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Captopril improves recovery of adenosine triphosphate during reperfusion of the ischemic isolated rat heart; a 31-phosphorus-nuclear magnetic resonance study

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Summary: The effect of captopril on energy-rich phosphates and pH during normothermic ischemic arrest, hypothermic cardioplegic arrest and subsequent reperfusion was investigated in the isolated rat heart using 31P-nuclear magnetic resonance. The hearts remained in the probe during all perfusion procedures and captopril (80 ml · l^{-1}) treatment was started directly after cannulation.

After normothermic ischemic arrest (15 min), the ATP content of captopril-treated hearts was not significantly different from that of untreated hearts (53 ± 9 % and 52 ± 8 %, respectively). Accumulation of inorganic phosphate at the end of ischemia was significantly less in treated hearts, suggesting a higher end-ischemic nucleotide content in treated hearts.

Hypothermic cardioplegic arrest (St. Thomas' Hospital solution, 4 °C) lasted for 3 h at 10 °C. Adenosine triphosphate in treated hearts was significantly lower at the end of ischemia; 36 ± 6 % compared to 53 ± 9 % for untreated hearts.

Adenosine triphosphate in untreated hearts recovered to 76 ± 9 % after normothermic ischemia and to 72 ± 7 % after hypothermic ischemia at the end of 30 min reperfusion. Captopril significantly improved adenosine triphosphate recovery in both treated groups; 89 ± 4 % after normothermic and 83 ± 4 % hypothermic ischemia.

We conclude that captopril has a beneficial effect on recovery of adenosine triphosphate both after normothermic and after hypothermic ischemia.

Key words: captopril in ischemia; captopril in cardioplegia; adenosine triphosphate; 31-phosphorus nuclear magnetic resonance

Introduction

Reducing ischemic damage by the addition of drugs to coronary infusates is an important way of obtaining effective myocardial protection under ischemic conditions.

A number of drugs can be employed for their specific effects against ischemia-induced metabolic changes (11) or against reperfusion-induced damage (5, 12).

For many drugs investigated for their cardioprotective properties, however, it is not clear which metabolic processes are particularly influenced. Captopril, an angiotensin-converting enzyme inhibitor, has been shown to reduce purine overflow upon reperfusion of ischemic hearts (3), suggesting that high energy phosphates in treated hearts were maintained at higher levels.

Whether or not this was indeed due to the maintenance of higher ATP levels during ischemia or whether reperfusion-induced damage and subsequent purine loss was coun-

teracted could not be concluded from those findings. Therefore this study was undertaken to monitor the metabolism of adenosine triphosphate (ATP) during ischemia and subsequent reperfusion with 31-phosphorus-nuclear magnetic resonance (31P-NMR).

In addition, the effects of temperature were studied, since some agents, e.g. calcium antagonists, are effective during normothermia (6) and not during hypothermia (14, 19). Therefore, in order to establish whether captopril maintains its beneficial effect during hypothermia we also compared normothermic and hypothermic conditions.

Materials and Methods

Perfusion model

Male Wistar rats (220–260 g) were anesthetized with ether and given 500 IU of heparin intravenously. After rapid excision, the hearts were cooled in ice-cold saline. The aorta was cannulated and retrograde perfusion according to Langendorff was started. Hearts were then allowed to beat spontaneously. The perfusion fluid was a modified Krebs-Henseleit buffer containing, in mM: NaCl 128.2; KCl 4.7; $CaCl_2$ 1.3; $MgCl_2$ 1.1; $NaHCO_3$ 20; glucose 10. In accordance with other investigators, no inorganic phosphate was added (9, 10, 13, 18). The perfusion fluid was equilibrated with 95 % O_2 and 5 % CO_2 in a reservoir at 40 °C, warranting an oxygenated coronary perfusion at 37 °C. The perfusion pressure was hydrostatically maintained at 80 cm H_2O. The hearts were placed in a 15-mm diameter NMR-tube, while the perfusion tubing within the NMR-tube was centralized and supported by teflon discs. The hearts were immersed in the perfusate and excess perfusate was continuously aspirated using a vacuum suction pump. Global ischemia was accomplished by interrupting flow (study A). In study B, hearts were arrested with the St. Thomas' Hospital cardioplegic solution (8), which contained, in mM: NaCl 110; KCl 16; $MgCl_2$ 16; $CaCl_2$ 1.2; $NaHCO_3$ 10.

The pH of the St. Thomas' Hospital solution was adjusted to 7.8 by titration with hydrochloric acid. The solution was then equilibrated with 100 % O_2 in a reservoir on melting ice for perfusion at a hydrostatic pressure of 80 cm H_2O.

NMR methods

Measurements were performed in a Bruker narrow bore 360 MHz magnet using a 15-mm home built 31P probe. Spectra were collected using 60° pulses and a relaxation delay of 2 s. A frequency lock was not required. Signal-to-noise enhancement was accomplished using a negative exponential equivalent to a resonance line broadening of 30 Hz. Peak areas were measured by line fitting of all peaks.

A change in temperature during the experiments in study B appeared to influence the tuning characteristics of the probe resulting in a higher signal-to-noise ratio at lower temperatures. It was therefore impossible to relate the intensities of spectra obtained at different temperatures during the experiment. The chemical shift of inorganic phosphate (P_i) relative to the resonance frequency of creatine phosphate (CP) was used to calculate intracellular pH by means of the following equation (1): $pH_i = pK - \log \frac{\delta o - \delta b}{\delta a - \delta o}$; δo represents the difference in measured resonance position of P_i and CP in parts per million (ppm) at a given pH. The constants used in the equation are pK = 6.90, δ_a = 3.290 ppm and δ_b = 5.805 ppm.

Experimental design

Study A

12 hearts were studied. Six served as controls and six were treated with captopril (80 mg · l^{-1}). After 20 min of equilibration the perfusion was stopped for 15 min. Subsequent reperfusion lasted for 30 min. During the entire experiment, the temperature of probe and perfusate was maintained at 37 °C. Captopril treatment was started immediately after

Table 1. Effect of captopril on accumulation of inorganic phosphate during normothermic (A) and hypothermic (B) ischemia. Values are obtained from NMR spectra and expressed in cm^2.

A	0–5 min	5–10 min	10–15 min	
Saline	2.2 ± 0.5	2.8 ± 0.7	3.6 ± 0.6	
Captopril	1.6 ± 0.1	2.2 ± 0.2	2.5 ± 0.2*	
B	0.5 h	1.0 h	2.0 h	3.0 h
Saline	1.4 ± 0.1	2.0 ± 0.2	2.7 ± 0.3	3.0 ± 0.4
Captopril	1.2 ± 0.1	1.8 ± 0.2	2.3 ± 0.3	2.9 ± 0.7

* Significant difference when compared with untreated hearts ($p < 0.05$)

cannulation of the aorta and was continued during reperfusion. During the last 10 min of the preischemic period, a control 31P-NMR spectrum (300 transients) was obtained. After the onset of ischemia, six 75 transient spectra (2.5 min) were obtained in immediate succession. Intracellular pH was calculated from 75 transient spectra. Intensities of the phosphorus metabolites were measured from 150 transient spectra obtained by addition of two 75 transient spectra, accumulated between 0–5 min, 5–10 min and 10–15 min, respectively. During reperfusion, two 300 transient spectra were obtained between 5–15 min and 20–30 min, respectively. Values of CP and ATP during ischemia and reperfusion were expressed as percentages of their preischemic values. No reliable control values of P_i could be obtained, because of very low P_i intensities prior to ischemia. Its accumulation during ischemia is expressed as the mean of integrated intensities for treated and untreated groups (Table 1). Examples of 300 and 150 transient spectra obtained during normothermic perfusion and ischemia are shown in Fig. 1.

Study B

12 hearts were studied of which six were treated with captopril ($80\ mg \cdot l^{-1}$). After 30 min equilibration, cardioplegic arrest was accomplished during a 4-min perfusion with the St. Thomas' Hospital solution. The temperature of this cardioplegic infusate was 4 °C; during administration the probe was cooled to 10 °C. The probe temperature was thermostatically maintained at 10 °C during 3 h of ischemia. Upon reperfusion, the temperature was raised again to 37 °C. Captopril treatment was started at the onset of perfusion and was continued throughout the experiment. Captopril ($80\ mg \cdot l^{-1}$) was also added to the cardioplegic solution. A 300 transient spectrum (10 min) was obtained prior to cardioplegic arrest, serving as a control for reperfusion measurements. Another 300 transient spectrum was obtained immediately after cardioplegic perfusion and served as control for six subsequent 300 transient spectra obtained at 30-min intervals during ischemia. During subsequent reperfusion (30 min) two spectra were obtained between 5–15 min and 20–30 min. Accumulation of P_i during ischemia was expressed as the mean of integrated intensities for the treated and untreated groups.

Statistical procedures

Results shown in the tables and graphs are means ± SEM. The means were compared by using the Student's t-test and $p = 0.05$ was taken as the limit of significance.

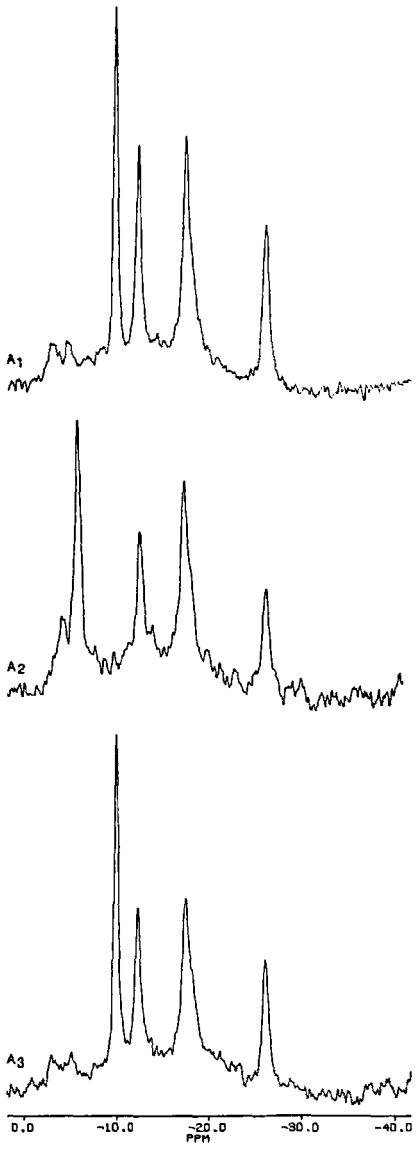

Fig. 1. Spectra obtained in study A; normothermic ischemia. A_1: equilibration (300 transients); A_2: 10–15 min of ischemia (150 transients); A_3: reperfusion (300 transients).

Results

Study A

Equilibration: Absolute intensities of ATP and CP in captopril-treated hearts were not different from those in untreated hearts when the means were compared. A reliable estimate

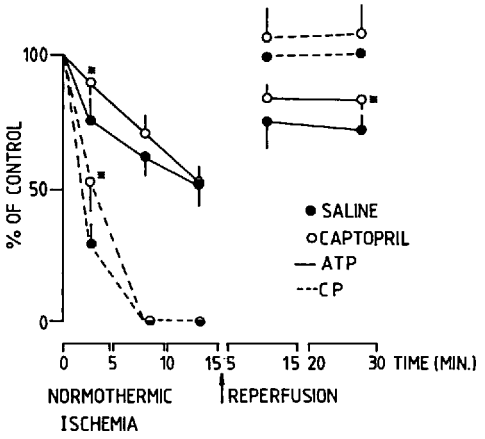

Fig. 2. Changes in ATP and CP for treated and untreated hearts during normothermic ischemic arrest and subsequent reperfusion are plotted against time.
* Significant difference when compared with untreated hearts ($p < 0.05$)

of the intracellular pH could not be obtained during equilibration since no P_i could be detected.

Normothermic ischemia: CP disappeared after 10 min of ischemia in both untreated and captopril-treated hearts. However, CP in captopril-treated hearts decreased significantly slower during the first 5 min (to $53 \pm 12\%$ and $30 \pm 8\%$, respectively; Fig. 2). ATP in untreated hearts decreased to $53 \pm 9\%$ of initial values at the end of ischemia. In captopril-treated hearts, ATP decreased more slowly initially, but end-ischemic levels were comparable to untreated hearts ($52 \pm 8\%$). Significant differences were apparent during the first

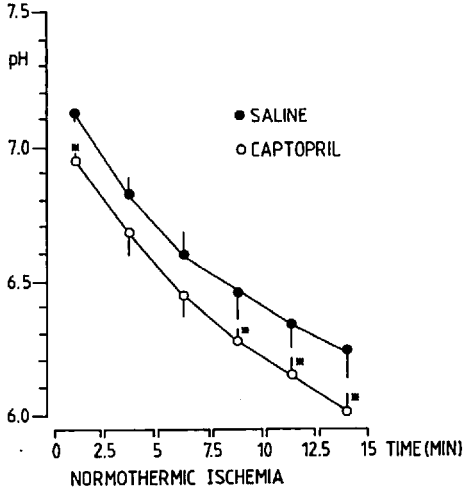

Fig. 3. Decrease of pH during normothermic ischemic arrest for treated and untreated hearts.
* Significantly lower pH when compared with untreated hearts ($p < 0.05$)

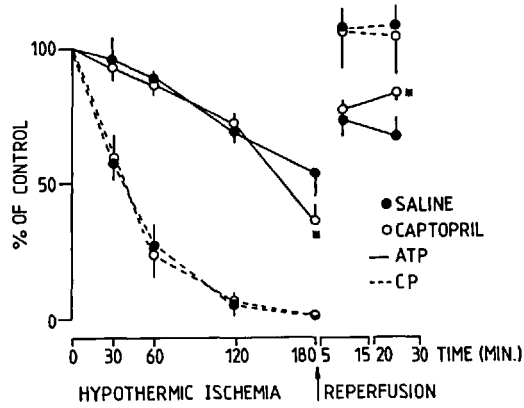

Fig. 4. Changes in ATP and CP for treated and untreated hearts during hypothermic cardioplegic arrest and subsequent reperfusion are plotted against time.
* Significant difference when compared with untreated hearts ($p < 0.05$)

5 min; 89 ± 5 % compared to 76 ± 9 % for untreated hearts (Fig. 2). Intracellular pH in captopril-treated hearts was already significantly lower at the onset of ischemia and decreased at the same rate as untreated hearts to a significantly lower end-ischemic level, 6.01 ± 0.07 and 6.24 ± 0.11 for untreated hearts (Fig. 3). Accumulation of inorganic phosphate was clearly less in treated hearts during the entire period of ischemia and significantly lower at the end of ischemia (Table 1A).

Reperfusion: ATP in untreated hearts returned to 75 ± 11 % of preischemic values after 5 min of reperfusion, followed by a slight fall to 72 ± 7 % at the end of reperfusion. CP recovered completely and remained constant during the entire reperfusion period (Fig. 2). ATP recovered to higher levels in treated hearts than in untreated hearts, which was significant after 20 min, 83 ± 4 % versus 72 ± 7 %. CP in treated hearts recovered above initial values during the entire reperfusion period (Fig. 2), but no significant differences were observed when compared to untreated hearts. Inorganic phosphate was not detectable upon reperfusion, and thus no intracellular pH could be obtained.

Study B

Equilibration and cardioplegic perfusion: No inorganic phosphate was detected and therefore intracellular pH could not be estimated during normothermic equilibration. Changes in the levels of the phosphorus metabolites due to oxygenated cardioplegic perfusion after equilibration could not be assessed, because of the changes in probe tuning characteristics, as mentioned in the Methods section. However, peak height ratios of all peaks measured immediately after arrest were similar to those during equilibration.

The intracellular pH was above physiological levels as a consequence of the alkaline cardioplegic solution (7.8). The pH was 7.58 ± 0.03 for treated hearts and 7.56 ± 0.1 for controls.

Hypothermic ischemia: Figure 4 shows that ATP decreased steadily and linearly in untreated hearts; after 3 h 53 ± 9 % remained. CP decreased relatively fast within the first hour to 27 ± 8 % and had almost disappeared after 2 h. Intracellular pH (Fig. 5) dropped linearly during the first 1½ h (0.92 units) continuing to decline less rapidly during the second half of the ischemic period (0.48 units) and resulting in an end-ischemic pH of 6.16 ± 0.04. The

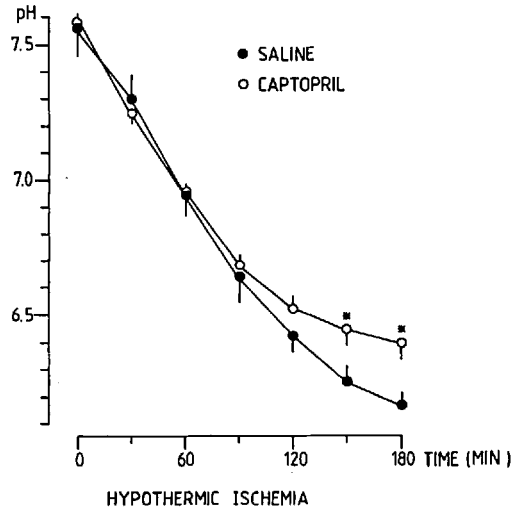

Fig. 5. Time course of tissue pH during hypothermic cardioplegic arrest after an initial perfusion for 4 min (arrow on abscissa).
* $p < 0.05$ when compared with untreated hearts.

decrease of ATP in captopril-treated hearts was similar to that in untreated hearts during the initial phase of ischemia, 72 ± 4 % compared to 69 ± 5 % for untreated hearts after 2 h. Thereafter, a further decrease in ATP in treated hearts resulted in a significantly lower end-ischemic ATP; 36 ± 6 % compared to 53 ± 9 % for untreated hearts (Fig. 4). Intracellular pH in captopril-treated hearts decreased similarly as in untreated hearts (0.90 units) during the first 1½ h. Thereafter captopril-treated hearts showed a more gradual decline in pH (0.29 units) resulting in a significantly higher end-ischemic pH 6.39 ± 0.07 compared to 6.16 ± 0.04 for untreated hearts (Fig. 5).

Table 1B shows that the accumulation of inorganic phosphate was similar in both groups. *Reperfusion:* ATP in untreated hearts recovered to 73 ± 7 % of preischemic values initially but showed a subsequent decrease to 67 ± 7 % at the end of reperfusion. The initial recovery of ATP to 77 ± 3 % in treated hearts was similar to untreated hearts, but in contrast to the subsequent decrease in untreated hearts, ATP in all treated hearts recovered even more at the end of the reperfusion period, resulting in a significantly higher ATP; 83 ± 3 % versus 67 ± 7 %.

CP, in both groups, recovered to above the initial level and was stable during the entire period showing no (significant) differences between treated and untreated hearts (Fig. 4). Inorganic phosphate was not detectable during reperfusion and therefore intracellular pH could not be obtained for either group.

Discussion

NMR spectroscopy has the advantages of monitoring ATP metabolism continuously while tissue integrity is maintained. However, it is difficult to calibrate signals obtained from intramyocardial phosphorus metabolites with absolute tissue concentrations, as it is necessary to calibrate for each heart individually. But it has been shown that changes in peak intensities correlate linearly with tissue concentrations (10).

In this study, we have shown that captopril improves the recovery of ATP during reperfusion, both after normothermic and after hypothermic ischemia.

During normothermic ischemia, ATP and CP levels decreased at a slower rate and accumulation of inorganic phosphate was significantly reduced compared to untreated hearts. This suggests that, at the end of normothermic ischemia, the degree of dephosphorylation in treated hearts was reduced and that more nucleotides were readily available for rephosphorylation to ATP upon reperfusion. However, the difference in levels of inorganic phosphate in both groups could also be due to a different membrane phospholipid metabolism or a difference in precipitation with free calcium, which is elevated during ischemia (11).

During hypothermic ischemia, captopril did not reduce the rate of ATP catabolism. ATP levels were actually even lower at the end of the hypothermic period when compared to untreated hearts. However, captopril treated hearts showed an improved maintenance of intracellular pH during this period. Apparently this pH effect is not the result of reduced hydrolysis of ATP (9, 18). A possible explanation for this phenomenon could be an inhibition of glycolytic flux, which would lead to a slower decrease of intracellular pH and an increased rate of ATP depletion (2).

Another observation is the lack of a relationship between end-ischemic ATP levels and the recovery at ATP during reperfusion under both conditions. Therefore, we conclude that prior to reperfusion ATP levels as such are poor markers for myocardial recovery, which was also found by others (13, 17). The amount of precursors available for rephosphorylation probably correlates much better with recovery of ATP levels. This underlines the importance of monitoring the loss of purines during ischemia and reperfusion (17).

The more efficient restoration of ATP in treated hearts could be due to an effect of captopril in the reperfusion phase. This could be due to a better preservation of ATP-generating enzyme systems (12, 15) or by counteraction of reperfusion-induced tissue injury (5, 12).

The ATP and CP levels were much better maintained during hypothermic cardiac arrest than during normothermia. For instance, a drop in ATP levels to approximately 50% occurred after 15 min normothermia, whereas this reduction took 180 min during hypothermic cardioplegia. Interestingly, captopril improved recovery of the heart, as measured by ATP and CP levels, under this condition of improved ischemic tolerance. Its effects on ischemic metabolism, however, appeared to be different under both conditions. During normothermic ischemia, effects of captopril are apparent during the initial phase of ischemia, whereas during hypothermic ischemia, effects appear in a later phase. The latter could be due to hypothermia and the use of the St. Thomas' Hospital solution, which make it difficult for any drug to superimpose an additional effect, especially during the initial phase. During normothermic ischemia, however, it is conceivable that any protective effect will already be observed early in ischemia. This is also seen with Ca entry blockers; besides their attributed effects during normothermic ischemia, it has been proposed that they also exert a cardioplegic effect by lowering oxygen demand and contractility (16). With captopril, such an effect seems unlikely, because previous studies have shown that captopril does not influence pressure rate index (3).

The effect of captopril might be due to another mechanism which is temperature dependent. Previous studies have shown that captopril influences the catecholamine overflow due to ischemia and reperfusion (4). It is conceivable that the effect of a reduced catecholamine overflow will be more pronounced under normothermic conditions than during hypothermia (Study B). During normothermic reperfusion, overflow of catecholamines will impair recovery. Therefore, captopril might be effective, especially during this phase, which is in accordance with our own findings. This mechanism must be considered

speculative until catecholamine efflux data from hypothermic cardiac arrest experiments are available.

In conclusion, the observed effects indicate that captopril may be beneficial as an additive to cardioplegic solutions and during normothermic ischemia followed by reperfusion. The latter effect makes captopril potentially useful as comedication during thrombolytic procedures, which are increasingly performed clinically. However, for a rational use of captopril in these settings, further elucidation of the underlying mechanism is needed.

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