Br. J. Pharmac. (1985), **86,** 181–189

Cardioprotection by the calcium antagonist PN 200-110 in the absence and presence of cardiodepression

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1 The globally-ischaemic Langendorff rabbit heart model has been used to study the cardioprotective effects of the dihydropyridine PN 200-110 (PN) at two doses, one having no negative inotropic effect and a higher dose causing a 62 ± 5% reduction in contractility.

2 Following 45 min no-flow global ischaemia, recovery was monitored for a period of 90 min reperfusion. Hearts were paced at a constant rate throughout experiments. Contractile force and coronary flow were recorded continuously. Tracer microspheres were injected at regular intervals to assess regional flow distributions, drill biopsies were taken to determine tissue high energy phosphate content, and enzyme leakage in the coronary effluent measured during the first 15 min of reperfusion.

3 Untreated hearts recovered 21 ± 2% of their initial contractile force and flow to all heart regions was reduced. In particular, endocardial flow fell to 20% of its pre-ischaemic level, with the ratio of flow to the endocardium (endo)/epicardium (epi) decreasing from ca. 1.0 to 0.4.

4 Hearts treated with 2×10^{-8} M PN (included in the perfusate from 30 min before ischaemia until 30 min after ischaemia) recovered 49 ± 2% of their initial, pretreatment contractile force, and following the ischaemia the endo/epi ratio was not significantly changed from the pre-ischaemic value.

5 The lower PN dose (3×10^{-10} M) afforded a lesser degree of protection, contractility recovering to 29 ± 4% of the initial level, with an endo/epi ratio of 0.7 after 90 min reperfusion.

6 The two PN doses afforded a similar degree of protection against enzyme leakage which was in both cases significantly less than in untreated hearts.

7 Myocardial ATP and creatine phosphate content was markedly reduced by the ischaemic episode. Neither PN dose modified this depletion.

8 These results suggest that whilst cardiodepression may well offer protection against ischaemic damage, this is not the sole mechanism wherby PN (and possibly other calcium antagonists) can protect the heart. Preservation of blood flow to the inner layers of the left ventricular wall is likely to be one of the major factors underlying the enhanced recovery shown by PN.

Introduction

Previous experimental studies have shown that a number of calcium antagonists can protect the heart against ischaemic damage, particularly when applied before the ischaemic insult (Nayler *et al.*, 1980, Watts *et al.*, 1980, Bourdillon & Poole-Wilson, 1982). However, the exact mechanism(s) involved in this 'protection' remain(s) unclear. In most cases the concentrations of calcium antagonists shown to offer cardioprotection also have marked negative inotropic activity. The energy-sparing effect of this reduction in heart work is a possible explanation for the protection afforded by these drugs. Indeed, the extent to which mechanical function recovers after a period of ischaemia has been shown to correlate well with the tissue ATP content at the end of the reperfusion period (Watts *et al.*, 1980; Nayler, 1982). However, more recent evidence has emerged that calcium antagonists can provide myocardial protection other than by an energy-preserving mechanism (Drake-Holland, 1982; Hamm & Opie, 1983; Henry & Wahl, 1983).

This study has used the dihydropyridine calcium antagonist PN 200-110 (PN; structure shown in Figure 1) to see if concentrations of this compound which do not cause a negative inotropic effect can protect the heart against ischaemic damage. PN is particularly suitable for this purpose since it is known to exhibit significant vascular effects at doses below those causing cardiodepression (Hof *et al.*, 1984a,b). The

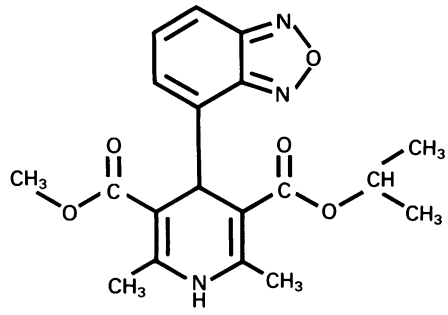

Figure 1 Chemical structure of PN 200-110 (PN).

isolated heart model used allows direct myocardial protection to be investigated in the absence of possible contributions from drug effects on the peripheral circulation. Furthermore, the rabbit hearts chosen for this study were sufficiently large to permit both regional blood flow distributions to be studied, and the removal of a limited number of biopsy samples without decreasing the force developed. By simultaneously assessing changes in myocardial contractility, regional distribution of coronary flow and energy metabolism, we have obtained results providing an insight into how calcium antagonists of this type might protect the ischaemic myocardium other than by cardiodepression, collateral development, or effects on the peripheral circulation.

Methods

Mongrel rabbits weighing 4.0 to 5.0 kg were anaesthetized with pentobarbitone (25 mg kg^{-1} i.v.) and pretreated with heparin (4 mg kg^{-1} i.v.). The animals were stunned by a blow on the head, exsanguinated and perfusion of the hearts started *in situ* under a constant pressure of 60 cmH_2O (44 mmHg) with Tyrode solution of the following composition (mM) NaCl 137, KCl 2.68, $CaCl_2$ 1.80, $MgCl_2$ 1.08, $NaHCO_3$ 11.9, NaH_2PO_4 0.41, glucose 1.0 $g\,l^{-1}$, gassed with a mixture of 95% O_2/5% CO_2. The hearts were dissected free and transferred to a non-recirculating Langendorff perfusion system. The temperature of the perfusate entering the heart was closely maintained at 37°C, with minimal temperature fluctuations during changes to/from drug-containing solutions or on reperfusion following the no-flow ischaemia. A strain gauge was sewn onto the left ventricle to measure contractile force and two platinum electrodes were placed on the right atrium. Hearts were paced throughout the experiments, including the period of ischaemia, at a constant rate of 3–4 Hz (adjusted for each individual heart to approximately 1.5 Hz above the unstimulated rate) using a Grass S44 stimulator (2.5 V, 2 ms duration). Perfusate leaving the heart was collected and a continuous estimate of the coronary flow derived from the weight of fluid with respect to time. Force, its first derivative *dF/dt*, heart rate and coronary flow were recorded on a Schwarzer 8 channel recorder.

Experimental protocol

Preliminary experiments were performed to compare the negative inotropic effects of PN, verapamil and diltiazem on the rabbit isolated heart. Hearts were perfused with normal Tyrode solution for 60 min before changing to Tyrode containing a drug. After 30 min perfusion the next concentration of the drug was introduced, with the force measurements being made at the end of each 30 min period. PN was prepared as a 10^{-3} M solution in 50% ethanol and diluted further with Tyrode solution. Experiments with PN were performed under sodium light as a precaution against photodecay.

For the ischaemia experiments, hearts were perfused for an initial period of 90 min, followed by 45 min no-flow global ischaemia and 90 min reperfusion. PN (0, 3×10^{-10} M or 2×10^{-8} M) was included in the perfusate for the 30 min before and following the ischaemia. Those hearts failing to resume normal rhythmicity after 17 min reperfusion were administered 0.2 ml of 2% lidocaine, after which all such hearts began to beat normally. Coronary effluent was collected over the first 15 min of reperfusion and stored at 4°C. Levels of creatine kinase (CK) and lactate dehydrogenase (LDH) were assayed spectrophotometrically within 1 day of collection using assay kits obtained from Sigma.

A high speed drill (core diameter ca. 1.5 mm) was used to take tissue biopsy samples from the left ventricle, starting at the apex, after 30 min control perfusion, after 40 min ischaemia and at the end of the experiment. Tissue samples were rapidly ejected by air pressure into ethanol/dry ice (−80°C) and subsequently stored frozen at −70°C. The extraction of metabolites from the biopsy samples was performed as described by Hearse (1984b). Briefly, following lyophilysation and weighing, the tissue samples were homogenized and the metabolites extracted on ice with 130 µl of 6% perchloric acid + 1 mM EDTA for ca. 20 min. The supernatant was neutralized with 2 mM $KHCO_3$ in 100 mM Tris buffer before being assayed. The entire extraction procedure was performed in specially designed tubes (Hearse, 1984b), so as to avoid any tissue loss. The adenosine 5′-triphosphate (ATP) and creatine phosphate contents of the extracts were determined fluorimetrically using the method described by Lowry & Passoneau (1972).

Tracer microspheres

The use of radioactive microspheres for the determination of regional blood flow in perfused hearts has previously been described in considerable detail (Hof *et al.*, 1981). Microspheres (15 μm diameter, 3M Company) labelled with ^{125}I, ^{141}Ce, ^{51}Cr, ^{85}Sr, ^{46}Sc or ^{95}Nb were vigorously shaken in an ultrasonic bath for 5 min before injection. At various stages of the experiment approximately 30000 microspheres of a particular isotope were injected into the perfusate at a point just before its entry into the heart. Before injection a paper filter was placed beneath the heart to collect those microspheres not trapped by the coronary vessels. These untrapped spheres were taken as an indication of flow other than through the coronary vessels when calculating the regional blood flow (Hof *et al.*, 1981). The injection of microspheres had no apparent effects on cardiac function. The order in which isotopes were injected was changed for each experiment to avoid any systematic errors arising.

At the end of the experiment the heart was divided into the left and right ventricles, the atria (including some connective tissue) and the septum. The left ventricle was further sectioned into the inner, mid and outer layers, the papillay muscles being included with the endocardial layer. Blood flow results for the middle layer of the left ventricle are not shown, since this was taken primarily to provide a better separation between the endocardial and epicardial layers.

Statistical analysis

Mean ± s.e.mean are shown for all figures, with $n = 7$ for untreated hearts, $n = 8$ for hearts treated with 3×10^{-10} M PN and $n = 6$ for hearts treated with 2×10^{-8} M PN. Statistical analyses were performed (Hewlett Packard 9815A) by the Mann Whitney U test, with $P < 0.05$ considered significant.

Drugs, chemicals and enzymes

The following were used: pentobarbitone (Nembutal; Abbott); creatine kinase, dithiothreitol, glucose-6-phosphate dehydrogenase, hexokinase and NADP (Boehringer); diltiazem (Gödecke); verapamil (Knoll); heparin Na (Sanabo); PN 200-110 (Sandoz); adenosine 5'-diphosphate (ADP), adenosine 5'-triphosphate (ATP), creatine kinase assay kit no. 46-UV, lactate dehydrogenase assay kit no. 340-UV (Sigma); xylocaine (Vifor).

Results

The negative inotropic effects of PN 200-110 and two structurally different calcium antagonists, verapamil and diltiazem, are shown in Figure 2. At high doses verapamil and diltiazem caused conduction disturbances and arrhythmias (shown with asterisks in figure), forcing the drug to be withdrawn before the 30 min treatment period had been completed. The approximate EC_{50} values for the negative inotropic effects of the three drugs, read directly from Figure 2, are PN 2×10^{-8} M, verapamil 2×10^{-7} M and diltiazem 1.5×10^{-6} M. The values for verapamil and diltiazem compare favourably with previous results on the rabbit heart (Cavero *et al.*, 1983). Based on these results, PN doses of 3×10^{-10} M and 2×10^{-8} M were selected for the following ischaemia experiments.

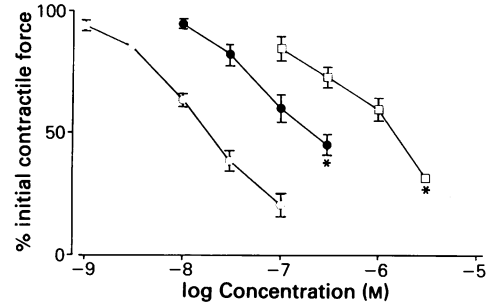

Figure 2 Dose-response curves for the negative inotropic effects of PN 200-110 (○; $n = 6$), verapamil (●; $n = 5$) and diltiazem (□; $n = 5$) on the rabbit isolated heart. The developed force after 30 min drug perfusion is expressed as a percentage of the initial baseline force for each heart. Asterisks indicate the withdrawal of the drug before completion of the 30 min perfusion (see text).

Effects of ischaemia on contractility

Before any drug treatment the mean developed force for the three groups of hearts (control, low and high dose PN) was 26.1 ± 1.6 g ($n = 21$), with no significant differences between the respective groups. After 30 min of drug treatment the lower PN dose (3×10^{-10} M) caused no significant cardiodepression, whereas the higher PN dose (2×10^{-8} M) reduced contractility by 62 ± 5%. After 45 min of global ischaemia and 17 min of reperfusion, a bolus injection of lidocaine was administered to 3/6 hearts treated with 2×10^{-8} M PN, 5/8 hearts receiving 3×10^{-10} M PN and 6/7 of the untreated hearts, to facilitate a return of normal heart rhythm.

With the initiation of reperfusion after the period of no-flow ischaemia, hearts underwent a contracture, as indicated by the change in diastolic tension with respect to the pre-ischaemic level. Figure 3 shows how the magnitude of this increase in tension was reduced in a dose-related manner by treatment of the hearts with PN. The recovery of contractile force with further reperfusion is shown in Figure 4. At the times when

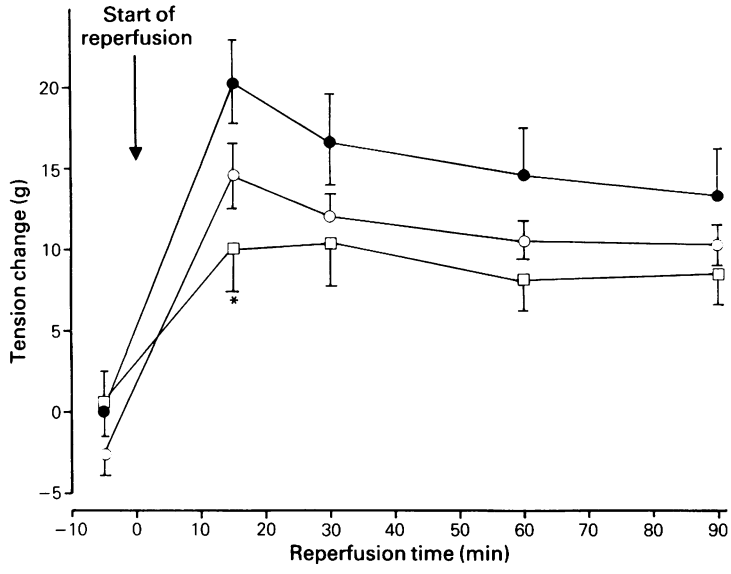

Figure 3 Diastolic contracture seen upon reperfusion after 45 min global ischaemia in untreated hearts (●; $n=7$) and those treated with 3×10^{-10} M PN 200-110 (PN) (○; $n=8$) and 2×10^{-8} M PN (□; $n=6$). Each point represents the mean and vertical lines s.e.mean of n observations. Asterisks indicate a significant difference from the controls, $P<0.05$.

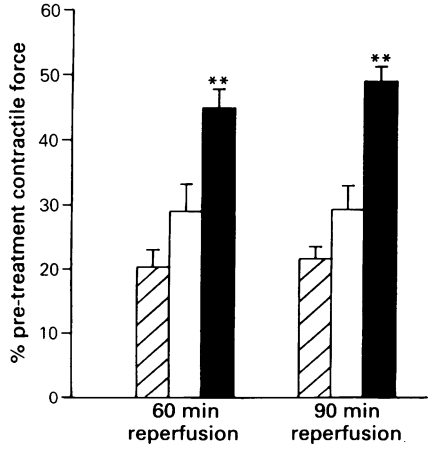

Figure 4 Recovery of contractility with reperfusion after 45 min global ischaemia in untreated hearts (hatched columns; $n=7$), and those treated for 30 min before the ischaemia until 30 min after with 3×10^{-10} M PN 200-110 (PN; open columns; $n=8$) and 2×10^{-8} M PN (solid columns; $n=6$). results are expressed as a percentage of the initial contractile force before any drug treatment. Asterisks indicate a significant difference from controls, $P<0.01$. In the subsequent figures the shading of the columns and n for each group is the same, with each column representing the mean and vertical lines s.e.mean of n observations.

these measurements were taken all hearts were being perfused with drug-free Tyrode solution, the drug-treated hearts being returned to this perfusion fluid after 30 min of reperfusion. The percentage recoveries shown in Figure 4 are calculated with respect to the initial contractile force before any drug treatment. After 60 min reperfusion the untreated hearts recovered only 20% of their original contractility, with little improvement seen 30 min later. Hearts treated with 2×10^{-8} M PN made a significantly better recovery, to 49% of their pretreatment level. Hearts treated with 3×10^{-10} M PN recovered 29% of their initial contractile force.

Effect of ischaemia on regional flow distribution

The administration of radioactive microspheres permits the total organ blood flow to be subdivided into flow to the various heart regions, and also to make allowance for 'leak' flow (e.g. as a consequence of insufficient heart valves or following biopsy sample removal). Before any treatment the initial flow values for the three groups of hearts were (in ml min^{-1} per 100 g tissue); control group 635 ± 19, low dose PN group 619 ± 38 and high dose PN group 593 ± 70. Subsequent drug application to the latter two groups caused no significant change in either the total coronary flow or in the regional flow distributions (Figure

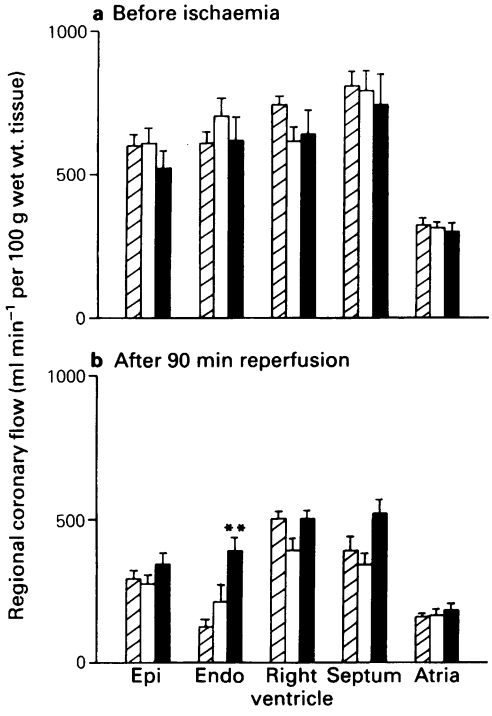

Figure 5 Regional coronary flow distribution obtained by injecting radioactive tracer microspheres, (a) before the ischaemia and, (b) after 90 min reperfusion. Flow to the various heart regions is expressed as ml min^{-1}per 100 g wet weight of tissue. Key to columns as for Figure 4. Epi = epicardium, Endo = endocardium. Asterisks indicate a significant difference from controls, $P<0.01$

5a). Following 45 min ischaemia and 90 min reperfusion, coronary flow was reduced by 53% in the untreated hearts, 55% in those treated with 3×10^{-10} M PN and by 30% in those treated with 2×10^{-8} M PN ($P<0.05$ compared with untreated hearts). The distribution of this flow between the different heart regions is shown in Figure 5b. Whilst the higher PN dose caused a slight improvement in recovery of flow to all regions, this only reached significance in the flow to the subendocardial layer of the left ventricle (endo). Flow values following 90 min reperfusion in hearts treated with 3×10^{-10} M PN were not significantly different from untreated hearts.

Figure 6 shows how the ischaemic episode affected the ratio of flow to the endocardial and epicardial layers of the left ventricle. Before the ischaemia, the baseline endo/epi ratio of ca. 1.0 was not significantly affected by PN application. however, after ischaemia this flow ratio fell to 0.4 in the untreated hearts, whereas hearts treated with 2×10^{-8} M PN recovered their initial endo/epi ratio. hearts treated with 3×10^{-10} M PN showed a gradual recovery of the ratio to a final value of 0.7 after 90 min reperfusion. Indeed, this latter group consisted of four hearts which recovered after 90 min reperfusion to an endo/epi ratio of 1.14 ± 0.15 and another four hearts where the ratio was 0.28 ± 0.05, suggesting that preservation of this flow ratio might possibly be an 'all or nothing' situation.

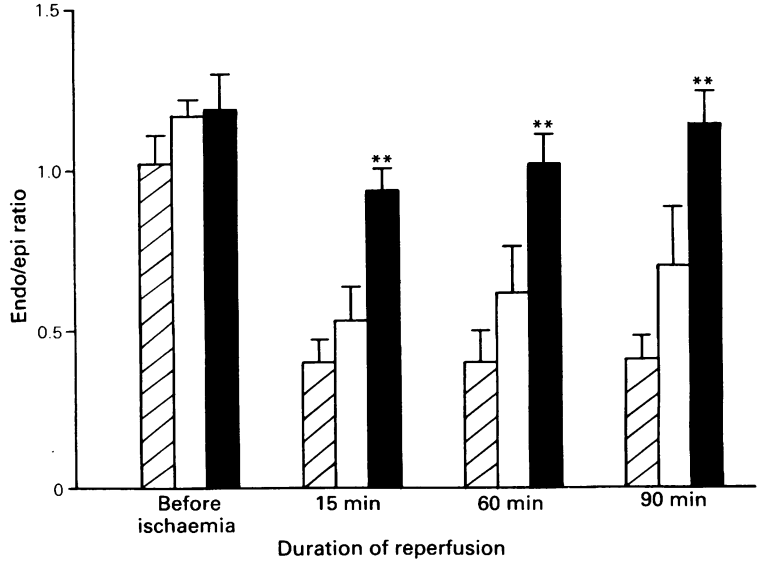

Figure 6 Flow distribution ratio between the endocardial (endo) and epicardial (epi) layers of the left ventricle. Regional flow was determined by injection of radioactive microspheres at the times shown. Key to columns as in Figure 4. In the drug-treated hearts PN 200-110 was present in the perfusate at the time of microsphere injection before the ischaemia and for the first 30 min of reperfusion. Asterisks indicate a significant difference from control hearts, $P<0.01$.

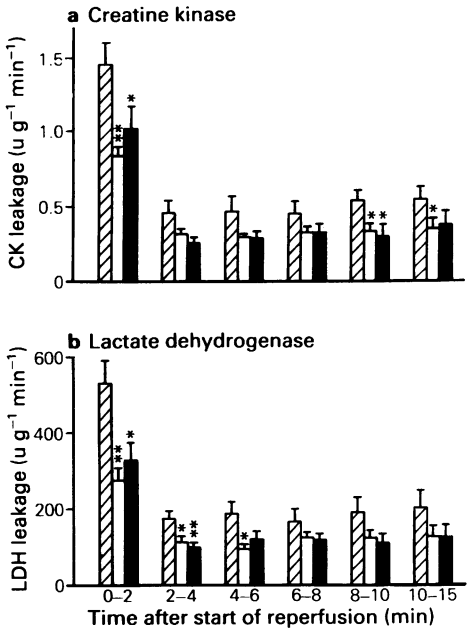

Figure 7 Leakage of (a) creatine kinase (CK) and (b) lactate dehydrogenase (LDH) into the perfusate leaving the hearts during the first 15 min of reperfusion following 45 min global ischaemia. Enzyme release is expressed as units released min^{-1} per g wet weight of heart. Key to columns as in Figure 4. Asterisks indicate a significant difference from controls; $*P<0.05$, $**P<0.01$.

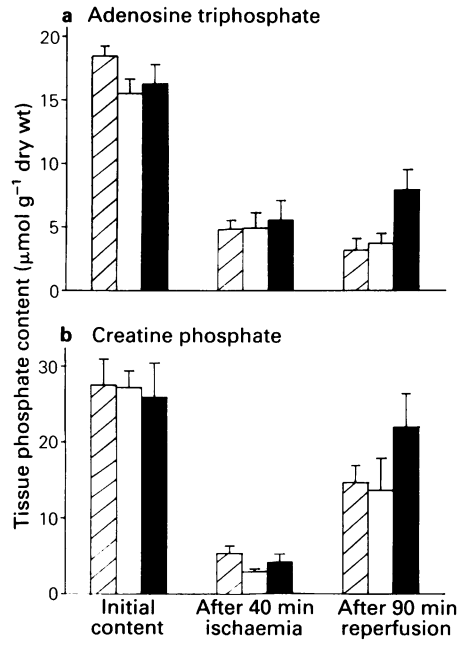

Figure 8 Myocardial high energy phosphate content determined in tissue drill biopsy samples taken from the hearts at the times indicated. Key to shading of columns as in Figure 4.

Biochemical changes due to ischaemia

Figure 7 shows the loss of creatine kinase (a) and lactate dehydrogenase (b) from the hearts during the first 15 min of reperfusion following ischaemia. The initial enzyme leakage was significantly reduced by treating the hearts with both doses of PN. Total enzyme loss over this 15 min period is shown in Table 1. The reduction in enzyme leakage was comparable for both PN doses.

Figure 8 shows the ATP and creatine phosphate contents of the heart tissue at various stages of the experiment. Before treatment there was no significant difference in phosphate contents between the three groups. After 40 min of ischaemia there was a marked reduction in phosphate contents of all hearts, which was not prevented by treatment with PN. After 90 min reperfusion, hearts treated with 2×10^{-8} M PN showed a slight improvement in ATP content. Untreated hearts and those treated with 3×10^{-10} M PN showed no recovery of ATP contents with reperfusion. All three groups showed an increase in creatine phosphate content with reperfusion, the recovery being greatest in those hearts treated with 2×10^{-8} M PN.

Table 1 Total enzyme release from the rabbit isolated heart during the first 15 min of reperfusion following ischaemia

	n	*Creatine kinase* (u g^{-1} wet wt. per 15 min.)	*Lactate dehydrogenase* (u g^{-1} wet wt. per 15 min.)
Untreated hearts	7	9.42 ± 1.25	3580 ± 480
PN 3×10^{-10} M	8	5.89 ± 0.59*	2090 ± 260*
PN 2×10^{-8} M	6	6.27 ± 1.08*	2200 ± 300*

PN = PN 200-110
*$P<0.05$.

Discussion

Recent advances in coronary surgery and thrombolysis now offer the possibility of re-establishing coronary blood flow to an infarcted heart region. Consequently, drugs which delay irreversible ischaemic damage, restrict the area of necrosis, or limit the damage caused by reperfusion could be particularly valuable in these instances. Understanding in detail how different functions, processes or structures are protected by calcium antagonists may ultimately help to establish in which clinical situations these drugs might be useful. In the present study we have found clear evidence for a preservation of subendocardial flow by PN, upon reperfusion after a period of ischaemia. Mechanical function, the ability to synthesize ATP, and enzyme leakage were also protected in the drug-treated hearts. However, the improved recovery appears not to be related to a preservation of tissue ATP stores during the period of ischaemia, since these were equally depleted in both the drug-treated and untreated hearts (Figure 8).

These results with PN are in agreement with a previous report by Drake-Holland (1982) on the cardioprotective action of lidoflazine in dogs; whilst the lidoflazine-treated hearts showed a better recovery from ischaemia than untreated hearts, at the end of the ischaemic period ATP and creatine phosphate levels had fallen to the same extent in both groups. Kinetic studies of ATP depletion in rat ischaemic hearts at 37°C by both biochemical techniques (Rosenberger *et al.*, 1984) and ^{31}P-nuclear magnetic resonance scanning (Ruigrok *et al.*, 1984) shows that, despite a somewhat slower depletion of phosphate stores in hearts treated with verapamil and nifedipine, there were virtually no differences in the phosphate levels between treated and untreated hearts with ischaemic periods of longer than 30 min. Ruigrok *et al.* (1984) also found that, upon reperfusion, a recovery of phosphate levels was seen only in the nifedipine-treated hearts.

Several recent findings further support the view that calcium antagonists can provide myocardial protection other than through energy-preserving mechanisms. Thus, Hamm & Opie (1983) found that some indices of myocardial damage, induced by regional ischaemia in the rat isolated heart, were reduced by treatment with nifedipine and diltiazem at concentrations not decreasing left ventricular work. Henry & Wahl (1983) have shown that diltiazem and nitrendipine (in doses known to be cardio-depressant) can reduce the hypoxic contracture of quiescent rabbit papillary muscles. Likewise, cardiodepressant doses of calcium antagonists administered to hearts at the onset of a low-flow ischaemic period, where the hearts are no longer contracting, also seem to afford protection (Henry *et al.*, 1977; Cavero *et al.*, 1983). It thus seems reasonable to conclude that preservation of ATP stores (through cardiodepression) does not explain the cardioprotective effects of the calcium antagonists. However, after 90 min reperfusion the hearts treated with the higher PN dose showed a better recovery of ATP levels than the controls (Figure 8). Hence the cardioprotective effects of calcium antagonists such as PN may be linked to preservation of the ATP generating capacity of the myocardial cells, for instance by a protection of mitochondrial function (Henry *et al.*, 1977; Nayler *et al.*, 1980; Nagao *et al.*, 1980). The correlation found between tissue ATP content and recovery of mechanical function after a period of reperfusion (Watts *et al.*, 1980; Nayler, 1982) could thus be a consequence of this.

Preservation of endocardial blood flow is probably the most important protective mechanism elicited by PN. After 90 min reperfusion of the untreated hearts the endocardial flow fell to 20% of the pre-ischaemic

Table 2 Recovery from ischaemia of hearts treated with 3×10^{-10} M PN 200-110

	Endo/epi ratio > 0.8 ($n = 4$)	*Endo/epi* ratio < 0.4 ($n = 4$)
Endo/epi flow ratio	1.14 ± 0.15	0.28 ± 0.05**
Endocardial flow (ml min^{-1}per 100 g)	342 ± 69	74 ± 22*
Epicardial flow (ml min^{-1} per 100 g)	300 ± 47	260 ± 43 (NS)
Contractile force (% recovery)	37.0 ± 5.3	21.1 ± 2.6*
Tissue ATP content (μmol g^{-1}dry wt.)	5.2 ± 0.8	2.2 ± 0.7*

The eight hearts receiving 3×10^{-10} M PN have been subdivided according to their endo/ epi ratio at the end of 90 min reperfusion. Due to the low n value a parametric non-paired t test was used to assess significance between the two groups, * $P < 0.05$, ** $P < 0.01$, NS, not significant.

level (Figure 5), whereas in the PN-treated hearts this flow was 75% (3×10^{-10} M PN) and 225% (2×10^{-8} M PN) greater. The ratio of flow distribution between the endocardial and epicardial layers of the left ventricle (Figure 6) was found to correlate well with the recovery of mechanical funtion for each group of hearts (Figure 4).

For the hearts treated with the lower PN dose it was found that 4 hearts fully recovered their pre-ischaemic endo/epi ratios, whereas the other four hearts showed no evidence of cardioprotection. The subdivision of the hearts treated with 3×10^{-10} M PN, based on their endo/epi ratios after 90 min reperfusion, is shown in Table 2. It is clear that both ATP content and recovery of contractile force are closely correlated to the preservation of endocardial flow in these hearts. At present it is not possible to say whether the ability to replenish ATP stores is a secondary consequence of the improved endocardial flow, or vice versa.

Previous studies in dogs with a ligated descending coronary artery have show that nifedipine does not modify the endo/epi flow distribution during the occlusion period (Henry *et al.*, 1978; Roan *et al.*, 1981). Lamping *et al.*, (1984) showed that following 30 min reperfusion of dog hearts which had undergone coronary ligation for 2 h, the endo/epi flow distribution in the ischaemic region was not significantly different between untreated and nifedipine-treated hearts. Treatment with the vasodilator nicorandil caused a worsening of this ratio on reperfusion compared with controls (Lamping *et al.*, 1984). Whilst differences between these models and that used for the present study make comparisons difficult, it appears that the effects of different calcium antagonists on regional myocardial blood flow during ischaemia and reperfusion may be complex and variable (see also Weintraub *et al.*, 1981).

It should be noted that before the ischaemia neither the endo/epi ratio nor the total coronary flow were significantly affected by PN treatment (Figures 5 and 6). These observations are at variance with previous *in vivo* studies in cats, where PN markedly increased coronary blood flow (Hof *et al.*, 1984a). The discrepancy is probably attributable to the high baseline flow values (around 600 ml min^{-1} per 100 g of tissue) in the present experiments with Tyrode-perfused isolated hearts, allowing little additional coronary reserve on which to see vasodilator effects. Hamm & Opie (1983) have described similar observations with verapamil, nifedipine and diltiazem in the rat isolated heart, where they too perfused the hearts with a solution containing no haemoglobin. Additionally, the 60% reduction in contractile force caused by the higher PN dose might obscure possible vasodilator properties of this compound.

Whilst this study does not distinguish between myocardial protection and postponement of ultimate cell death, it is reasonable to assume that cardiac function will only recover if blood flow is indeed restored (Hearse, 1984a). Hence an ability of PN (and possibly other calcium antagonists too) to preserve flow to the subendocardial layer of the left ventricle, where the initial ischaemic damage occurs (Reimer *et al.*, 1977; Regitz *et al.*, 1984), could offer true protection to the myocardium. This might also explain why certain calcium antagonists have been found to offer cardioprotection only when administered before the ischaemic event (Watts *et al.*, 1980; Bourdillon & Poole-Wilson, 1982). Once the subendocardial vessels have already become constricted, drug access to these regions may become limited, and their success as cardioprotective agents accordingly reduced.

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(*Received February 16, 1985*
Accepted May 7, 1985.)