European Heart Journal (1983) **4** (*Supplement C*), 109–113

Protective effect of nifedipine in myocardial ischemia assessed by phosphorus-31 nuclear magnetic resonance

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KEY WORDS: Phosphorus-31 nuclear magnetic resonance, myocardial ischemia, calcium antagonist, nifedipine, intracellular pH, adenosine triphosphate, creatine phosphate.

Calcium antagonists may protect the myocardium against the consequences of ischemia. Phosphorus-31 nuclear magnetic resonance (^{31}P NMR) was used to study the effect of nifedipine on intracellular acidosis and high energy phosphate depletion during global ischemia. Isolated rat hearts were paced (300 beats/min), perfused with a modified Tyrode solution for 30 min, made totally ischemic for 30 min (37°C) and then reperfused for 30 min. When required, nifedipine (1 mg/l) was added to the perfusion fluid 10 min before ischemia.

During ischemia intracellular pH fell from 7·11±0·03 (mean±S.E.M.) to 5·88±0·04 in the untreated hearts (n=6), and from 7·11±0·03 to 5·95±0·02 in the treated hearts (n=6). During the first 20 min of ischemia, intracellular pH was significantly higher in the treated than in the untreated hearts ($P<0·001$). Myocardial creatine phosphate (CP) content was depleted after 15 min of ischemia in the untreated hearts, and after 20 min of ischemia in the hearts treated with nifedipine. Myocardial adenosine triphosphate (ATP) content was depleted after 20 min of ischemia in the untreated hearts; ATP content in hearts that received nifedipine amounted to 23·5±6·2% of control after 30 min of ischemia. In contrast with the untreated hearts, the nifedipine-treated hearts showed a rapid recovery of CP content during reperfusion.

The results indicate that nifedipine protects the myocardium against the metabolic consequences of ischemia and reperfusion.

Myocardial ischemia evokes a variety of changes, including a depletion of endogenous high-energy phosphate stores[1], intracellular acidosis[2], accumulation of calcium[3] and the development of contracture[1]. Nifedipine given prior to or at the onset of a period of ischemia has been shown to maintain tissue CP at ATP[4,5] and to prevent calcium accumulation and contracture[3]. In addition, nifedipine prevented the massive uptake of calcium during reperfusion and promoted the recovery of myocardial contractility[3].

Phosphorus-31 nuclear magnetic resonance (^{31}P NMR) is a non-destructive method permitting repetitive measurements of intracellular pH[6] as well as the CP and ATP content[7] in the isolated, intact heart. The present study was designed to test the effect of nifedipine on intracellular acidosis and high-energy phosphate depletion in the isolated rat heart during 30 min of normothermic global ischemia.

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Materials and methods

PERFUSION METHODS

Male Wistar rats that weighed 400–450 g were anesthetized with diethyl ether and heparinized. Their hearts were removed and subsequently perfused at 37°C using the Langendorff technique[8] at a constant pressure of 10·0 kPa (75 mm Hg). The perfusate had the following composition (mmol/l): NaCl, 124; KCl, 4·7; $CaCl_2$, 1·3; $MgCl_2$, 1·0; $NaHCO_3$, 24·0; Na_2HPO_4, 0·5; glucose, 11·0. After equilibration with 95% O_2–5% CO_2, the pH was 7·40±0·05. The heart rate was maintained at 300 beats/min by left ventricular pacing with a KCl-wick electrode, connected to a Grass S88 stimulator. After 30 min of control perfusion, the

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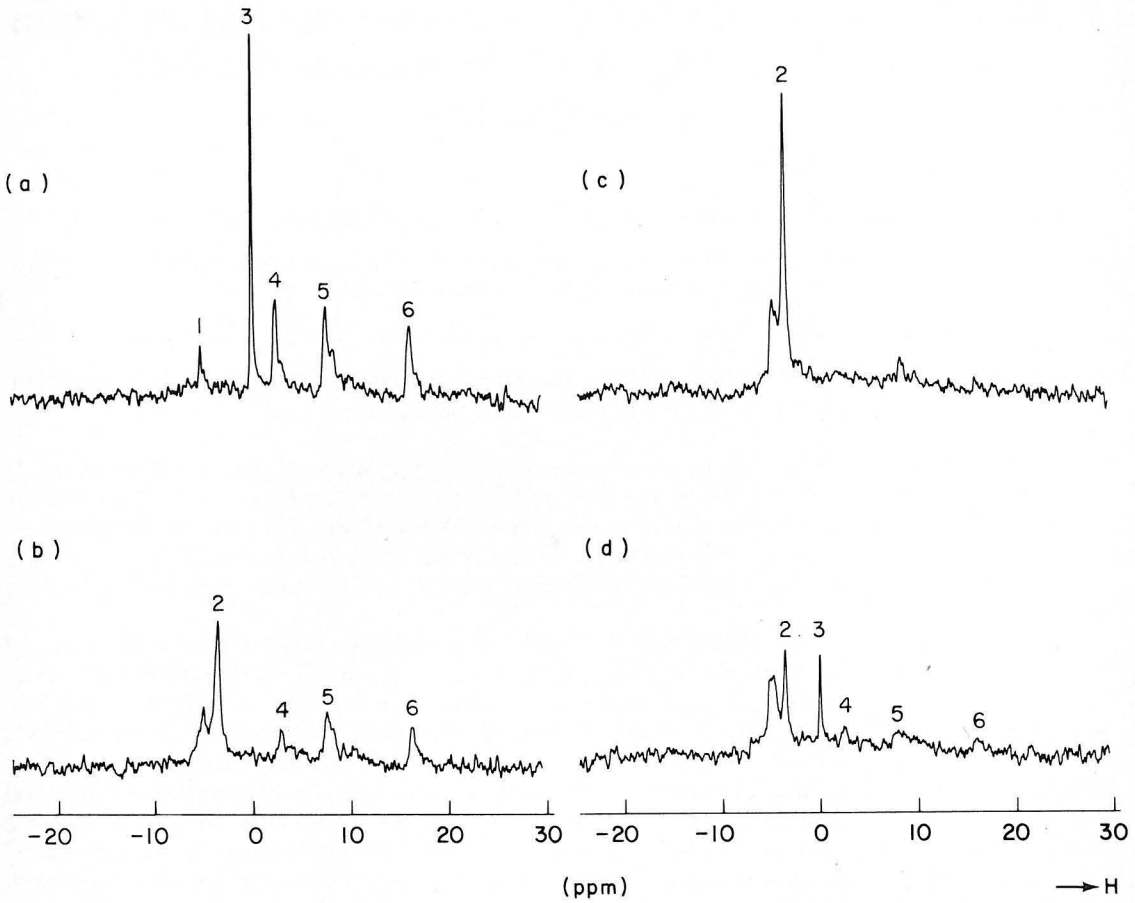

Figure 1 ^{31}P NMR spectra obtained (a) between 20 and 30 min of control perfusion, (b) between 5 and 15 min of total ischemia, (c) between 20 and 30 min of total ischemia and (d) between 20 and 30 min of reperfusion. The spectra were obtained from 260 radiofrequency pulses. The arrow indicates the direction of increasing field strength. Numbered peaks include (1) extracellular inorganic phosphate (Pi(ext)), (2) intracellular inorganic phosphate (Pi(int)), (3) creatine phosphate (CP), and (4) the $\gamma-$, (5) $\alpha-$and (6) $\beta-$phosphate groups of adenosine triphosphate (ATP). Note the marked increase of the Pi(int) peak and the absence of the high-energy phosphate peaks at the end of the ischemic period.

hearts were made totally ischemic for 30 min and then reperfused for 30 min. Myocardial temperature was maintained at 37°C throughout the experiment. When appropriate, nifedipine (1 mg/l) was added to the perfusion fluid 10 min before ischemia. Bayer AG (Leverkusen, F.R.G.) supplied ampoules containing a 0·1 mg/ml solution of nifedipine (solvent: ethanol/polyethylene glycol/water, 15/15/70). Care was taken that the nifedipine-containing perfusate was not exposed to light[9].

NUCLEAR MAGNETIC RESONANCE METHODS

Spectra of ^{31}P NMR at 81·0 MHz were recorded without proton decoupling and ^{2}H-lock on a Bruker WP200 spectrometer using a 5 kHz spectral width, a 90° pulse angle, an acquisition time of 0·82 s and a pulse repetition rate of 2·32 s. Accumulated free induction decays were obtained from 130 or 260 transients on submerged rat hearts in a total volume of 10 ml in 20 mm tubes and exponentially multiplied resulting in 10 Hz line broadening. Zero ppm was assigned to the resonance position of CP at pH 7·0.

Intracellular pH was measured from the chemical shift of the Pi(int) peak, using a titration curve obtained from a solution containing ATP (10 mmol/l), CP (10 mmol/l), Pi (10 mmol/l), NADPH (10 mmol/l), glucose-6P (10 mmol/l), and $MgCl_2$

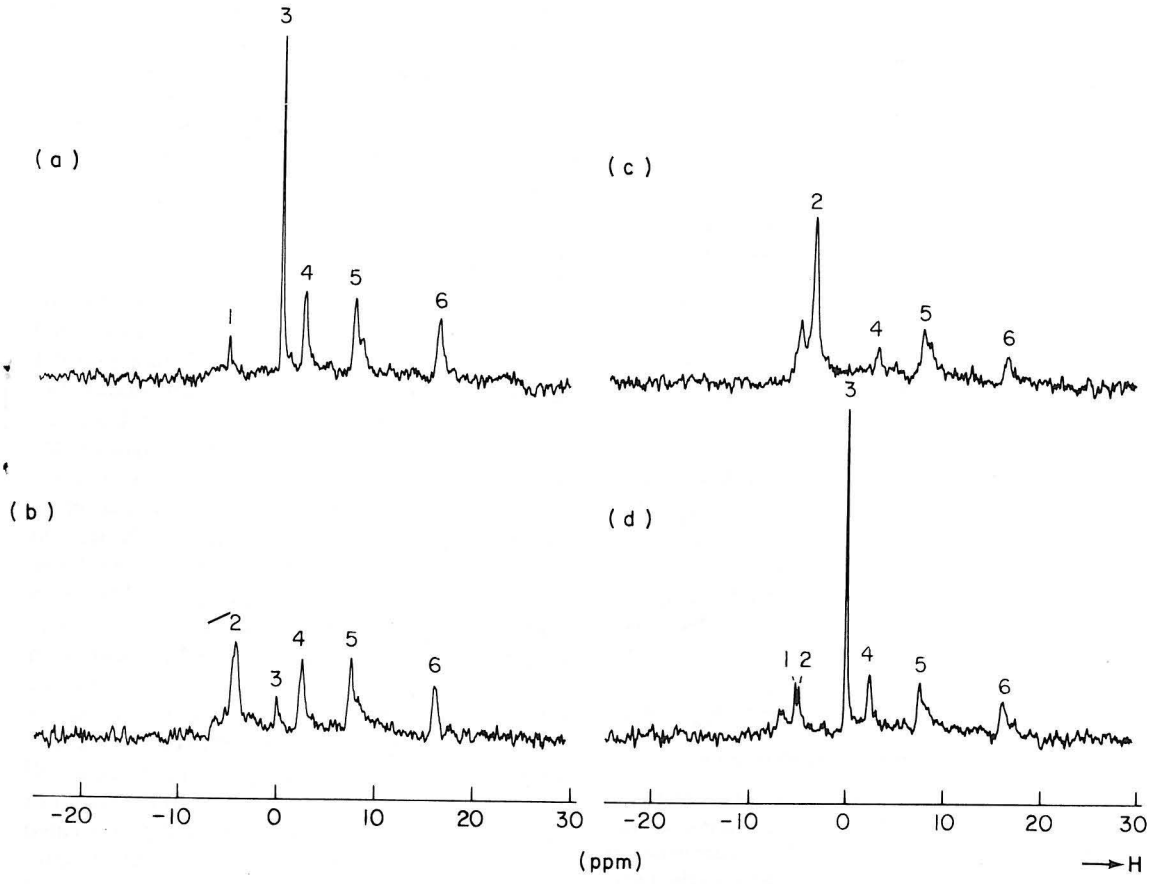

Figure 2 ^{31}P NMR spectra obtained (a) between 20 and 30 min of control perfusion in the presence of 1 mg/l nifedipine, (b) between 5 and 15 min of total ischemia, (c) between 20 and 30 min of total ischemia and (d) between 20 and 30 min of reperfusion. The spectra were obtained from 260 radiofrequency pulses. The arrow indicates the direction of increasing field strength. Numbered peaks: see legend to Fig. 1. Note the relative preservation of the β–ATP peak at the end of the ischemic period (spectrum c), and the decline of the Pi(int) peak and return of the CP peak during reperfusion.

(10 mmol/l). Myocardial CP and ATP levels were measured by integrating the CP and β-ATP signals. CP and ATP levels are expressed as percentage of the pre-ischemic control levels. Results are expressed as mean±S.E.M. of six experiments. Tests of significance were made using the unpaired t-test, taking $P=0{\cdot}05$ as the limit of significance.

Results

Figures 1 and 2 show typical ^{31}P NMR spectra of rat hearts during control perfusion, ischemia, and reperfusion. Figures 1(a) and 2(a) represent control spectra, obtained during perfusion without and with nifedipine, respectively. During 30 min of total ischemia, there was complete depletion of myocardial high-energy phosphates in the untreated heart (Fig. 1 b and c). In the treated heart, CP and ATP levels decreased less rapidly (Fig. 2 b and c). In contrast with the untreated heart, the nifedipine-treated heart showed a marked decline of the Pi(int) peak and a concomitant return of the CP peak during reperfusion (Figs 1 d and 2 d).

In the untreated hearts intracellular pH fell from 7·11±0·03 during control perfusion to 5·88±0·04 at the end of the 30 min period of ischemia (Fig. 3). When 1 mg/l nifedipine was added to the perfusion fluid 10 min before the onset of ischemia, intracellular pH fell from 7·11±0·03 to 5·95±0·02 during ischemia. Intracellular pH after 30 min of

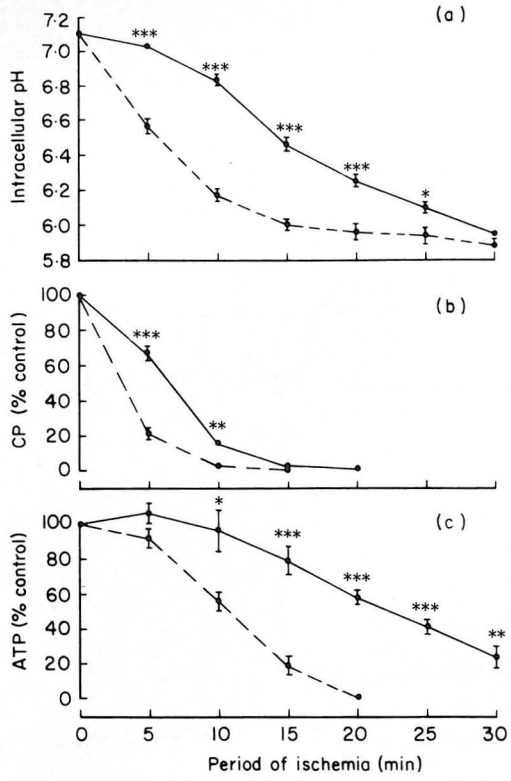

Figure 3 Time course of intracellular pH (a), and myocardial CP (b) and ATP (c) levels during 30 min of normothermic total ischemia. CP and ATP levels are expressed as percentage of the pre-ischemic control levels. In the treated hearts (——), 1 mg/l nifedipine was added to the perfusion fluid 10 min before the onset of ischemia. – – – – – = Control. Measurements were obtained from 130-pulse spectra (5 min), collected at 0–5, 5–10, 10–15, 15–20, 20–25 and 25–30 min of ischemia. Each point is the mean ± s.e.m. of six experiments. $*P<0{\cdot}05$; $**P<0{\cdot}005$; $***P<0{\cdot}001$.

ischemia did not differ significantly between the treated and untreated group. Until 25 min of ischemia, however, intracellular pH was significantly higher in the treated hearts ($P<0{\cdot}001$ until 20 min of ischemia; $P<0{\cdot}05$ at 25 min of ischemia).

During ischemia, CP levels decreased rapidly in both groups. Until 10 min of ischemia, however, the rate of decrease in CP content was lower in the treated than in the untreated hearts ($P<0{\cdot}001$ at 5 min of ischemia; $P<0{\cdot}05$ at 10 min of ischemia) (Figs 1b, 2b and 3). Myocardial CP content was depleted after 15 min of ischemia in the untreated hearts, and after 20 min of ischemia in the hearts treated with nifedipine.

After 20 min of ischemia, myocardial ATP content of the untreated hearts was depleted (Fig. 3), whereas the treated hearts still contained $58{\cdot}6 \pm 4{\cdot}4\%$ of the pre-ischemic control level ($P<0{\cdot}001$). After 30 min of ischemia, ATP content in hearts that received nifedipine amounted to $23{\cdot}5 \pm 6{\cdot}2\%$ of control (Figs 2c and 3).

Discussion

The results of the present study show that addition of 1 mg/l nifedipine to the perfusion fluid during 10 min before the onset of ischemia, protects the rat heart against some of the consequences of 30 min of normothermic global ischemia. Evidence of this protection was provided by a significantly lower rate of development of intracellular acidosis and a significantly lower rate of decrease in myocardial CP and ATP content during ischemia. At the end of the ischemic period, both treated and untreated hearts were depleted of CP. This is in agreement with the results of Flaherty *et al*[7], who used ^{31}P NMR to study the effect of administration of a hyperkalemic cardioplegic solution during 60 min of hypothermic global ischemia in rabbit hearts. Although the rates of CP decline were dissimilar in the different groups of their study, all hearts were equally depleted of CP after 60 min of ischemia. Our finding that the nifedipine treated hearts contained $23{\cdot}5 \pm 6{\cdot}2\%$ of control after 30 min of ischemia and showed a rapid recovery of CP and ATP levels during reperfusion (Fig 2 a and d) lends support to the suggestion of Flaherty *et al.*[7] that preservation of ATP content is the better metabolic correlate of functional recovery. We are aware that nifedipine was added in a high dose, and that the protective effect we observed may be a consequence of the cardioplegic effect of the drug.

Our data show a qualitative resemblance to the results of Nayler *et al.*[4]. In their study rabbits were injected subcutaneously twice daily with 2 mg/kg nifedipine for 4 to 5 days. The hearts were then isolated and made ischemic for 90 min. Pretreating the rabbits with nifedipine resulted in a significant preservation during normothermic ischemia of myocardial CP and ATP stores measured with the freeze-clamp method.

Magee *et al*[10] compared the effect of a cardioplegic dose of nifedipine, given at the onset of 90 min of hypothermic ischemic arrest, to hypothermia alone and to hypothermia with potassium cardioplegia. Isolated feline hearts were used in their study and the cardioplegic dose consisted of 10 ml

of perfusate containing 100 μg of nifedipine. In our study, the hearts received a comparable dose of nifedipine during 10 min of perfusion with fluid containing 1 mg/l nifedipine before the onset of 30 min of normothermic ischemic arrest. Magee *et al.*[10] demonstrated that nifedipine in a cardioplegic dose resulted in preservation of myocardial structure and function that was similar to that obtained with potassium cardioplegia.

In conclusion, our results indicate that nifedipine protects the myocardium against some of the metabolic consequences of ischemia and reperfusion. ^{31}P NMR is a valuable tool for studying interventions designed to protect the myocardium.

The authors are very appreciative of the excellent technical assistance of D. de Moes and P. van der Meer.

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