a thoracotomy was performed through the left lateral fifth intercostal space of each animal. The technique used was adapted from that described by Szekeres *et al.* (1976) and Csik *et al.* (1983), using only an electrophysiological indicator at the onset of the post-ischaemic recovery phase to represent oxygen/metabolic debt. Instead of inducing partial obstruction to flow through the mid-region of the left anterior descending artery (LAD), by means of constricting a flexible cannula system linking autoperfused blood to a femoral or carotid artery, a partially snared ligature was applied. Sufficient obstruction to flow was caused to allow a 2 min period of left atrial electrical pacing of the heart at 50–70 beats min^{-1} above its resting rate to induce ischaemic ST segment changes in epicardial unipolar electrograms recorded from up to eight electrodes sutured to the surface of the left ventricle (LV) within the underperfused vascular area. With partial occlusion of the LAD continuously sustained throughout the experiment, these ischaemic changes were reversible when the heart was allowed to return to spontaneous beating. After four to five repeated cycles (2 min pacing, 5 min initial rest, drug administration with a further 5 min rest) of ischaemic insult, each heart was conditioned to give reproducible control traces. Tests were then made to determine whether pre-test treatment with nicardipine, verapamil, or nifedipine could inhibit stress pacing-induced ST segment changes recorded for one heart beat within the first ten cycles of cardiac recovery.

(ii) *Measurement of gross cardiac conduction in dogs* In three anaesthetised adult male beagles (12.9–20.5 kg), with a mid-sternal thoracotomy, paired bipolar epicardial electrodes were sutured to the right atrium (RA) and right ventricle (RV) to permit the timing of arrival of impulses, spontaneous or electrically induced at different pacing frequencies, from the atrium to the ventricle (atrioventricular [A-V] conduction time) and between points either in the atrium (intra-atrial, [I-A]) or in the ventricle (intra-ventricular [I-V]) (Baum *et al.*, 1971). The electrograms were recorded at paper speeds calibrated to run accurately at 1000 mm s^{-1} (1 mm = 1 ms). When

required, direct electrical pacing of the atria or ventricle was effected by stimulating electrodes sutured to the right atrial appendage and to the apex of the RV using impulses from a Grass S-88 physiological stimulator set at 8 V, 1 ms duration with variable cycle lengths 320, 280, 250, 230 ms giving rate equivalents of 188, 214, 240, and 261 beats min^{-1} respectively.

Monitoring of abdominal aortic blood pressure was obtained by a catheter introduced into the left femoral artery. Doses of nicardipine, given i.v. were increased cumulatively under repeated test conditions from 0.5–666.5 $\mu g\ kg^{-1}$ in all three dogs and further to 1666.5 $\mu g\ kg^{-1}$ in one of the dogs.

(iii) *One-week LAD occlusion in dogs* The method and measurements have been described for a similar study (as for procedure (v), Alps *et al.*, 1983*b*). This study was conducted using 12 adult male beagle dogs (15.4–20.6 kg). Nicardipine was administered daily to six animals for 16 weeks at an oral dose of 1–2 mg kg^{-1}. The other six dogs served as a control group receiving placebo treatment. Electrocardiograms were recorded for each dog before treatment, immediately before surgery for LAD occlusion, and 1 week later. The external diameter of the LAD was measured prior to ligation using adjustable callipers. Oral treatment, as above for both groups, was continued daily during the week of post-ligation survival.

At 1 week post-LAD ligation, invasive measurement of the general haemodynamic function was made in all surviving dogs. The external diameter of the LAD was measured prior to a lethal overdose of sodium pentobarbitone. The heart was removed, washed, photographed and placed overnight in a refrigerator at 4°C. On the following day the stub of the LAD proximal to the ligature and the circumflex artery were cannulated separately. Red-pigmented vinylite (10% in acetone) was injected into the circumflex artery at a pressure of 100 mm Hg using a Boreas vacuum compressor. The upper portion of the LAD was injected similarly with the basic white-pigmented vinylite (8% in acetone). The heart was rephotographed, and detailed drawings were made of the epicardial distribution of collateral vessels. The surface-area presentation of resolving necrotic tissue within the superficial area of the occluded LAD supply was traced directly and planimetered by the method of counting squares (mm^2). Then the heart was immersed in concentrated hydrochloric acid and placed in a fume cupboard to macerate over a period of 1–2 weeks. The coronary artery casts were cleaned and photographed, and a micrometer screw gauge was used to measure the diameter of the coronary anastomoses; the number of prominent junctions was noted.

(iv) *Acute 6h LAD ligation in baboons* A detailed account of the method has been reported (Alps *et al.*, 1983*a*). The following three different dosing schedules with nicardipine were used to treat three groups (groups 2, 3, 7) of six anaesthetised animals (6.0–11.0 kg) of either sex, with thoracotomies, subjected to a 6 h occlusion of the LAD. Group 2 received pre-ligation treatment with 10 $\mu g\ kg^{-1}$ nicardipine i.v. and post-ligation maintenance at 2 $\mu g\ kg^{-1}$ every 15 min for 6 h. Group 3 received post-ligation treatment (10 $\mu g\ kg^{-1}$ i.v.) delayed for 1 h followed by maintenance (2 $\mu g\ kg^{-1}$ every 15 min for 5 h). Group 7 received 6 months pre-ligation treatment (1–2 mg kg^{-1} orally once daily).

Two other groups (1 and 6) of six untreated animals were used to study a standard 6 h infarct; in group 6, radiopaque barium in gelatin was injected post-mortem into the coronary arteries to allow X-ray examination of the anatomical vascular area at risk (VARP) in the sectioned hearts. A similar X-ray procedure was carried out for hearts from group 7. Comparative observations on two further groups (4 and 5) of six baboons treated after ligation with nifedipine or verapamil respectively, as in group 3, are referred to in the discussion.

Before fixing in 10% buffered formal saline, all hearts were sliced into ten transverse sections, approximately 5–6 mm thick, and incubated with nitro blue tetrazolium (NBT) to stain non-specific cellular dehydrogenases present in metabolically viable myocardium. The heart slices were traced on to acetate sheet photocopies of 1 mm^2 graph paper to delineate the anatomical landmarks of the free LV and RV walls and the septum, to allow planimetry of infarcted areas. The VARP was determined similarly for those hearts injected with barium gel.

(v) *Three months LAD occlusion in dogs* A detailed account of the method has been reported (Alps *et al.*, 1983*b*). Three groups (X, Y, Z) of 12–14 adult male beagles (13.0–20.0 kg) subjected to thoracotomy and LAD ligation, with survival to 3 months, were treated as follows:

Group X received 1-month oral pre-ligation treatment (1–2 mg kg^{-1} daily); i.v. treatment

commencing 1 h post-ligation (10 $\mu g\ kg^{-1}$ followed by 2 $\mu g\ kg^{-1}$ every 15 min for 5 h); oral treatment post recovery (1–2 mg kg^{-1} daily) for 3 months. Group Y received 1 month oral placebo; i.v. saline vehicle every 15 min for 5 h commencing 1 h post-ligation; oral placebo for months. Group Z received 1 month oral placebo as in group Y; i.v. and oral drug treatment post-ligation as for group X.

For comparative purposes, measurements on internal cardiac anatomy also were available from 11 normal dog hearts (designated group C).

Invasive recording of general haemodynamic function was made before LAD ligation and the monitoring was continued throughout surgery until repair of the thoracotomy was complete, and was repeated at 3 months during the terminal procedure. Before administration of a lethal dose of sodium pentobarbitone, 60 ml of a 5% solution of triphenyl tetrazolium chloride (TTC) was injected i.v. to delineate scar tissue in the heart. As with NBT, TTC stains metabolically viable tissue containing nonspecific cellular dehydrogenases.

Before fixing the tissue in 10% buffered normal saline, radiopaque barium in gelatin was injected into the coronary arteries and the ventricles were packed with wads of cotton wool to preserve lumen shapes. X-rays were taken of the whole hearts in antero-posterior and lateral planes. Each heart was sliced into 15 transverse sections, each about 3–5 mm thick. Sections 2–15 were X-rayed and traced on to clear acetate sheets with the boundaries of the scar tissue clearly outlined.

In sections 2–15 of the LV, measurements were made of the thickness of the anterior wall at its thinnest region (B) excluding the trabeculae carnii, the two heads of the anterior papillary muscle (A_1, A_2), the posterior papillary muscle (C), and the posterior wall (D). In each section indices also were determined for (*a*) LV wall thinning (B/D), (*b*) LV dilatation (ratio of endocardial length of infarcted anterior LV segment to endocardial length of noninfarcted posterior segment), (*c*) transmural scarring (ratio of width of scar in thinnest region B to thickness of anterior wall B), (*d*) circumferential scarring (ratio of intramural length of the scar to the intramural length of the anterior LV segment containing the scar).

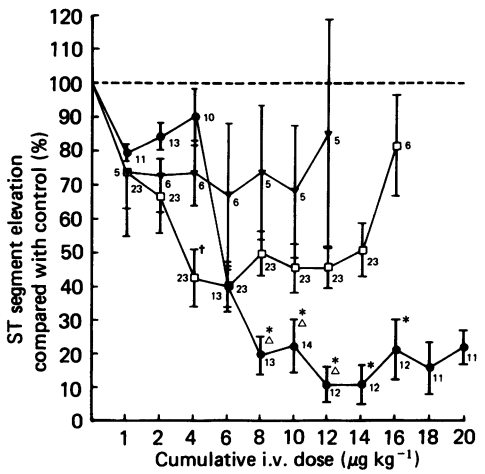

Figure 1 Effect of nicardipine (●, six dogs), nifedipine (□, four dogs) and verapamil (▼, three dogs) in decreasing the ST segment elevation induced by electrical pacing of myocardium rendered ischaemic by partial occlusion of the left anterior descending coronary artery. Each point represents the mean ± s.e. mean. Figures accompanying data points indicate total numbers of electrodes used for each mean value. * $P < 0.05$ for nicardipine compared with nifedipine, △ $P < 0.05$ for nicardipine compared with verapamil, † $P < 0.05$ for nifedipine compared with nicardipine.

Results

The effect of nicardipine, nifedipine, and verapamil in decreasing ST segment elevation induced by electrical pacing of myocardium rendered ischaemic by partial occlusion of the LAD

The results obtained for the three drugs are shown in Figure 1. The total number of measurable electrode recordings obtained for each group at each dose level is indicated. Only levels showing a control response greater than 2 mV were chosen for inclusion. Electrocardiograms from a representative nicardipine experiment are shown in Figure 2 to illustrate how the ST segment elevation induced by electrical pacing of ischaemic myocardium was reduced by increasing i.v. doses of the drug.

It can be seen that nicardipine offered slight protection in the dog angina model at low doses of 1–4 $\mu g\ kg^{-1}$ i.v., increasing markedly to reach a peak of activity at cumulative 12–14 $\mu g\ kg^{-1}$, then decreasing slightly.

Nifedipine offered greater protection than nicardipine at 1–4 $\mu g\ kg^{-1}$, and peak activity was attained at 4–6 $\mu g\ kg^{-1}$. This degree of protection persisted overall to a cumulative dose of

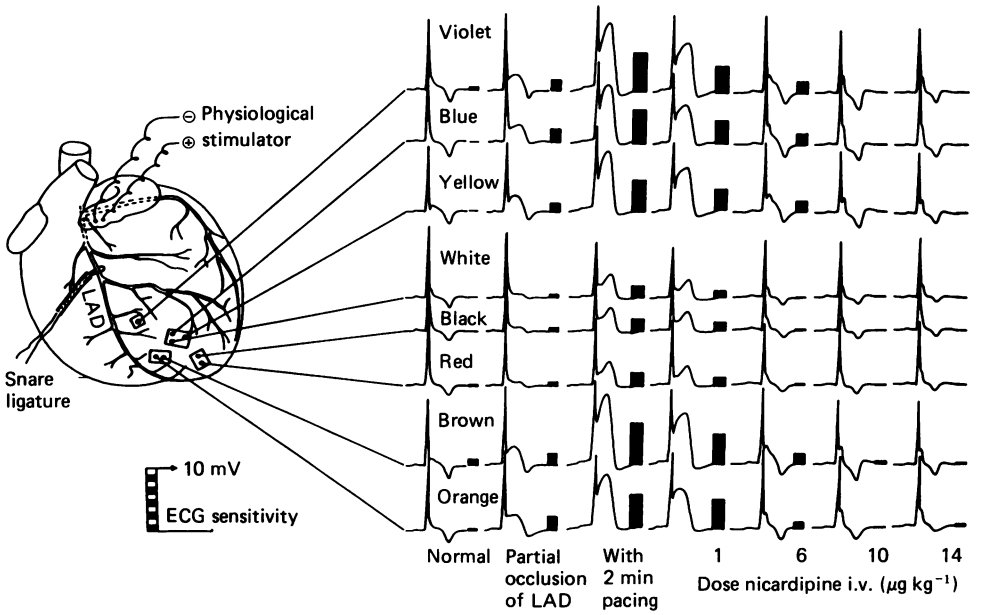

Figure 2 Diagrammatical representation of the epicardial electrode placements over an area of myocardium in the dog heart rendered ischaemic by partial occlusion of the left anterior descending coronary artery (LAD). Increasing, cumulative, i.v. doses of nicardipine progressively reduced the elevations in the ST segment (black bars) induced by electrical stress pacing.

12–14 $\mu g\ kg^{-1}$ before lessening. However, the peak activity of nifedipine was significantly less than that attained with the higher doses of nicardipine.

Verapamil behaved similarly to nifedipine at doses of 1–2 $\mu g\ kg^{-1}$, at which level peak activity was attained. As there was great variability in the results obtained from the few experiments involved and as the high standard errors incurred preclude any sensible analysis, we can only state that activity was detectable.

Effect of nicardipine on gross cardiac conduction in dogs

Spontaneous heart rate Synchronously recorded ECG traces from one of the three dogs studied at spontaneous heart rate are shown in Figure 3. The effects of cumulative i.v. doses of nicardipine from 0.5 to 666.5 $\mu g\ kg^{-1}$ on I-A, I-V and A-V conduction in these animals are shown in Table 1 together with observations on heart rate (spontaneous cycle length) and blood pressure.

Overall, cumulative doses up to 166.5 $\mu g\ kg^{-1}$ had little effect on I-A conduction. A small increase from baseline was observed in two of the three dogs at cumulative 666.5 $\mu g\ kg^{-1}$, and in one of these animals a further increase occurred at 1666.5 $\mu g\ kg^{-1}$. I-V conduction was not affected over the whole dose range in any animal.

A-V conduction was not appreciably affected over the cumulative i.v. dose range 0.5–66.5 $\mu g\ kg^{-1}$, although a slight tachycardia occurred in response to a fall in blood pressure. At higher doses, lengthening of A-V conduction reflected bradycardia (increased cycle length) with further falls in blood pressure. A-V dissociation occurred in one dog at cumulative 666.5 $\mu g\ kg^{-1}$, but not in the one animal receiving 1666.5 $\mu g\ kg^{-1}$.

Electrical pacing The results from one of the dogs are shown in Figure 4. Neither I-A nor I-V conduction was affected in the three dogs at any pacing cycle length (320–230 ms) over the whole dose range 0.5 to cumulative 666.5 $\mu g\ kg^{-1}$. In the absence of drug, A-V conduction increased physiologically with increase in pacing rate (decreased cycle length) in all dogs as a compensatory inhibition. Small increases or decreases occurred in A–V conduction in the different dogs over the dose range 0.5 to cumulative 66.5 $\mu g\ kg^{-1}$. The dog featured in Figure 4 showed 2:1 A-V heart block at the cumulative 166.5 $\mu g\ kg^{-1}$ i.v. dose of nicardipine, when its heart rate was driven above 188

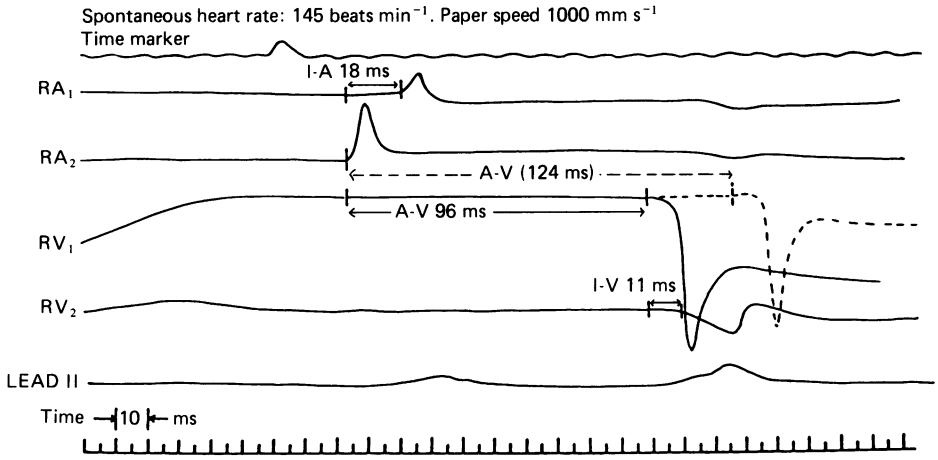

Figure 3 Representative right atrial (RA_1, RA_2) and ventricular (RV_1, RV_2) epicardial electrograms recorded at spontaneous heart rate from a dog before (continuous lines) and after (interrupted lines) nicardipine treatment at cumulative 166.5 $\mu g\ kg^{-1}$ i.v. At this dose level intra-atrial (I-A) and intraventricular (I-V) conduction times were not altered. Atrioventricular (A-V) conduction was lengthened (96–124 ms) and heart rate decreased (145–121 beats min^{-1}). Lead II also recorded.

Table 1 Effect of nicardipine on intra-atrial, intra-ventricular and atrioventricular conduction (mean ± s.e. mean) in the dog heart ($n = 3$) at spontaneous heart rate

Cumulative i.v. dose ($\mu g\ kg^{-1}$)	*Conduction time (ms)*			*Spontaneous cycle length (ms)*	*Heart rate (beats min^{-1})*	*Abdominal aortic blood pressure (mmHg)*	
	I-A	*I-V*	*A-V*			*Systolic*	*Diastolic*
Baseline (no drug)	14.0±1.5	9.3±0.9	99.2±2.6	418±30	145±11.0	158±6.0	97±8.7
0.5	14.2±1.4	9.3±0.9	99.7±3.0	419±31	145±11.0	160±6.0	97±8.0
1.5	14.0±1.5	8.8±0.4	99.3±2.7	414±25	146±9.0	153±11.0	94±10.0
6.5	13.3±0.6	8.8±0.6	99.2±2.9	411±22	147±7.7	147±19.3	80±12.6
16.5	13.3±0.9	9.0±0.5	98.0±2.6	401±30	151±10.5	148±16.4	70±5.8
66.5	13.3±1.3	9.2±0.4	103.0±3.2	481±77	131±18.1	117±26.4	56±7.8
166.5	14.7±0.9	9.2±0.4	112.0±8.0	511±108	121±23.0	102±21.3	38±13.9
666.5	16.0±2.1	9.2±0.4	142.5±31.5 ($n = 2$)	581±81	107±13.0	77±8.3	33±11.1

beats min^{-1}, and episodes of A-V dissociation at 66.5 $\mu g\ kg^{-1}$ at a heart rate of 261 beats min^{-1}; this did not occur at cumulative 666.5 $\mu g\ kg^{-1}$ in the other two animals. One of the latter dogs did show A-V dissociation at all pacing frequencies when cumulative 1666.5 $\mu g\ kg^{-1}$ was administered.

Effect of nicardipine in dogs surviving 1-week occlusion of the LAD

Ligation of the LAD and autopsy of the heart Immediately on ligation in five of six drug-treated dogs, but in none of the controls, there was retrograde filling of the portion of the LAD distal to the ligature, from clearly visible surface anastomoses with branches of the left circumflex artery (LCA). The LAD itself was significantly ($P < 0.01$) larger in external diameter (3.5±0.13 mm) at pre-ligation than in untreated dogs (2.4±0.15 mm).

The myocardium supplied by the LAD in these dogs remained of good colour, whereas in all control animals the tissue quickly became cyanotic because of vessel collapse. The sixth drug-treated dog, which did not show retrograde

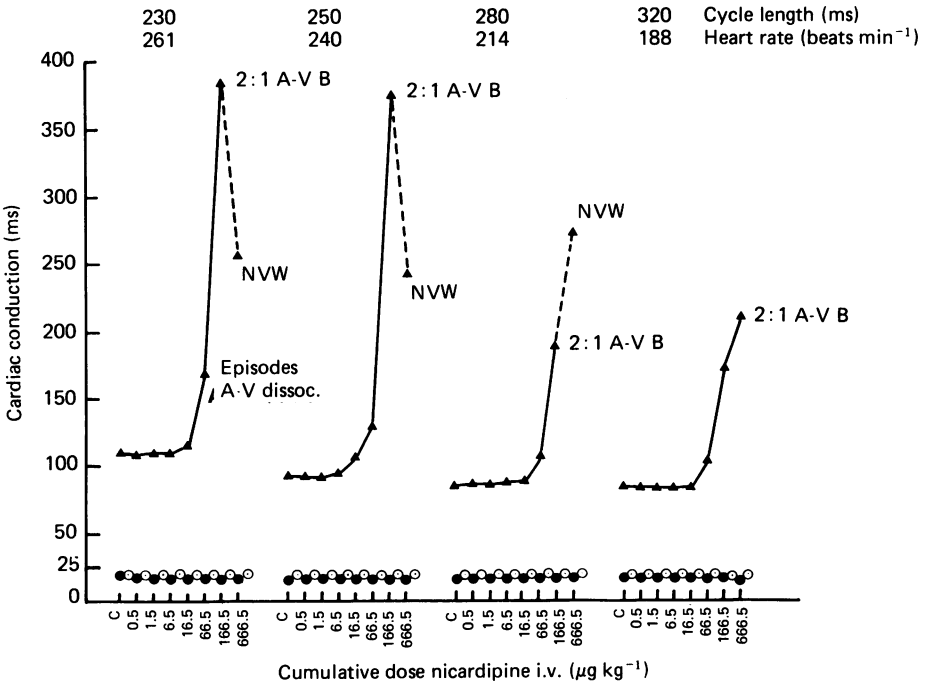

Figure 4 Effect of nicardipine on intra-atrial (●), intraventricular (○) and atrioventricular (▲) conduction and cardiac rhythm in a dog heart at increasing frequencies of electrical pacing. The points for intraventricular data have been slightly displaced rightward for convenience. Note occurrence of 2:1 heart block and appearance of new ventricular waveform (NVW) when atrioventricular pathway was blocked.

filling of the LAD, developed ventricular fibrillation (VF) 20 min after ligation. It was not possible to defibrillate this animal using DC shock. VF also occurred in two control dogs within 2–3 min, and one did not respond to electrical defibrillation.

At post-mortem examination 1 week after LAD occlusion, it appeared that in the drug-treated hearts the LAD proximal to the ligature was further increased in external diameter (4.3 ± 0.30 mm, $P < 0.001$) compared with pre-ligation measurement and was larger still ($P < 0.01$) compared with that in control hearts (2.40 ± 0.19 mm). It was also evident that the LAD distal to the ligature was patent and functional in all dogs of both groups, and this artery in the nicardipine-treated hearts (4.3 ± 0.30 mm, $P < 0.001$) was much larger than in control hearts (1.30 ± 0.12 mm).

There was no significant difference between group mean heart weights (control group, 170.1 ± 6.6 g; nicardipine group 169.9 ± 9.7 g) or group mean bodyweights (control group, 17.5 ± 0.7 kg; nicardipine group, 16.8 ± 0.5 kg).

Planimetered areas of necrotic tissue presented at the epicardium in the area of the occluded vascular bed were significantly smaller in nicardipine-treated dogs ($34.7 \pm 9.8\%$, $P < 0.05$–0.02) than in untreated animals ($67.8 \pm 10.5\%$).

Observations on the electrocardiogram and cardiac rhythm Electrocardiograms recorded from left lateral precordial positions in both groups of dogs demonstrated alterations in electrical activity in the LV characteristic of infarction. Traces illustrating these changes for a representative control (*a*) and for a nicardipine pre-ligation treated dog (*b*) are depicted in Figure 5. The net increase in Q/S waveform negativity and decrease in the R wave positivity in leads V_1–LV_4 were measured for each group as shown in Figure 6. A greater, although not significant, Q/S gain and a significant R wave loss ($P < 0.05$) was found in the control dogs compared with drug-treated animals. Using regression analysis in four of five dogs in both groups, good correlation was established between percentage epicardial surface

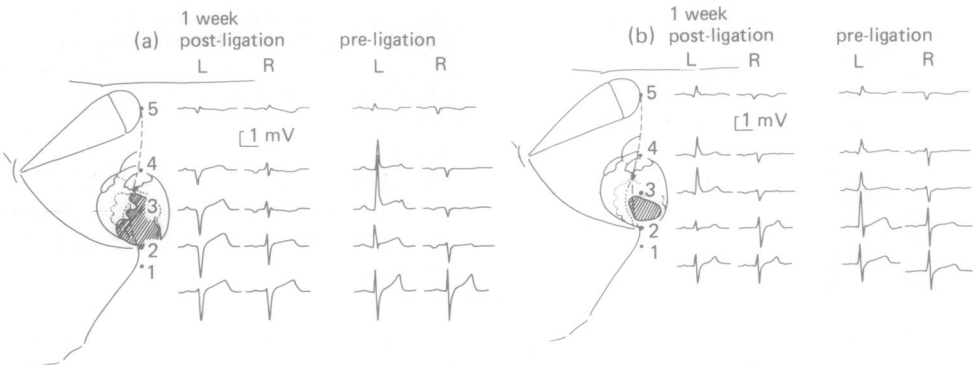

Figure 5 Left (L) and right (R) precordial electrocardiograms recorded from dogs prior to ligation of the left anterior descending coronary artery and again 1 week later. Recording sites, following the vertical caudal edge of the triceps muscle, are related anatomically; 1 (V_1), to midsternum; 2 (L or RV_2), to point of elbow; 3 (L or RV_3), to midway between point of shoulder and elbow; 4 (L or RV_4), to point of shoulder; 5 (L or RV_5), to caudal edge of scapula. The irregular dotted line signifies the ischaemic area and diagonals denote the surface area of fibrosis within. (a) control dog showing complete loss of R wave, development of Q/S wave in left precordials post-ligation, and large area of fibrosis. (b) nicardipine-treated dog showing good retention of R wave, no Q/S wave development and smaller area of fibrosis.

damage and increased Q/S negativity in each dog (control group, $r = 0.992$; nicardipine group, $r = 0.945$).

Ventricular dysrhythmias occurred significantly earlier in control dogs (5.9 ± 2.9 min) than in nicardipine-treated animals (186.1 ± 58.2 min, $P < 0.02$). Multiple premature ventricular systoles of multifocal origin, as the predominant dysrhythmia, appeared within 45 min to 7 h in control dogs which did not develop VF, but not until 5–10 h in nicardipine-treated animals. Whereas these time spans were not significantly different, the duration of more severe ventricular dysrhythmia was longer in control dogs (57.1 ± 5.5 h) compared with nicardipine-treated animals (33.0 ± 2.7 h, $P < 0.01$). Sinus rhythm had returned in all dogs in both groups within 80–130 h after LAD occlusion.

More i.v. lignocaine was used in control dogs (15.1 ± 2.8 mg kg^{-1}) than in nicardipine-treated dogs (8.4 ± 1.4 mg kg^{-1}) during the 12 h following LAD occlusion, but the difference was not significant.

Effect of nicardipine on general haemodynamic function At the time of the terminal procedure 1 week after LAD occlusion, the general haemodynamic variables shown in Table 2 were recorded for nicardipine-treated and control dogs. Measurements in intact animals showed that systolic blood pressure, heart rate, LV contractile force (dP/dt P^{-1} max.) and the derived cardiac effort index were significantly lower in the treated dogs. Left ventricular end diastolic pressure (LVEDP) and aortic diastolic pressure were significantly higher in untreated animals only after thoracotomy.

Vinylite casts of the left anterior descending and circumflex arteries Red vinylite injected selectively into the LCA appeared in the portion of the LAD distal to the ligature. Red vinylite also penetrated the distal LAD in control group hearts, but the vessel and its side branches were very thin. Representative casts from a nicardipine-treated dog (*a*) and a control dog (*b*) are shown in Figure 7. The number of observed surface collateral vessels (200–400 μm diameter) joining branches of the occluded LAD and patent LCA at all levels from the ligature to the apex of the heart was greater (33.6 ± 6.8, $P < 0.02$) in nicardipine-treated dogs compared with untreated animals (10.8 ± 3.1). The hearts from nicardipine-treated dogs also showed a significantly greater number (13.2 ± 2.1, $P < 0.05$) of larger collaterals (500–700 μm diameter) than did those of untreated animals (5.6 ± 2.2), principally linking the distal LAD and LCA at the mid and apical regions of the heart and the distal LAD to the septal branch of the proximal LAD.

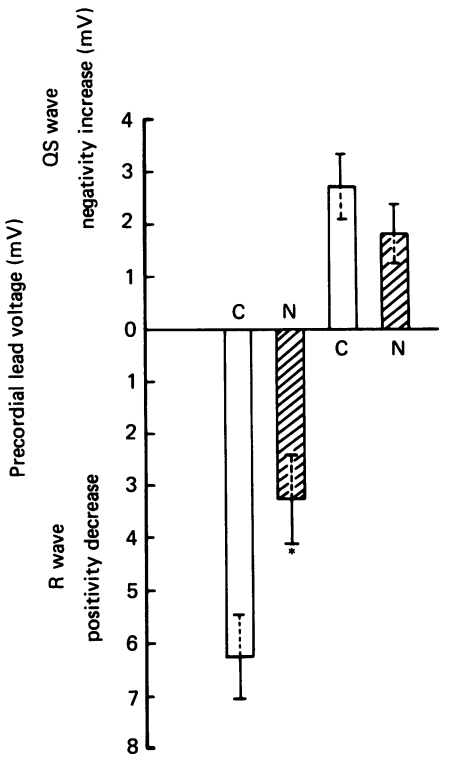

Figure 6 Net losses in R and gains in QS waveform voltages in left precordial electrocardiograms (LV_4, LV_3, LV_2, V_1) in dogs 1 week following high level occlusion of the left anterior descending coronary artery. C, Untreated control group ($n = 5$); N, nicardipine oral-treated (1–2 mg kg^{-1} for 4 months pre-ligation followed by 1–2 mg kg^{-1} daily for 1 week post-ligation) group ($n = 5$). * $P < 0.05$ for nicardipine-treated dogs compared with untreated animals.

The effect of nicardipine on acute myocardial infarction in baboon hearts subjected to 6 h occlusion of the left anterior descending coronary artery

Topographical features of the epicardial and transmural distribution of the LAD and LCA observed in baboon hearts The illustration in Figure 8 depicts the variations encountered in the distribution of the left coronary artery and shows the high level of ligation applied in relation to the particular vascular area supplied by this vessel and its branches. The manner of 'breadloaf' sectioning from cardiac apex to base into ten transverse slices also is shown. The contributions of the various branches of the LAD and LCA to regional myocardial blood supply in the LV and those of the right coronary artery are represented diagrammatically in Figure 9(a). This information has been determined from previous unpublished studies. The dependence of the apical region of the LV myocardium for most of its blood supply from the LAD is very evident. In most hearts (patterns (b) and (c) in Figure 8), a substantial first diagonal branch arose at a high level from the LAD, thus providing a potentially large standardized vascular area at risk to ischaemic damage.

The myocardial lesion in untreated baboon hearts Six-hour ligation of the LAD in hearts from groups 1 and 6 caused complete transmural depletion of nonspecific cellular dehydrogenases at all levels of the heart, affecting predominantly the anterolateral region of the LV wall, a large area of the interventricular septum, and extending into the RV wall, as represented diagrammatically for group 6 in Figure 9(b) (for sectional quantification of the infarct see Alps *et al.*, 1983a). The lesion was particularly large in the apical region of the heart, with wide involvement of the epicardium and endocardium. Of the total available myocardium in groups 1 and 6 (5169 ± 128 mm^2 and 7706 ± 390 mm^2 respectively) the lesion occupied $41.5 \pm 2.3\%$ and $41.2 \pm 4.8\%$

The histological examination of myocardium taken from infarcted tissue failing to stain with NBT revealed changes characteristic of early ischaemic damage (Alps *et al.*, 1983a); there was also a distinct but interdigitated border between such tissue and normal-appearing myocardium taking the stain.

Effect of nicardipine treatment on baboon hearts subjected to 6 h occlusion of the LAD All three nicardipine dosage regimens in groups 2, 3, and 7 markedly limited the spread of the infarct in the LV and RV including the septum. A representative diagram depicting typical distribution of the infarct in treated hearts is presented in Figure 9(c). Although lateral spread of the lesion was limited within the apical third of the heart, the differences in the relatively small areas involved compared with the middle and basal areas of the heart were not statistically significant. The lesion occupied $22.1 \pm 3.4\%$, $21.0 \pm 3.2\%$ and $19.7 \pm 2.0\%$ of the total available myocardium in groups 2, 3, and 7 (respectively 5689 ± 329 mm^2, 6275 ± 337 mm^2, 5336 ± 262 mm^2). These values all were significantly smaller compared with those for groups 1 ($P < 0.01$) and 6 ($P < 0.001$).

Table 2 General haemodynamic measurements (mean ± s.e. mean) made on anaesthetised dogs 1 week post-LAD occlusion

	Control dogs ($n = 5$)	*Nicardipine pre-LAD ligation-treated dogs* (n = 5)
Left ventricular systolic pressure (mmHg)	153.0 ± 13.4	109.5 ± 6.1**
Left ventricular end diastolic pressure (mmHg)		
Aortic systolic pressure (mmHg)	3.5 ± 0.6	3.5 ± 0.6 NS
	7.3 ± 1.7	3.0 ± 0.5*
Pre-thoracotomy	199 ± 10.8	144.2 ± 10.9***
Post-thoracotomy	189 ± 10.7	143.8 ± 9.3**
Aortic diastolic pressure (mmHg)		
Pre-thoracotomy	122.4 ± 8.0	98.8 ± 7.6 NS
Post-thoracotomy	133.6 ± 5.8	105.6 ± 5.5***
LV dP/dt max. (mmHg/s)	3360 ± 721	2210 ± 144 NS
LV dP/dt P^{-1} max. (s^{-1})	204.0 ± 7.5	144.0 ± 9.3***
Heart rate (beats min^{-1})	169.2 ± 12.2	115.2 ± 11.0**
Cardiac effort index (LV syst. pressure × heart rate) (mmHg beats min^{-1})	25992 ± 3280	12739 ± 1625***

Student's t-test where * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$ for nicardipine-treated dogs compared with untreated control animals.

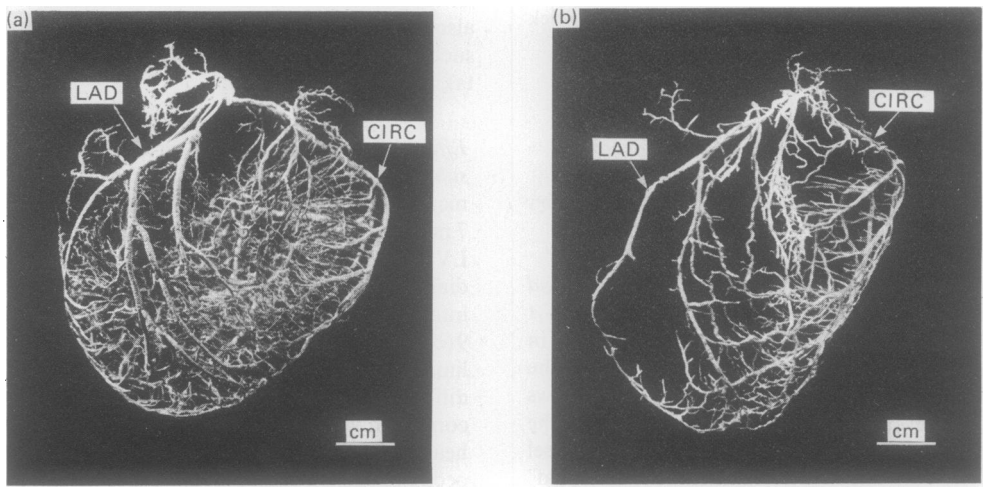

Figure 7 Vinylite casts of the left anterior descending (LAD) and circumflex (CIRC) coronary arteries from dogs surviving 1 week occlusion of the LAD. (*a*) representative oral nicardipine-treated dog (1–2 mg kg^{-1} daily for 4 months followed by 1–2 mg kg^{-1} daily for 1 week post-ligation); (*b*) representative untreated dog. Surviving drug-treated dogs, as exemplified in (*a*) developed a more extensive collateral circulation than untreated dogs.

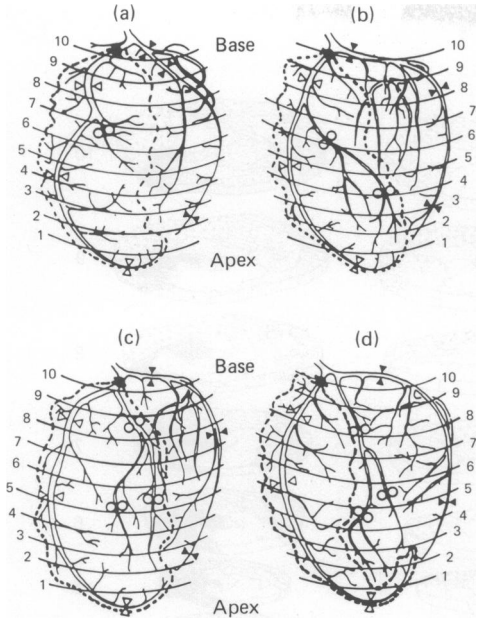

Figure 8 Diagrammatical representations of the variations (a–d) encountered in the distribution of the left coronary artery in baboon hearts in relation to the high level of ligation, the manner of 'breadloaf' sectioning and the vascular area supplied by this vessel. ✎ , site of ligation; ⋈, left anterior descending coronary artery; ∞, first diagonal; ⋈, left circumflex; ---, boundary of occluded vascular bed.

Vascular area at risk and resulting infarction in baboon hearts subjected to 6 h ligation of the LAD Group mean total heart values for VARP and the extent of myocardial infarction in baboons treated orally with nicardipine for 6 months (group 7) compared with untreated control animals (group 6) are shown in Table 3. Comparative sectional values are shown in Table 4. Both for the whole hearts and at each sectional level, the VARP values were not statistically significantly different in the two groups, and they matched the anatomical distributions of the LAD and its first diagonal branch, as previously outlined for the normal baboon heart in Figure 9(a). In these same areas it can be seen that in the hearts of untreated animals, the lesion (white area) extended beyond the boundaries (interrupted lines) of the VARP, whereas in the hearts of animals treated for 6 months, spread of the lesion was contained substantially to within the VARP.

The effect of nicardipine on the healing of myocardial infarcts in the hearts of dogs surviving 3-months' occlusion of the LAD

Pathoanatomical features of the healed infarcted dog heart In Table 5 are shown the mean whole heart values for the five basic thickness measurements of the anterior ventricular wall (B) and posterior wall (D), anterior (A_1) and anterolateral (A_2) papillary muscle heads, and posterior papillary muscle (C), for all hearts determined from slices 2–15 inclusive in each group (as reported by Alps *et al.*, 1983b). As depicted schematically in Figure 10, serial sectioning of the hearts revealed marked changes in gross anatomical landmarks within the LV chamber during the healing of the ischaemic lesion. Thinning of the LV anterior wall and loss of associated papillary muscles occurred in all groups of infarcted hearts, but to a significantly lesser extent in animals treated with nicardipine. Some apical thickening of the posterior LV wall and associated papillary muscle was evident in all infarcted hearts, but overall, the values were not significantly different for the various groups compared with normal hearts. From the derived indices for LV wall thinning and dilatation (Table 6, Alps *et al.*, 1983b), significantly less overall damage was evident in the X- and Z-treated groups compared with the untreated Y group.

The range of changes incurred in the LV geometry of infarcted hearts, taking for example three different levels (12, 7, and 3) below the LAD ligature, are shown diagrammatically in Figure 11. The seven grades of LV pathoanatomical change featuring different lumen shapes can be related to the different groups of dogs at 3 months post-ligation, where grade 1 represents normal hearts from Group C, grades 2–4 the X group, grades 3–5 the Z group and grades 5–7 the Y group.

Discrete, well-defined collagenous scars, interspersed with varying amounts of normal myocardium, were observed in all infarcted hearts (Table 7). Whereas the lesion in the placebo-treated Y group was predominantly transmural at the apex and middle third of the ventricle, there were appreciable layers of epicardial and endocardial muscle sandwiching the scars of the drug-treated X and Z groups. Vascular proliferation was extensive in the area of scar tissue; from preliminary observations of slice 3 from all infarcted hearts, this feature was more pronounced in hearts from the X and Z groups than in those of the Y group.

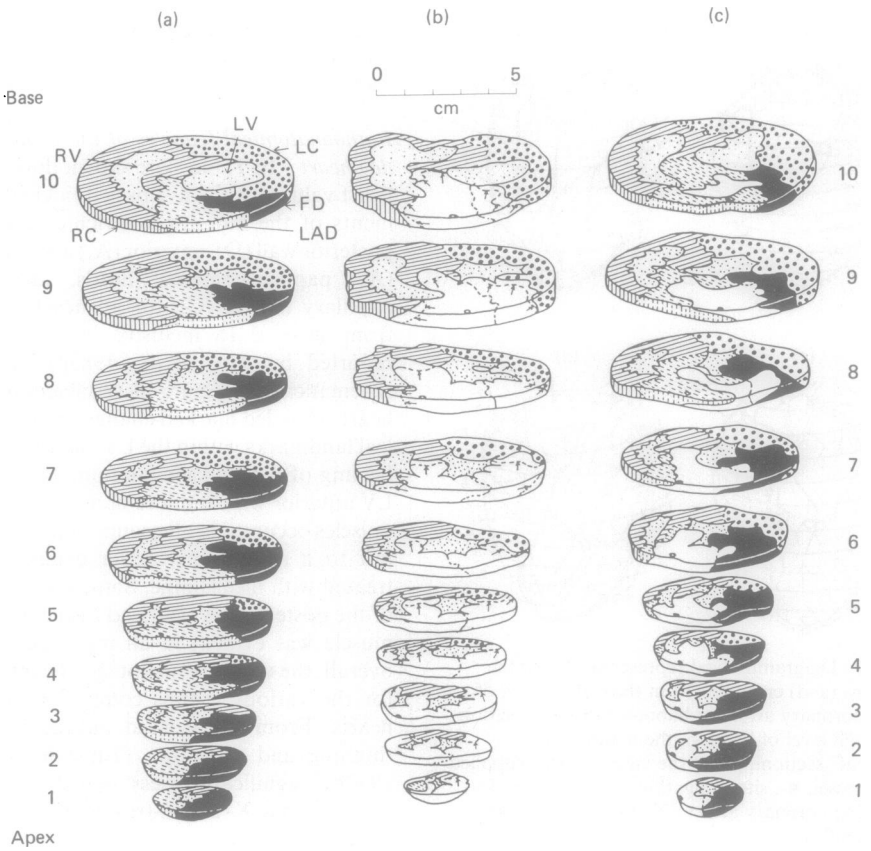

Figure 9 Diagrammatical representation of (a) the sectional contributions of the various branches of the left anterior descending (LAD) coronary artery (▨), its first diagonal (FD) (■), the left circumflex (LC) (⊞), and the right coronary (RC) artery (▤) to regional myocardial blood supply in the left (LV) and right (RV) ventricles of the normal baboon heart (typical *Papio hamadryas*); ▩, lumen. (b) distribution of typical myocardial lesion (□) in the untreated infarcted baboon heart extending beyond the boundaries (---) of the LAD and FD into the areas of the LC and RC. (c) limited distribution of myocardial infarct lesion (□) in nicardipine-treated animals. Lesion confined within boundaries of LAD and LC.

Effect of nicardipine on general haemodynamics in dogs surviving 3-months' occlusion of the LAD

A summary of the essential effects of nicardipine on general haemodynamics in LAD-ligated dogs is presented in Table 8.

Prior to thoracotomy, the diastolic component of carotid artery pressure in the X group was significantly lower than in either the Y or Z groups. The X group systolic component was also lower, but significantly so only when compared with the Y group. The differential in diastolic pressure was maintained between the X and Y groups through the 3 months. A significant hypotensive effect induced by nicardipine in the Z group was observed when pressure was recorded 2 h after ligation and again at 3 months in response to continued dosing with the drug. A similar situation occurred with respect to left ventricular systolic pressure.

In the X group LVEDP was apparently unaffected by pre-ligation treatment with nicardipine prior to LAD occlusion. Following ligation LVEDP did not rise as markedly as in the Y and Z groups. In contrast to the Y group, the pressure fell in the X group 1h after nicardipine treatment. In response to nicardipine treatment the pressure also fell significantly in the Z group to a level equal to that recorded for the X group. The values for LVEDP were normal and not significantly different for the X, Y, and Z groups after 3 months.

The heart rate was slightly but significantly

Table 3 Group mean total heart values for extent of infarction of vascular area at risk post-mortem in baboons

Group	*Group mean areas (mm^2)*			*Group mean % infarction of vascular area at risk*
	Vascular area at risk post-mortem	*Total available myocardium*	*Total infarct*	
Untreated group 6	2923±412 (38.4±5.3%)	7706±390	3140±319 (41.2±4.8%)	110.4±5.7
Nicardipine oral treatment group 7	2298±152 (44.0±4.6%)	5336±262	1020±95 (19.7±2.0%)**	44.0±3.3***

Student's *t*-test where ** $P < 0.01$, *** $P < 0.001$ for nicardipine-treated hearts compared with untreated animals.

Table 4 Effect of nicardipine treatment (group 7) on percentage sectional myocardial vascular area at risk post-mortem in baboon hearts subjected to 6 h occlusion of the LAD compared with untreated hearts (group 6)

	Group (n = 6) *mean* (±*s.e. mean*) *percentage sectional values*			
	Vascular area at risk post-mortem		*Infarction of vascular area at risk post-mortem*	
Section level	*Untreated control animals*	*Nicardipine-treated animals*	*Untreated control animals*	*Nicardipine-treated animals*
10	34.2±7.1	27.1±8.6	108.6±5.7	36.8±11.4***
9	31.4±3.6	32.9±4.4	108.5±7.9	28.2±8.0***
8	38.9±6.7	40.3±5.8	118.6±7.4	53.2±6.2***
7	38.7±4.4	47.1±6.9	114.4±7.6	41.6±10.2***
6	40.4±6.2	50.0±6.3	111.1±6.8	54.0±8.8***
5	40.0±6.3	48.2±5.9	112.6±9.1	55.2±6.7***
4	44.4±8.5	54.7±5.0	109.9±9.4	43.9±7.0***
3	41.8±10.1	51.6±9.1	102.4±12.5	47.2±8.5**
2	55.4±12.5	63.2±11.1	106.3±9.1	37.1±10.5***
1	61.8±11.3	65.9±14.4	99.3±12.3	44.5±14.6*

Student's *t*-test where * $P < 0.02$, ** $P < 0.01$, *** $P < 0.001$ for nicardipine-treated hearts compared with untreated controls.

higher in the X group after 1-month pre-ligation treatment with nicardipine compared with the untreated Y and Z groups. This differential was maintained between the X and Y groups throughout the study, whereas it tended to narrow for the X and Z groups, perhaps because of a slight reflex tachycardia in the Z group in response to nicardipine's hypotensive effect.

The X, Y, and Z groups showed similar degrees of depression and recovery in left ventricular dP/dt max. in response to LAD ligation. There was no obvious effect on this feature prior to LAD ligation in the X group that could be ascribed to nicardipine treatment. Although the difference was not statistically significant, LV dP/dt max. did tend to be lower immediately post-ligation compared with the Y and Z group values. The tendency for the reverse to occur at 3 months was evident when percentage values were compared with those recorded pre-ligation.

Changes in left ventricular contractile force are regarded as being more accurately reflected by changes in dP/dt P^{-1} max. than in dP/dt max. Whereas the changes in this feature were very similar in a time-related manner in the 2 h post-ligation observation period, this was not the case after 3 months. Terminally, superior LV contractile force was generated in the X and Z groups compared with the Y group.

Table 5 Whole heart values (mm) for LV measurements (mean ± s.e. mean) in dog hearts 3 months after infarction compared with normal hearts

Group	*Anterior wall* (*B*)	*Anterior papillary muscle heads* (A_1)	*Anterior papillary muscle heads* (A_2)	*Posterior wall* (*D*)	*Papillary muscle* (*C*)
C ($n = 11$)	10.3 ± 0.4	17.5 ± 0.7	15.8 ± 0.6	10.6 ± 0.4	16.8 ± 0.5
X ($n = 9$)	8.5 ± 0.6** *	13.4 ± 0.9** *	14.0 ± 0.9 *	11.5 ± 0.3	16.8 ± 0.5
Y ($n = 8$)	5.5 ± 0.4**	8.7 ± 0.8**	11.4 ± 0.6**	11.1 ± 0.5	16.8 ± 0.8
Z ($n = 10$)	7.4 ± 0.7** *	12.5 ± 1.2** *	13.4 ± 0.7** *	11.1 ± 0.4	16.7 ± 0.6

Student's *t*-test where * $P < 0.05$ for groups X and Z compared with group Y; ** $P < 0.05$ for groups X, Y and Z compared with group C.

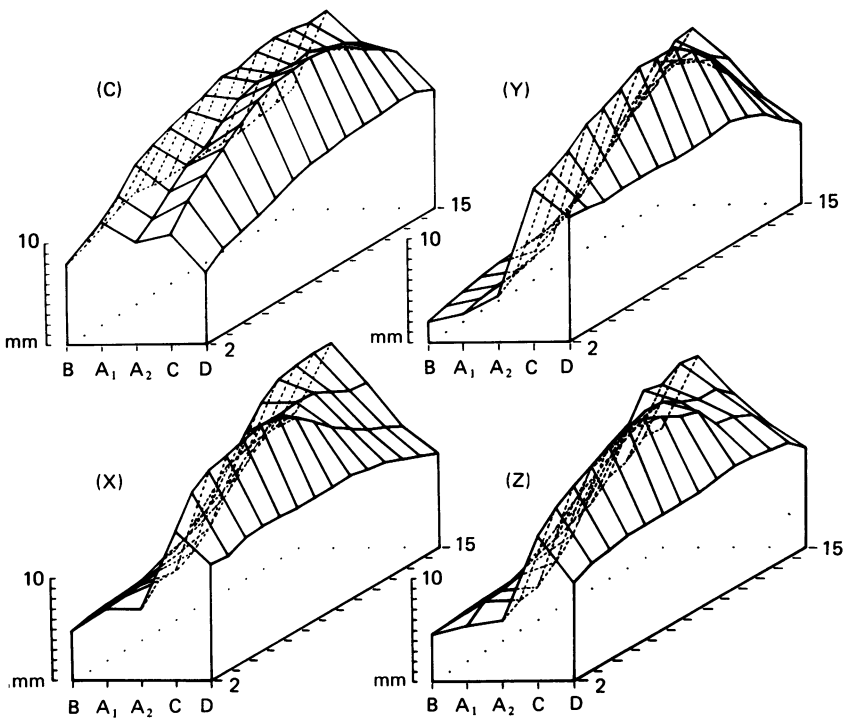

Figure 10 Schematical representation of the five left ventricular internal measurements made sectionally for infarcted (X), (Y), (Z) groups respectively and normal (C) group hearts, featuring the anterior wall, B, the anterior papillary muscle heads A_1 and A_2, the posterior papillary muscle, C, and posterior wall, D. The 'collapse' of the anterior wall and associated papillary muscle heads of the block representing the wholly untreated Y group hearts is marked compared with the treated X and Z group hearts.

Table 6 Whole heart values for indices of LV thinning and dilatation (mean ± s.e. mean) in dog hearts 3 months after infarction compared with normal hearts.

Group	*Index of LV thinning*	*Index of LV dilatation*
C ($n = 11$)	1.00 ± 0.04	0.92 ± 0.03
X ($n = 9$)	0.80 ± 0.05**	1.40 ± 0.10**
Y ($n = 8$)	0.56 ± 0.10**	2.26 ± 0.32**
Z ($n = 10$)	0.68 ± 0.05**	1.34 ± 0.08**

Student's *t*-test where * $P < 0.05$ for groups X and Z compared with group Y; ** $P < 0.05$ for groups X, Y and Z compared with group C.

Table 7 Whole heart values for indices of LV scarring (mean ± s.e. mean) in dog hearts 3 months after infarction

Group	*Transmural index*	*Circumferential index*
X ($n = 9$)	0.34 ± 0.03*	0.36 ± 0.03*
Y ($n = 8$)	0.75 ± 0.05	0.51 ± 0.02
Z ($n = 10$)	0.28 ± 0.05*	0.32 ± 0.04*

Student's *t*-test where * $P < 0.05$ for groups X and Z compared with group Y.

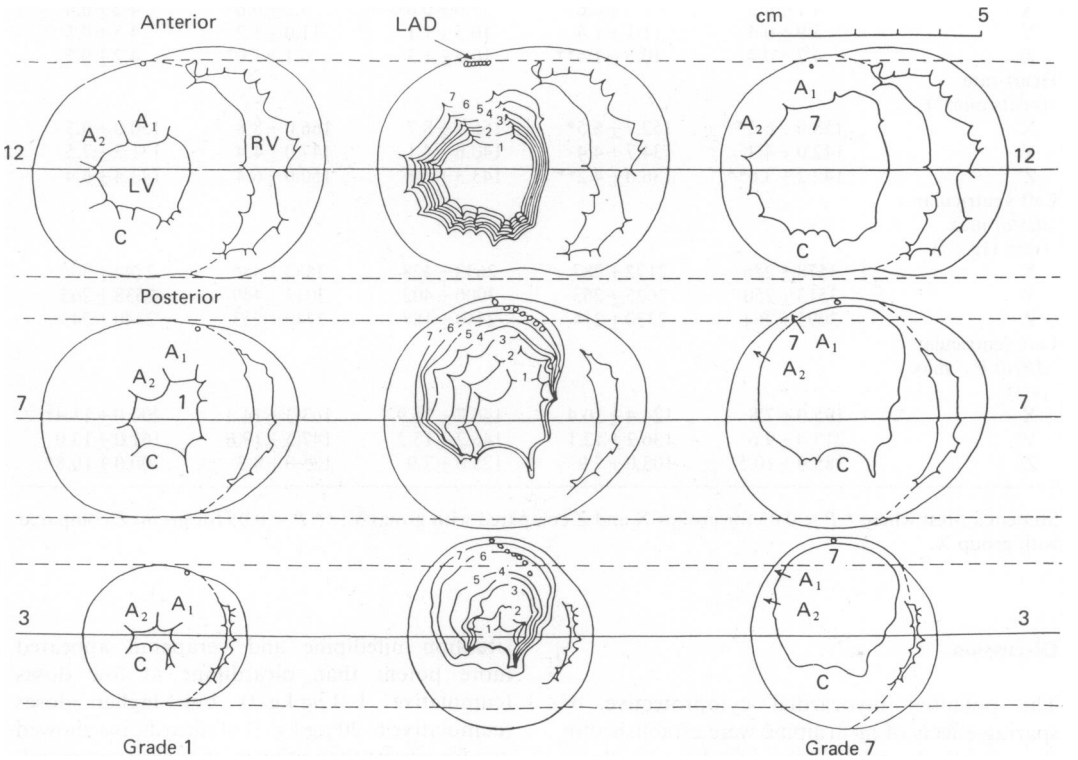

Figure 11 Diagrammatical representation of the possible range of changes affecting the left ventricular (LV) internal anatomy of the dog heart following permanent occlusion of the left anterior descending coronary artery (LAD) for 3 months. RV represents right ventricle. Note leftward shift in position of LAD as LV chamber dimensions change. Section 12 is located just below the site of ligation; 7 represents the mid-ventricular level; 3, the apical region. The seven grades of change represented here are purely arbitrary but relate to the different experimental groups, where 1 = group C, 2–4 = group X, 3–5 = group Z, 5–7 = group Y.

Table 8 General haemodynamic measurements in dogs surviving occlusion of the LAD for 3 months

Variable	*Pre-thoracotomy*	*Post-ligation*			*Terminal*
		30 min	*1 h*	*2 h*	*3 months*
Carotid artery blood pressure (mm Hg)					
Systolic X	103.2±3.9*	—	—	87.9±2.4	85.2±5.0*
Diastolic	55.3±4.8*	—	—	48.4±5.3*	56.6±4.4*
Systolic Y	116.5±4.5	—	—	87.9±5.2	103.4±3.7
Diastolic	83.9±4.3	—	—	71.6±4.6	79.5±3.5
Systolic Z	109±3.2	—	—	80.4±2.4	83.8±2.2*
Diastolic	79.4±3.7**	—	—	54.0±2.9**	54.2±2.5*
Left ventricular systolic pressure (mm Hg)					
X	118.7±3.5*	90.4±6.1	94.1±5.9*	102.2±7.9*	102.7±6.3
Y	133.0±5.1	103.9±2.0	113.6±6.0	124.4±5.5	111.3±4.3
Z	134.9±3.8**	95.3±4.0	105.6±3.5	97.2±4.6*	96.0±4.0*
Left ventricular end diastolic pressure (mm Hg)					
X	4.1±0.5	7.3±0.6*	7.6±0.8*	5.2±0.6*	4.2±0.4
Y	3.9±0.4	11.1±1.4	10.5±1.1	11.0±1.2	4.5±0.5
Z	4.7±0.4	10.5±1.4**	10.4±1.3	6.1±1.0*	4.2±0.5
Heart rate (beats min^{-1})					
X	158.0±6.2*	152.7±6.5*	156.7±8.7	166.0±8.8	150.0±9.5
Y	142.0±4.3	134.7±4.4	140.6±4.1	147.0±4.4	131.3±7.5
Z	142.2±3.6**	136.0±4.2**	143.3±6.6	150.0±6.4	143.4±6.4
Left ventricular dP/dt max. (mm Hg s^{-1})					
X	3578±226	2172±287	2633±428	3433±486	2744±306
Y	3513±250	2625±263	3000±402	3013±449	2538±265
Z	3095±264	2320±214	2440±188	2740±253	2340±243
Left ventricular dP/dt P^{-1} max. (s^{-1})					
X	195.0±7.8	124.4±10.4	146.7±15.9	163.3±14.3	200.0±11.4*
Y	215.0±9.6	136.2±12.1	146.2±15.2	147.5±19.6	161.0±13.0
Z	185.0±10.5	108.0±7.9	125.0±7.9	135.0±8.3	200.0±10.8*

Student's *t*-test where * $P < 0.05$ for groups X and Z compared with group Y; ** $P < 0.05$ for group Z compared with group X.

Discussion

The potential myocardial cytoprotective or sparing effects of nicardipine were established in the electrical stress-pacing studies in dogs. Reduction of the gross electrophysiological evidence of imbalance of oxygen supply and demand in myocardium lacking coronary arterial reserve was demonstrated over the i.v. coronary dilator dose-range known to increase markedly the blood supply to normal hearts. In this situation nifedipine and verapamil appeared more potent than nicardipine at low doses (cumulative 1–4 $\mu g\ kg^{-1}$) but higher doses (cumulative 6–20 $\mu g\ kg^{-1}$) of nicardipine showed greater activity than either nifedipine or verapamil. In this model the activity of nicardipine reached a plateau at about 8–12 $\mu g\ kg^{-1}$, and it cannot be resolved from this particular study whether the apparent beneficial effect involved extracardiac features favouring cardiac pump unloading or direct actions on myocardial metabolism.

It was important to determine whether or not nicardipine had any potentially undesirable effect on gross cardiac conduction at i.v. doses showing activity in the dog. This was evidently not the case since cumulative i.v. doses from 0.5 to 166.5 $\mu g\ kg^{-1}$ nicardipine had virtually no effect on I-A or I-V conduction and very little effect on A-V conduction at spontaneous heart rate. Thus, at i.v. doses well below those that did increase A-V conduction, nicardipine afforded protection in the dog.

Even at fast pacing frequencies (up to 240 beats min^{-1}), A-V conduction was not greatly increased following doses up to cumulative 66.5 $\mu g\ kg^{-1}$. It is interesting that Nakaya & Kanno (1982) found an i.v. dose of 30 $\mu g\ kg^{-1}$ nicardipine to be a potent coronary vasodilator in dogs, increasing regional myocardial blood flow (RMBF) and not reducing the conduction delay induced by myocardial ischaemia. However, verapamil at 300 $\mu g\ kg^{-1}$, which also caused coronary artery dilatation and increased RMBF, but to a lesser extent than nicardipine, markedly improved conduction delay. A dose of nicardipine 300 $\mu g\ kg^{-1}$ did improve myocardial conduction delay and, like verapamil at the same dose level, increased A-V conduction. Nakaya *et al.* (1981) had previously carried out studies on nifedipine and showed that it acts similarly to nicardipine.

Calcium entry blockers should enhance the functional size of the coronary collateral vessels, limit the size of infarcts, and enhance the chances of survival (Schmier *et al.*, 1975; Kanazawa, 1975) in both humans and dogs suffering from severe myocardial ischaemia. Whether or not chronic administration of coronary dilators can influence the proliferation or development of coronary collateral arteries has remained highly controversial ever since such effects were suggested in dogs for dipyridamole (Meesmann *et al.*, 1964; Meesmann & Bachman, 1966), lidoflazine (Schaper *et al.*, 1965, 1969), and nifedipine (Schmier *et al.*, 1975; Kanazawa, 1975).

The abundance of coronary arteries in the epicardium of the normal dog heart, and the propensity for this species to develop such vessels may facilitate the study of calcium entry blockers in experimental ischaemic heart disease. This was the view that prompted the other studies described here, and the results obtained from the dog influenced the selection and scheduling of the i.v. doses employed in both dogs and baboons. In those studies where nicardipine was administered orally the dose level of 1–2 mg kg^{-1} selected for chronic administration was based for comparative purposes on that used for nifedipine in similar studies by Schmier *et al.* (1975) and Kanazawa (1975).

It is well established that most of the coronary collateral anastomoses in the normal canine heart lie subepicardially compared with a predominant subendocardial location in the human and pig heart (Schaper, 1971). The location in the dog heart of the collateral vessels on the flat epicardial surface makes it possible to count and measure their size.

The vinylite casts from the nicardipine-treated dog hearts taken 1 week after LAD occlusion suggested the existence of a well-developed collateral circulation that was more extensive than that of untreated dogs. This finding was similar to that observed for nifedipine by Schmier *et al.* (1975) and Kanazawa (1975). The superior development, or perhaps compensation, of the collateral circulation associated with nicardipine treatment was believed to be related to the preservation of myocardium in the acutely ischaemic area and to its electrocardiographic integrity, as evidenced by the smaller loss in precordial ECG R waves and generation of smaller Q/S waves.

According to Reid *et al.* (1971), ECG mapping techniques in patients allow the natural history of acute myocardial infarction to be followed. Similar observations have been made epicardially in dogs subjected to acute coronary artery occlusion (Hillis *et al.*, 1976a, b).

The general observations on the use of lignocaine to offset development of ventricular dysrhythmia in dogs treated pre-ligation with nicardipine for 4 months suggested that nicardipine could offer protection lasting several hours after LAD ligation. This was also a feature noted in baboons with ligation for 6 h, but only in animals previously treated for 6 months prior to LAD ligation. Nicardipine injected i.v. immediately pre-ligation or delayed for 1 h was not protective. Observations made in the X group dogs treated for 1 month prior to LAD ligation, but not reported here in detail, showed that such treated dogs were not apparently protected to any extent that differed from untreated dogs.

At 1 week post-LAD occlusion there was no evidence of general haemodynamic dysfunction in the nicardipine-treated dogs. In fact, the measurements made were representative of those reported for normal dogs (Ettinger & Suter, 1970). This feature is of great importance when related later in this discussion to the terminal

findings in dogs surviving 3 months' occlusion of the LAD, especially in view of the abnormal haemodynamic function recorded for untreated dogs surviving 1 week LAD occlusion.

The mechanical advantage imparted to the heart by drugs capable of limiting the extent of cardiac cell necrosis following myocardial infarction is obvious. However, the methodological limitations inherent in many studies which are reported to show such protective action for various drugs have been pointed out by Chambers *et al.* (1983).

Most short-term studies have failed to distinguish between a sustained reduction versus a delay of injury (Hearse & Yellon, 1981). A major problem here would appear to be one of establishing a model of standard arterial occlusion with minimal variation in the degree of injury in a small group of animals. In order to overcome some of these problems, baboons, which anatomically appear to have a similar coronary collateral circulation to the human, were used (Alps *et al.*, 1983a). This model was considered to provide a good standard acute lesion for a small group of animals.

It is known that the extent of total infarction of the left ventricle correlates directly with the duration of ligation. At 6 h the lesion reaches a maximum as a percentage of the left ventricular volume (Barker *et al.*, 1980). According to Fishbein *et al.* (1981), the histochemical staining technique using tetrazolium salts to estimate the ultimate size of infarcts has obviated the need for experiments to be prolonged solely so that acute infarcts can be demonstrated histologically. This is true so long as it is realised that the long-term consequences of drug intervention at the acute infarct stage cannot be predicted using this method.

However, using the tetrazolium-staining procedure, observations made on the effect of acute intervention with nicardipine in animals subjected to a 6 h occlusion of the LAD strongly suggested that this drug can at least delay myocardial necrosis; this occurred to the same extent irrespective of whether treatment was pre-ligation (oral or i.v.) or delayed until 1 h post-ligation (i.v.).

In untreated baboons, damage to myocardial tissue failing to react to tetrazolium staining could be identified histologically to the extent that the area of the lesion exceeded the VARP by about 10% ($110.4 \pm 5.7\%$) at 6 h. The boundary between the lesion and tissue of normal appearance was also clearly delineated by the limit of barium gel penetration into the VARP. Whereas the VARP in the group treated with nicardipine for 6 months ($44.0 \pm 4.6\%$) was not significantly different from that in the untreated group ($38.4 \pm 5.3\%$), the lesion in the treated group was confined to only $44.0 \pm 3.3\%$ of VARP.

As to how the drug protected the myocardium, pre-ligation treatment obviously allowed drug access to the myocardium via unoccluded arteries. With regard to post-ligation treatment, the radiographic technique at post-mortem did not reveal the presence of a microcirculation linking the patent and occluded arterial beds, which would be capable of conveying active drug into the ischaemic area at risk. Nevertheless, the presence of such a microcirculation must be a strong possibility, since Lubbe *et al.* (1974), using radiolabelled microspheres in their studies on myocardial blood flow in LAD-ligated baboon hearts, have shown that a significant supply of blood can reach the ligated LAD bed from the LCA system.

It may be that the effect of nicardipine in reducing afterload contributed to the protective action on the baboon heart, in spite of an accompanying tachycardia. The importance of afterload reduction in the management of myocardial infarction is discussed later with regard to the nicardipine treatment of dogs surviving 3 months' occlusion of the LAD.

Treatment with nifedipine and verapamil has produced some reduction in potential infarct size in the baboon infarct model (Alps *et al.*, 1983a), but the results were not statistically significant. This is understandable for verapamil, since the i.v. dose used was relatively low compared with doses cited by other workers as protective (Smith *et al.*, 1975; Reimer *et al.*, 1977). It is more difficult to account for the weak effect of nifedipine, since protection for ischaemic myocardium has been shown, *in vivo* (Henry *et al.*, 1978) and *in vitro* (Henry *et al.*, 1977). Geary *et al.* (1982) have reported, however, that nifedipine had no appreciable activity in their baboon reperfusion infarct model.

The difficult question concerning the consequences of continued drug treatment on infarct resolution and healing was addressed using the dogs surviving 3 months' occlusion of the LAD. Ideally, it would have been more informative to study baboons under these conditions, but this was precluded by the much easier requirements for housing, post-operative management, and handling of good group sizes of dogs. The potential success of acute intervention in treating

myocardial infarction is believed to be related to the onset and duration of the ischaemic process before treatment is commenced (Maroko *et al.*, 1980), and this is a feature that can be controlled under experimental conditions.

There is, however, limited information available about the consequences of prolonging any particular treatment to infarct healing. Expansion of the infarct is a gross change occurring within the first few days of myocardial infarction before resorption of necrotic tissue, and this complicates healing of the infarct. The extent of ventricular wall thinning and cardiac dilatation persisting after infarction depends very much on whether infarct expansion occurs, and any added weakness in the region of the scar adds to the risk of cardiac rupture (Schuster & Bulkley, 1979).

The importance must be emphasised of attempting to control pre-ligation haemodynamic responses, and post-ligation responses of infarction, in order to influence favourably the heart to remain in a normal geometric configuration during its ischaemic crisis and then to heal morphologically with relatively normal topographical relationships. A vital factor must be the reduction in cardiac work by unloading the cardiac pump during its most vulnerable period.

It was clear in the chronic dog study that nicardipine treatment pre-ligation for 1 month significantly modified the responses of the cardiovascular system compared with untreated animals. Systemic arterial vascular unloading occurred, but LV contractility was not changed.

A very important finding during the first hour after LAD ligation was the way in which the LVEDP was not raised to anywhere near the same extent in treated dogs (X group) compared with the animals (Y and Z groups) given placebo before ligation. This effect, demonstrating a pharmacological reduction in venous return, or a lowered preload, also was induced in the Z group when nicardipine treatment, commenced 1 h after LAD ligation. Afterload and preload reductions were maintained in treated animals during the observed period of treatment. In addition, nicardipine's pharmacological properties (Takenaka *et al.*, 1976; Clarke *et al.*, 1983; Eglen *et al.*, 1983) are considered to interact beneficially at the oral and i.v. dose levels used in both the acute baboon (Alps *et al.*, 1983a) and chronic dog models (Alps *et al.*, 1983b).

Apart from the beneficial effects imparted by nicardipine in appropriately balancing vascular unloading effects on the cardiac pump, the preservation of the myocardium in the epicardial and endocardial regions is undoubtedly related to its direct cytoprotective effect, in the manner described for other calcium entry blocking drugs (Nayler, 1980; Grinwald & Nayler, 1981). In this situation, energy-related intracellular metabolic activities are maintained and the integrity of the ultrastructure is preserved where excessive Ca^{2+} influx into ischaemically damaged cells, which have temporarily lost their selective membrane permeability, is inhibited. Thus, nicardipine can allow time for resolution of a greater area of reversibly injured tissue.

All of these effects must be considered in relation to the eventual limited disposition of scar tissue and the minimized ventricular wall thinning and chamber dilatation that occurred in treated animals. From the human autopsy observations reported by Schuster & Bulkley (1979), it is considered that these animal findings closely reflect the haemodynamic events influencing disproportionate dilatation or expansion of the ischaemically-damaged human heart in its markedly weakened state; they also relate to the abnormal general haemodynamic features seen in dogs 1 week after LAD occlusion.

An extensive vascular proliferation was an interesting histological observation associated with the quality of scar formation in nicardipine-treated dogs, particularly those in the X group of the 3 month survival study. This feature reflects the limited observations on apparent collateral artery development in animals treated pre-ligation with nicardipine for 4 months; it is considered sufficiently important to warrant quantitative evaluation of the data.

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