Br. J. Pharmac. (1984), **83**, 943–949

Energy conservation by nisoldipine in ischaemic heart

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1 We studied the effect of the calcium entry blocker nisoldipine on ATP catabolism in the rat heart, perfused according to Langendorff. Even 1 nM nisoldipine induced vasodilatation; concentrations of 30 nM and higher caused significant negative inotropy.

2 The drug had a very strong affinity for silicon rubber tubing.

3 Myocardial ischaemia was induced by lowering the perfusion pressure, which reduced flow without nisoldipine by 85%. The efflux of purine nucleosides and oxypurines rose 14 fold. Nisoldipine reduced this efflux of ATP catabolites dose-dependently. The highest concentration, 300 nM, suppressed ischaemic purine production completely.

4 The action of the drug was antagonized by an increase in Ca^{2+}-concentration in the perfusion fluid.

5 We also showed the protective effect of nisoldipine on adenine nucleotides in freeze-clamped hearts. A concentration of 20 nM partially prevented the reduction of ATP and adenylate energy charge due to ischaemia.

6 We conclude that relatively low doses of nisoldipine effectively prevent ATP breakdown in ischaemic rat heart.

Introduction

Nisoldipine is one of the newer dihydropyridine derivatives, which act as powerful vasodilators by inhibiting transmembrane calcium influx. This Ca^{2+}-entry blocker reduces afterload and is an extremely potent coronary dilator (Kazda *et al.*, 1980). It seems to be a potential drug for the treatment of ischaemic heart disease (Vogt *et al.*, 1980).

Little is known about the effect of nisoldipine on myocardial metabolism. Takahashi & Kako (1983) found that nisoldipine suppresses the ischaemia-induced increase in phospholipid breakdown of cardiac sarcolemma. In the anaesthetized dog, Kazda *et al.* (1980) found no changes in myocardial lactate metabolism due to the drug. We tested whether it could prevent nucleotide catabolism in the ischaemic rat heart. Adenosine 5′-triphosphate (ATP) produces adenosine 5′-diphosphate (ADP) and adenosine 5′-phosphate (AMP) in the heart; an increased concentration of catabolites of the latter – adenosine, inosine, (hypo) xanthine and urate – occurs in the myocardial efflux during ischaemia (Schoutsen *et al.*, 1983). In an effort to elucidate the extent to which catabolism could be avoided and high-energy phosphates preserved, the efflux of these compounds was measured in the presence of various concentrations of the Ca^{2+}-entry blocker. Nisoldipine proved to be an effective inhibitor of ATP breakdown during myocardial ischaemia.

Methods

Heart perfusion

Male Wistar rats (230 to 380 g), with free access to food and water, were anaesthetized with 30 mg pentobarbitone i.p. Hearts were rapidly removed and cooled in ice-cold 0.9% w/v NaCl solution until beating ceased. Then retrograde perfusion of the aorta was started with a modified Tyrode buffer, gassed with 95% O_2 plus 5% CO_2 (pH 7.4, 37°C). Unless otherwise indicated the buffer contained (mM): D-glucose 10, NaCl, 128, KCl 4.7, $CaCl_2$ 1.4, $NaHCO_3$ 20, NaH_2PO_4 0.4, $MgCl_2$ 1.0. The perfusion temperature was measured in the aortic cannula. Pacing frequency was 300 beats min^{-1}. Other details of the perfusion are given elsewhere (De Jong *et al.*, 1984). Ischaemia was induced by lowering the perfusion pressure from 9.6 to about 2.2 kPa for 15 or 30 min. Where indicated, reperfusion took place for 15 min. Silicon rubber tubing, used in the perfusion apparatus, was supplied by Rubber-Technisch

Handels- en Adviesbureau, Hilversum, The Netherlands. Outer and inner dimensions were 6 and 4 mm, respectively.

Myocardial function

Apex displacement was measured as described previously (Stam & De Jong, 1977). This method gives relative values; the displacement found 5 min after the start of the perfusion was taken as 100%. Alternatively, developed tension was monitored with a P23Db transducer (Statham, Hato Rey, Puerto Rico) connected to the apex of the heart. Five minutes after the start of the perfusion, these hearts were adjusted to a resting tension of about 15 g.

Coronary flow

Perfusate was collected in a graduated cylinder for one minute periods at the end of normoxia, ischaemia and reperfusion.

Assay of purines

Perfusate samples were mixed with NaN_3 (0.02% final concentration) and kept on ice until analysis, usually within 12 h. Adenosine, inosine, hypoxanthine, xanthine, and urate were determined by high-performance liquid chromatography (h.p.l.c.), as described previously (Harmsen *et al.*, 1981; De Jong *et al.*, 1984).

Assay of adenine nucleotides

In freeze-clamped hearts, adenine nucleotides were determined by h.p.l.c. according to Harmsen *et al.*, (1982). From these nucleotides the adenylate energy charge, ([ATP] + 0.5 [ADP])/([ATP] + [ADP] + [AMP]), was calculated.

Chemicals

All chemicals were of the highest grade available. Water was purified with the Millipore-Ro4/Milli-Q System (Millipore, Bedford, MA). Nisoldipine (isobutyl methyl 1,4-dihydro-2,6-dimethyl-4 (2-nitrophenyl)-3,5-pyridinedicarboxylate; Bayer, Wuppertal, GFR) was solved in absolute ethanol. The stock solution (2.5 mM) was diluted by adding perfusion buffer and vigorous stirring. Nisoldipine solutions were kept in the dark.

Statistical analysis

Two-way analysis of variance was employed. Further evaluations were made using Scheffé's method for multiple comparisons (Snedecor & Cochran, 1967).

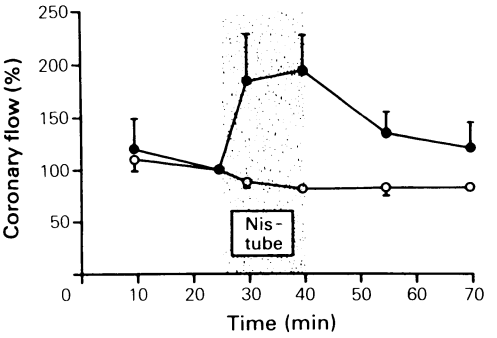

Figure 1 Increase in coronary flow due to residual nisoldipine (Nis) from perfusion tubing. For 15 min 100 nM of the drug, dissolved in perfusion medium, was pumped through 20 cm of silicon rubber tubing (internal diameter 4 mm) at a rate of 10 ml min^{-1}. Subsequently the tubing was washed for 1 min with four tube volumes of medium, and mounted in the perfusion apparatus. All hearts were perfused with standard medium for 25 min bypassing the tubing. Then the medium was forced to enter for 15 min the aorta either via the pretreated (●) tubing or via tubing which had been flushed similarly with standard medium (○). A significant ($P<0.001$) increase in flow, due to nisoldipine released from the tubing was observed. Flow data were calculated relative to the 25 min value; they are presented as means with vertical lines showing s.d. ($n=5$).

$P<0.05$ (two-tailed) was considered statistically significant.

Results

Affinity of nisoldipine for tubing

From initial experiments we got the impression that our standard wash-out procedures were insufficient to remove nisoldipine from the perfusion apparatus. The experiment depicted in Figure 1 showed that the drug had a very strong affinity for silicon rubber tubing. We pumped medium with nisoldipine through a piece of tubing, washed it, and tested to see whether this tubing affected coronary flow. Even after 15 min of perfusion, flow was doubled by the tubing treated with nisoldipine ($P<0.001$). We also noted some effect on apex displacement: it decreased by about 20% with this tubing ($P=0.009$; results not shown). In the studies, described below, we used only rubber tubing to connect glass pieces, with almost no contact between tubing and perfusate.

Coronary flow

From Figure 1 it is clear that very small amounts of nisoldipine induced vasodilatation. This is further

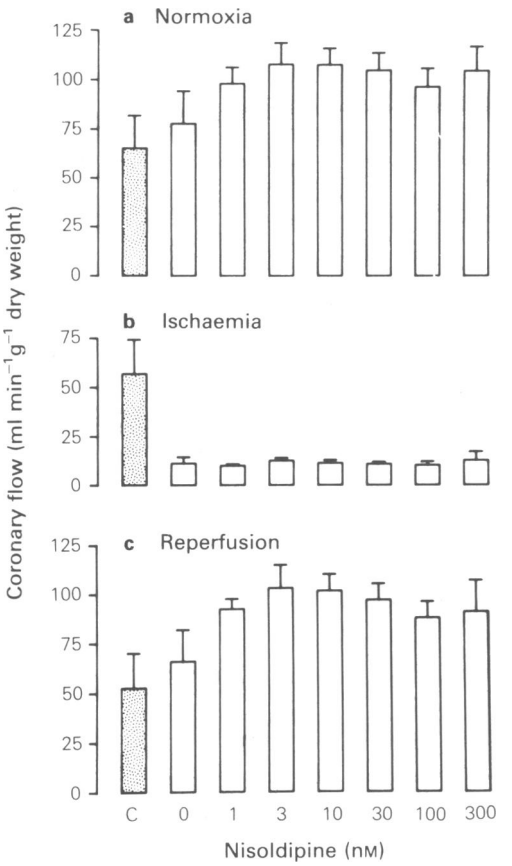

Figure 2 Vasodilatation by nisoldipine in (a) normoxic, (b) ischaemic and (c) reperfused hearts. At the end of the periods indicated, coronary flow data (in $ml\,min^{-1}g^{-1}$ dry weight) were obtained from hearts perfused for 20 min under normoxic conditions (perfusion pressure 9.6 kPa), followed by 15 min of ischaemia (pressure lowered to 2.2 kPa), and 15 min of reperfusion at 9.6 kPa. Where indicated, treatment with nisoldipine started after 5 min of perfusion. Stippled columns (C) = control perfusion, with neither nisoldipine nor ischaemia. Columns show means and vertical lines s.d. ($n = 5-7$).

documented in Figure 2. Even 1 nM gave an increase in flow; all doses caused vasodilatation in normoxic hearts ($P < 0.001$). When the perfusion pressure was reduced from 9.6 ± 0.1 to 2.2 ± 0.2 kPa, coronary flow dropped to about 11 $ml\,min^{-1}\,g^{-1}$ dry weight. This is about 85% less than flow in non-ischaemic control hearts. Nisoldipine did not cause significant differences in flow during ischaemia. However, in reperfused hearts the powerful vasodilator properties of nisoldipine were again apparent ($P < 0.001$). Already the lowest concentration (1 nM) caused an increase in flow.

Purine efflux

Nisoldipine, in a dose-range of 1 to 300 nM, did not significantly affect the efflux of adenosine, inosine, hypoxanthine or xanthine from normoxic hearts. For the four purines, mentioned above, the relative amounts were 2%, 39%, 35% and 24%, respectively. The total amount was 6 ± 3 $nmol\,min^{-1}\,g^{-1}$ dry weight after a 30 min perfusion period (Figure 3). The production of these purine nucleosides and oxypurines increased 14 fold as a result of ischaemia (Figure 3). The relative amounts found in the effluent of the ischaemic heart were 15% (adenosine), 51% (inosine), 20% (hypoxanthine) and 14% (xanthine). Nisoldipine suppressed purine efflux in a dose-dependent manner ($P < 0.001$). At the highest drug concentration, purine production was comparable to non-ischaemic control values. The reperfusion values were similar to those observed before the induction of ischaemia: no effect of nisoldipine.

We measured urate efflux in addition to the purine production mentioned above. It amounted to about 60 and 10% of total purines in the effluent of nor-

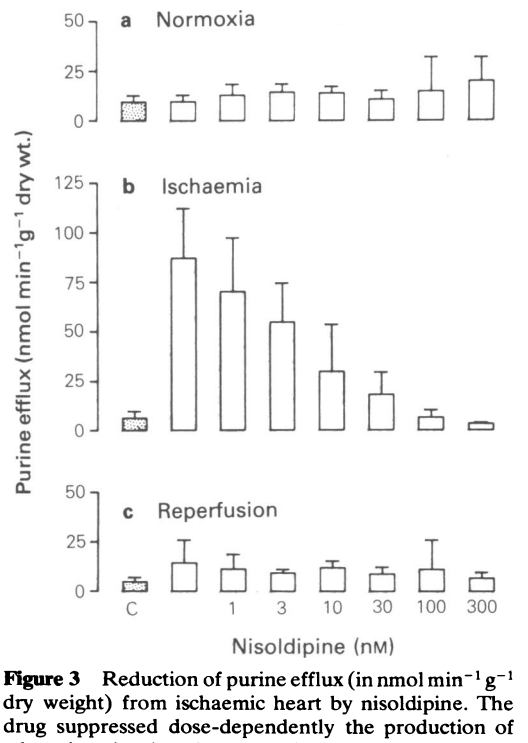

Figure 3 Reduction of purine efflux (in $nmol\,min^{-1}\,g^{-1}$ dry weight) from ischaemic heart by nisoldipine. The drug suppressed dose-dependently the production of adenosine, inosine, hypoxanthine and xanthine from ischaemic tissue. At the highest concentration of nisoldipine purine release was comparable to that from non-ischaemic controls (C, stippled columns). Other details are given in the legend to Figure 2.

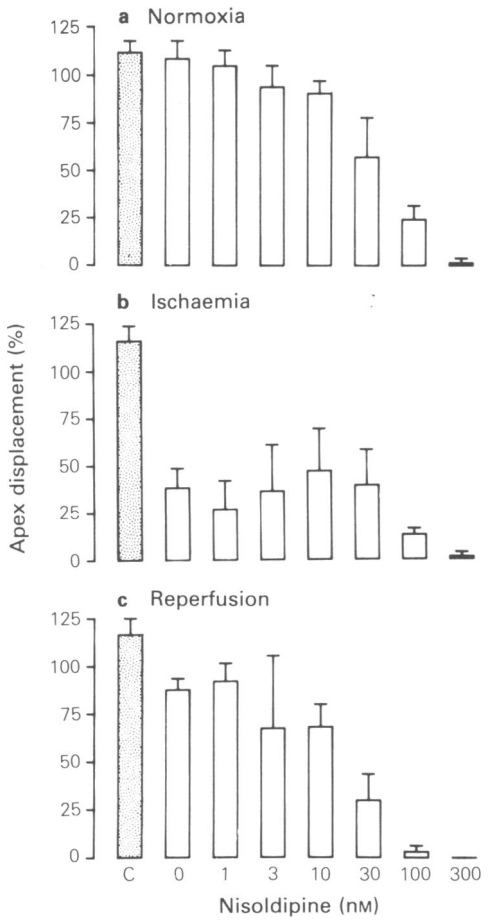

Figure 4 Effect of nisoldipine on apex displacement (%) of isolated perfused hearts. The value found 5 min after the start of the perfusion (before any drug was given) was taken as 100%. For other details, see legend to Figure 2. Please, note the dose-dependent negative inotropy before and after flow reduction. During ischaemia only the highest drug concentration reduced apex displacement significantly. Stippled columns (C) = non-ischaemic controls.

moxic and ischaemic heart, respectively. Nisoldipine (300 nM) also completely suppressed urate production from ischaemic heart. As the urate peaks in the high-performance liquid chromatograms were not always of a high quality, we decided to present only the data on the other purines in detail.

Myocardial function

We used apex displacement routinely as a measure of myocardial function. Figure 4 shows the dose-dependent ($P<0.001$) negative inotropic effect of nisoldipine in these isolated hearts. In the normoxic hearts, 30 nM nisoldipine decreased apex displacement by about 50%. A ten times higher concentration resulted in minimal contractile behaviour. Nisoldipine up to 30 nM did not affect apex displacement in ischaemic hearts. Apex displacement in these hearts was about 35% of non-ischaemic control values. Negative inotropy during ischaemia was seen with 100 and 300 nM nisoldipine. Apex displacement with 300 nM was significantly ($P<0.001$) smaller than that with the other doses. Apex displacement recovered to about 77% of control values without nisoldipine or with 1 nM of drug. Higher concentrations again gave dose-dependent negative inotropy ($P<0.001$).

In a number of hearts, we used developed tension to characterize myocardial function. Nisoldipine, in a concentration of 20 nM, decreased developed tension by 25% in normoxic hearts ($P<0.001$, data not shown). However, in both treated and untreated ischaemic hearts, developed tension was only a few % of that in non-ischaemic hearts ($P<0.001$, Figure 5). Nisoldipine treatment of the ischaemic heart did not significantly affect developed tension.

ATP breakdown

We studied the effect of nisoldipine on myocardial ATP content. In hearts with an initial preload of about 15 g, purine efflux was 13 ± 8 nmol $min^{-1} g^{-1}$ weight. This increased to 128 ± 20 nmol min^{-1} g^{-1} due to ischaemia ($P=0.001$, Figure 5). Twenty nM nisoldipine reduced this efflux to 42 ± 12 nmol min^{-1} g^{-1}, which is still significantly different from the aerobic control ($P=0.05$, $P<0.001$ vs. non-treated). The hearts were freeze-clamped and the adenine nucleotides analysed. ATP content decreased by 49% due to ischaemia ($P=0.004$, Figure 6). With the relatively low dose of nisoldipine used, a non-significant decrease of 27% was found ($P=0.11$; $P=0.12$ vs. non-treated). Similar observations were made on adenylate energy charge; this ratio dropped by 18% ($P=0.001$) as a result of ischaemia (Figure 6), whereas this was only 6% with nisoldipine treatment, a non-significant decrease ($P=0.21$; $P=0.02$ vs. non-treated).

Perfusion with high calcium

When the Ca^{2+}-concentration in the perfusion medium was raised from 1.4 to 5.0 mM, apex displacement in the normoxic heart went up 1.6 fold ($P<0.001$, Figure 7). The negative inotropic effect of nisoldipine (100 nM) was less pronounced ($P=0.05$), both relatively and absolutely, than in hearts perfused with a low calcium concentration,

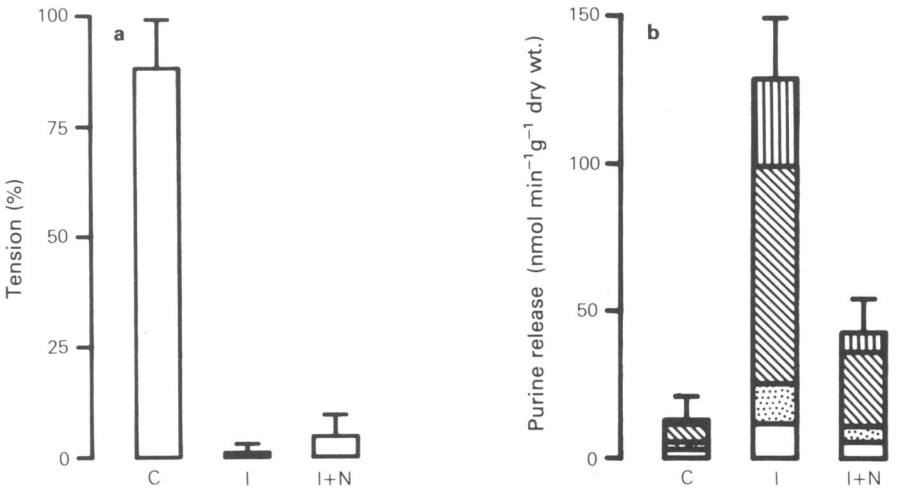

Figure 5 Effect of nisoldipine on developed tension (a) and purine efflux (b) from ischaemic myocardium. Rat hearts were perfused for 50 min under standard conditions, initially with a resting tension adjusted to about 15 g (control group: C). After 20 min of normoxic perfusion, 30 min of ischaemia (I) was induced in other hearts. Coronary flow was reduced by about 89%. Hearts in a third group were also made ischaemic, but they were perfused with medium containing 20 nM nisoldipine (I + N). This treatment began 15 min after the start of the perfusion, thus nisoldipine was already present before flow was reduced. Data, collected at the end of the perfusion, are presented. Developed tension at $t = 14$ min (22 ± 7 g) was taken as 100%. Mean values are given with vertical lines showing s.d. ($n = 4$). Striped section, adenosine; hatched section, inosine; stippled section, xanthine.

Despite the large differences in function of the normoxic heart, no significant differences in purine efflux were observed (Figure 8).

In contrast to the findings above, no significant differences in apex displacement were seen after 15 min of ischaemia, regardless of Ca^{2+}-concentration or the presence of nisoldipine (Figure 7). However, purine efflux from the ischaemic heart was increased by the higher Ca^{2+}-concentration ($P < 0.001$). Nisoldipine 100 nM suppressed purine production almost completely at low [Ca^{2+}], and reduced it by half at high [Ca^{2+}] (Figure 8).

Discussion

Nisoldipine showed a high affinity for silicon rubber tubing (Figure 1). We speculate that the isobutyl

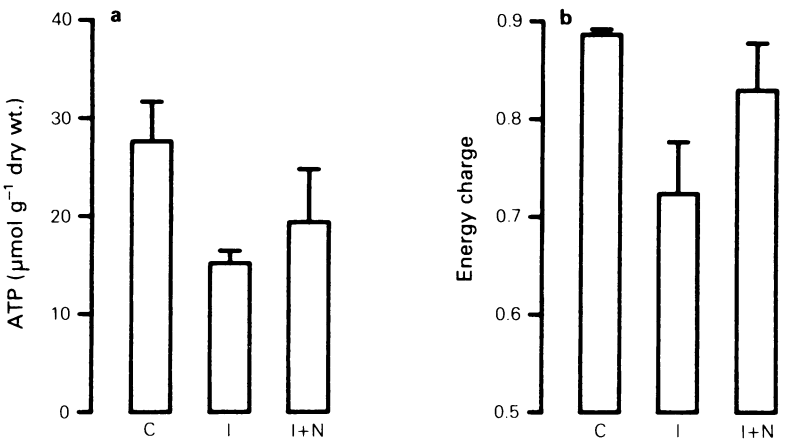

Figure 6 Protective effect of nisoldipine on ATP content (a) and adenylate energy charge (b) in ischaemic myocardium. Hearts were freeze-clamped after a control perfusion (C), or after a period of ischaemia (I). Where indicated by I + N, 20 nM nisoldipine was present. For other details, see legend to Figure 5.

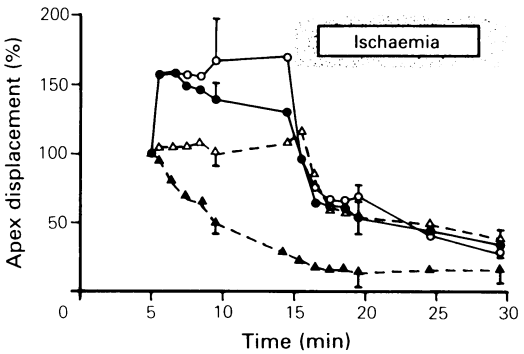

Figure 7 Effect of an increased Ca^{2+}-concentration in the perfusion medium on apex displacement (%). The value found 5 min after the start of the perfusion with standard medium was taken as 100%. Then perfusion took place with standard medium (1.4 mM $CaCl_2$) in the absence (△) or presence of 100 nM nisoldipine (▲), or medium containing 5.0 mM $CaCl_2$ in the absence (○) or presence of drug (●). Ischaemia was induced after 15 min normoxic perfusion by lowering the perfusion pressure from 9.6 to 2.3 kPa. Each point represents the mean and vertical lines s.d. ($n = 4$).

group in nisoldipine is responsible for this property. Our finding could have implications for the use of nisoldipine in, for instance, heart surgery.

Kazda *et al.* (1980) described the powerful vasodilator properties of nisoldipine. They noted that negative inotropy in guinea-pig isolated hearts is only apparent when higher doses are used. From a comparison of Figures 2 and 4, it is also clear that in rat heart effects on the vasculature are seen with much lower doses of nisoldipine than those on the myocardium.

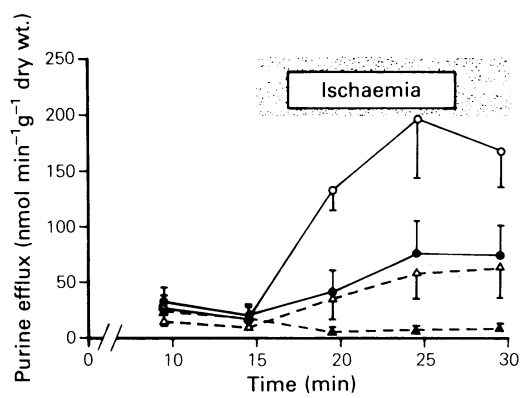

Figure 8 Effect of an increased Ca^{2+}-concentration in the perfusion medium on purine efflux (nmol min^{-1} g^{-1} dry weight). See legend to Figure 7. (△) Low Ca^{2+}, (▲) low Ca^{2+} + nisoldipine, (○) high Ca^{2+}, (●) high Ca^{2+} + nisoldipine.

In other studies, including those on calcium entry blocking agents, we used the efflux of purine nucleosides and oxypurines as a marker for myocardial ischaemia (see De Jong, 1979; De Jong *et al.*, 1982; 1984). Also in this study, ATP breakdown correlated well with the efflux of these purines (compare Figures 5 and 6). Nisoldipine proved to be an effective inhibitor of adenine nucleotide catabolism during ischaemia (Figures 3 and 5). About 50 nM reduced purine production by 50%. We estimate that the I_{50} is about 6 and 180 times higher for nifedipine and diltiazem, respectively.

It is likely that the protective effect of nisoldipine was caused by a reduction in contractile behaviour due to less Ca^{2+}-influx. Although at various doses of nisoldipine, apex displacement and developed tension in the ischaemic heart did not correlate with purine production (compare Figures 3 and 4, and see Figure 5), there is a strong correlation between normoxic function and ischaemic purine efflux (Figure 9). This occurs similarly with nifedipine and diltiazem (De Jong *et al.*, 1982; 1984). In addition, the sup-

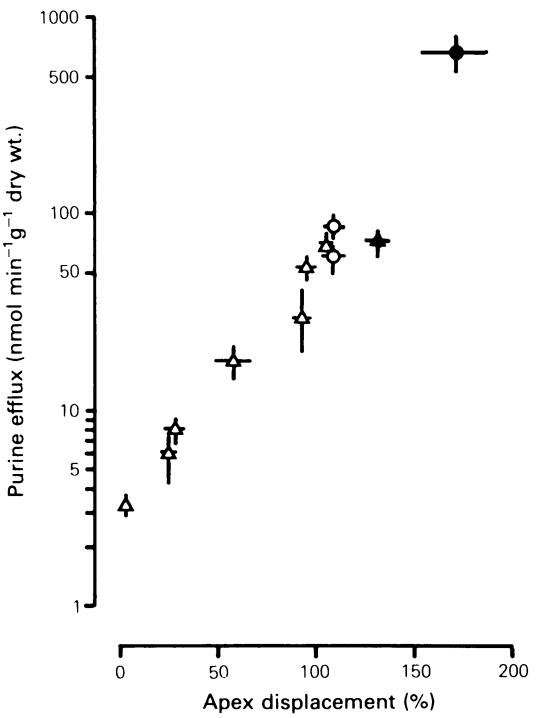

Figure 9 Correlation between normoxic function and ischaemic energy metabolism. Data were adapted from Figures 3, 4, 7 and 8. Open and closed symbols: 1.4 and 5.0 mM $CaCl_2$ in perfusion medium, respectively. Triangles: various concentrations of nisoldipine in perfusion medium. Each point shows mean and vertical and horizontal lines s.e. mean ($n = 4-7$).

pression of ATP breakdown by nisoldipine is antagonized by increased Ca^{2+}-concentrations in the perfusion medium (Figure 8).

In a recent editorial, Drake-Holland & Noble (1983) rejected the idea that the protective effect of calcium antagonists is due to the negative inotropism and salvage of creatine phosphate and ATP. According to these authors, recovery from ischaemia is not correlated with preservation of high-energy phosphate scores. However, from our, and other, studies (e.g., Nayler, 1982), it seems clear that these drugs could prevent ATP breakdown under a number of conditions. Whether they increase recovery after an ischaemic insult is less obvious.

Pang & Sperelakis (1983) showed that nitrendipine, an analogue of nisoldipine, enters cardiac muscle cells and is accumulated, probably through binding to internal sites. Diltiazem not only inhibits the Ca^{2+}-influx, but also acts intracellularly (Saida & Van Breemen, 1983). It protects (partially) against ATP breakdown, when it is administered during ischaemia (De Jong *et al.*, 1984). Diltiazem, verapamil and nifedipine have no influence on beef heart phosphodiesterase (Daly *et al.*, 1983), but Norman *et al.* (1983) found that nisoldipine and four other dihydropyridine Ca^{2+}-entry blockers selectively inhibit a myocardial cyclic AMP phosphodiesterase. Therefore, the possibility cannot be excluded that Ca^{2+}-entry blockers, such as nisoldipine, could directly affect the enzymatic machinery, thereby conserving energy during myocardial ischaemia.

We greatly appreciate the encouragement by Prof. P.G. Hugenholtz and secretarial help of Mrs M.J. Kanters-Stam. We are indebted to Dr R. Gross (Bayer, Wuppertal, GFR) for his suggestions and gift of nisoldipine.

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(*Received February 24, 1984. Revised July 19, 1984.*)