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The Phosphate Pool of Isolated Dog Heart During Global Ischaemia: Comparison of Two Cardioplegic Solutions with ^{31}P NMR Spectroscopy

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Summary

^{31}P NMR spectroscopy was used to study the time course of changes in the concentration of high-energy metabolites and intracellular pH in the dog myocardium during hypothermic ischaemia at 9 °C in Bretschneider (HTK-B) and St. Thomas' Hospital (StTH) cardioplegic solutions. It was found that ATP and phosphocreatine degrade slowlier in HTK-B than in StTH, with phosphocreatine depletion occurring within 7.9 ± 1.4 h in HTK-B and within 6.2 ± 1.4 h in StTH. The values are virtually identical with the time intervals at which ATP concentration falls below the critical level (60 % of initial ATP concentration). In agreement with biochemical analysis, a higher concentration of phosphomonoesters was noted until the 180th minute of ischaemia in HTK-B, a finding suggesting more rapid glycogen degradation in HTK-B. Even though HTK-B contains a high concentration of histidine buffer, higher values of intracellular pH were found during ischaemia in StTH. The effect of extracellular concentration of sodium ions on intracellular pH is discussed.

Key words:
^{31}P NMR spectroscopy – Ischaemic heart – Cardioplegia

Introduction

One of the main problems in heart transplantation is the limited preservation time of myocardial tissue. The reported markers of myocardial viability are high energy phosphates, especially ATP which seems to be the determinant factor of tissue injury (Hearse *et al.* 1981). It is generally believed that the ATP content in the heart at the end of ischaemia makes it possible to predict the rate of mechanical function restoration during reperfusion of the heart (Flaherty *et al.* 1982, Whitman *et al.* 1989). The threshold of reversibility is considered as a decrease in the ATP

Part of the study was presented at the 10th Congress of Pathological and Clinical Physiology in Prague (Hájek *et al.* 1989).

content to approximately 60 % of the initial concentration (Hearse *et al.* 1977, Preusse *et al.* 1982).

Depletion of cellular adenosine nucleotide pool and a decrease in high energy phosphates below a critical level during ischaemia can cause irreversible damage to cardiac cells. Some more recent studies indicate that while, under specially modified experimental conditions, the ATP content at the end of ischaemia may drop by more than 90 %, the recovery of ventricular function reached during reperfusion was 92 % (Rosenkranz *et al.* 1986, Neely *et al.* 1984). However, these conditions are not encountered in clinical situations. Depletion of high energy phosphate stores can be influenced to a great extent by temperature, employed cardioplegic solutions, and/or by multidose cardioplegia. The main reason for using cardioplegic solutions is to accelerate myocardium cooling, arrest cardiac activity and lower basal consumption of myocytic energy. The aim of this study was to compare the time changes in myocardial high energy phosphates and intracellular pH during hypothermic ischaemia after the administration of cardioplegic solutions with a very different ion composition and buffer capacity, i.e. Bretschneider (HTK-B) (Gebhard *et al.* 1984) and St. Thomas' Hospital (StTH) (Ledingham *et al.* 1987) cardioplegic solutions using ^{31}P NMR spectroscopy. In the initial stages of ischaemia, concentration changes of ATP, glycogen and lactate were also followed by biochemical analysis. We wanted to determine the extent to which a change in the extracellular environment can alter the course of intracellular processes caused by ischaemia. The study was also designed to develop a methodology of measurement of ^{31}P NMR spectra of organs using a surface coil in a whole-body magnet.

Material and Methods

a) Animal preparation

Experimental animals were dogs of either sex weighing 20–30 kg (2 experimental groups, each with 7 animals). Anaesthesia: thiopental i.v., controlled ventilation with a mixture of O_2 + N_2O + halothane. Cardioplegia: 850 ml of HTK-B or StTH solutions. Monitoring: ECG, temperature of the left ventricular musculature.

b) Surgical technique

1 mg/kg heparin was administered i.v. After sternotomy a cardioplegic cannula was introduced from the brachiocephalic trunk into the aorta, with the subclavian artery and azygous vein ligated. Next, the superior and inferior venae cavae were ligated and an opening was cut between the ligatures to drain the cardioplegic solution. After clamping the descending aorta, cardioplegia was started with a solution at 4 °C flowing from a bottle with an overpressure equipment. 850 ml of solution flowed within 2.5–4 min and cardiac muscle was cooled down to 7–11 °C. Cardiac action disappeared within 6–30 s. Thereafter the heart was removed by cutting the vessels at the origin and placed in a cool plegic solution. The composition of the used cardioplegic solutions is shown in Tab. 1.

c) Biochemical analysis

Samples for biochemical analysis were excised from the left ventricle at the beginning of the ischaemic period and 180 min afterwards, approximately 1 g of wet weight in both cases. These samples were immediately freeze-clamped with Wollenberger tongs and stored under liquid nitrogen until assayed.

Table 1
Composition of Bretschneider (HTK-B) and St. Thomas' Hospital (StTH) cardioplegic solutions (in mmol/l)

	HTK-B	StTH
NaCl	15.0	110.0
KCl	9.0	16.0
$MgCl_2 . 6 H_2O$	4.0	16.0
$CaCl_2$		1.2
$NaHCO_3$		10.0
histidine.HCl . H_2O	18.0	
histidine	180.0	
kaliumhydrogen-2-oxoglutarate	1.0	
tryptophan	2.0	
mannitol	30.0	
pH	7.2	7.8
osmolarity	280	285–300

Glycogen was extracted with boiling in 30 % KOH after the delipidation of the tissue by chloroform : methanol (2 : 1) solution and assayed as glucose by the glucose-oxidase method. ATP and lactate were measured in 0.6 mol/l perchloric acid extract using enzymatic techniques (Lamprecht and Trautschold 1963). The results were recalculated per dry weight obtained from heart tissue samples extracted by 0.6 mol/l perchloric acid and dehydrated into constant weight at 105 °C.

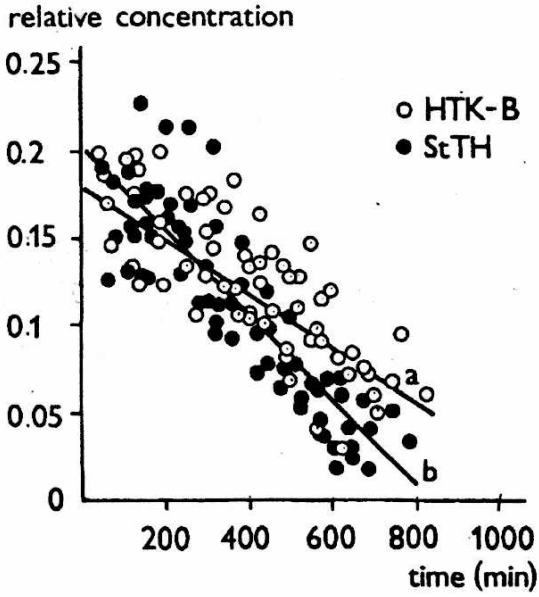

Fig. 1
Time course of relative concentration of ATP in the dog myocardium. The relative concentration of ATP was determined from β-ATP signal intensity. The dependence can be described as follows:
(HTK-B group, line a) $c_{ATP} = -1.6 \times 10^{-4} t + 0.18$ ($r = 0.77$),
(StTH group, line b) $c_{ATP} = -2.4 \times 10^{-4} t + 0.20$ ($r = 0.88$).

d) Spectroscopic measurement

^{31}P NMR spectra were obtained on a Magnetom 1.5 (Siemens) whole-body system. A surface coil of 8 cm diameter was used for transmission and signal detection. The measurements were carried out with the following parameters: flip angle 90°, pulse repetition time 2 s, spectral width 2000 Hz (1k data points), number of scans 512.

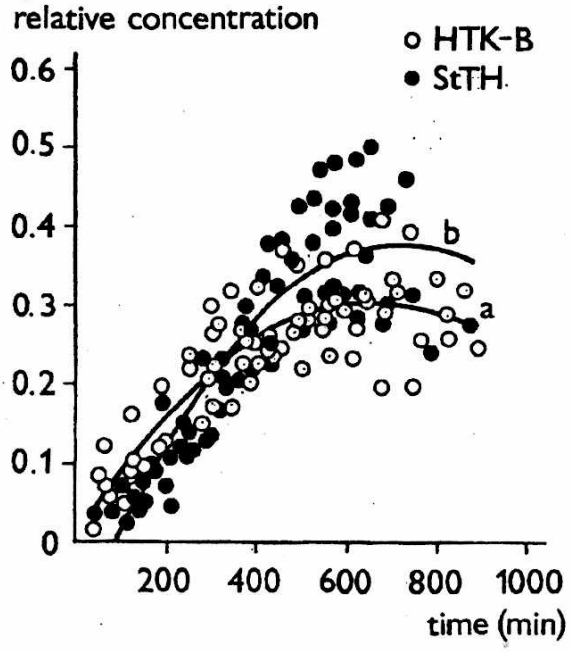

Fig. 2
Time course of relative concentration of phosphomonoesters in the dog myocardium. The dependence can be described by following equations:
(HTK-B group, curve a) $c_{PME} = -8.0 \times 10^{-7} t^2 + 9.9 \times 10^{-4} t + 2.9 \times 10^{-3}$,
(StTH group, curve b) $c_{PME} = -9.3 \times 10^{-7} t^2 + 1.4 \times 10^{-3} t - 1.1 \times 10^{-1}$.

The relative concentrations of metabolites (Fig. 1 and 2) are not corrected for saturation of resonance signals. To eliminate the effects of partial saturation each experiment with TR = 2 s was followed by measurement with TR = 20 s. Concentration changes during measurement time (17 min each experiment) were the main reasons for the failure to calculate the saturation factors with sufficient precision. Since this study is based on a comparison of relative metabolite concentration, the results should not be affected in this respect on the assumption that there are no differences in saturation coefficients (and relaxation times) in both cardioplegic solutions.

The FID was multiplied by an exponential function resulting in line broadening of 20 Hz. Smoothing function was used and baseline correction was applied to the spectra.

Intracellular pH was calculated from the equation (Dawson *et al.* 1977)

$$pH = 6.88 + \log [(\delta - 3.35)/(5.60 - \delta)] \qquad (1)$$

where δ is the chemical shift difference of inorganic phosphate and phosphocreatine.

The content of metabolites was expressed as the relative concentration (no external standard for absolute quantification was used) determined from the relative integral intensity of a given signal. ATP analysis was made by calculating the relative integral intensity of the β-ATP signal which corresponds only to ATP (Fig. 3). The data were statistically analyzed using Student's t-test ($\alpha = 0.95$). Results are expressed as mean ± standard deviation.

Hearts in the cardioplegic solution were placed in a polystyrene foam box the inside of which was cooled with ice. The temperature was maintained at 9 ± 1 °C. The surface coil was centered over the left myocardial ventricle.

Results

^{31}P NMR spectra (Fig. 3) illustrate the time changes of metabolite concentrations in the dog myocardium. The relative ATP concentration decreased linearly during the period of observation (Fig. 1). The decrease was faster in StTH cardioplegic solution (slope $-2.4 \times 10^{-4} \pm 0.2 \times 10^{-4}$, r = 0.88) than in HTK-B (slope $-1.6 \times 10^{-4} \pm 0.2 \times 10^{-4}$, r = 0.77) with a higher concentration of ATP maintained in HTK-B. Although the experimental data seem to be rather scattered this difference is statistically significant.

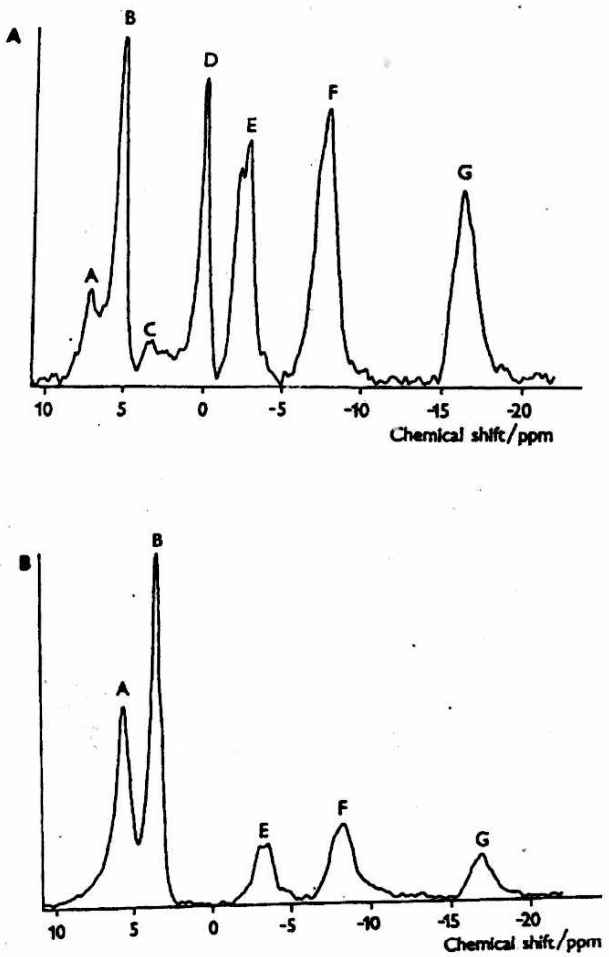

Fig. 3
^{31}P NMR spectra of the dog myocardium in StTH cardioplegic solution at 1.5 T: a) cca 2 h after the onset of ischaemia, b) cca 8.5 h after the onset of ischaemia. Spectra were obtained with 512 acquisitions and 2 s pulse repetition time. A – phosphomonoesters, B – inorganic phosphate, C – phosphodiesters, D – phosphocreatine, E – γ-ATP, β-ADP, F – α-ATP, α-ADP, NADH, G – β-ATP.

The results of biochemical analyses performed at the start and after 180 min of ischaemia also document the difference between both groups in the decreased ATP levels. In hearts perfused with HTK-B, the ATP content almost did not decline from the initial value of 24.6 ± 0.7 μmol/g d.w. In the StTH group, ATP content decreased significantly from 24.2 ± 1.4 μmol/g d.w. by 4.7 ± 0.96 μmol/g d.w.

Precision of the determination of zero phosphocreatine concentration was limited especially by the duration of the experiment (17 min) and the signal to noise ratio at low phosphocreatine concentrations. Phosphocreatine is depleted significantly sooner in StTH (6.2 ± 1.4 h) than in HTK-B (7.9 ± 1.4 h).

The time dependence of the concentration of monoesters (glucose-6-phosphate and fructose-6-phosphate prevail) (Fig. 2) was fitted with a quadratic function $at^2 + bt + c$ (where t is time in min). Calculated parameters a, b, c for HTK-B are -8.0×10^{-7}, 9.9×10^{-4}, 2.9×10^{-3} (standard deviation of concentration 0.05), for StTH -9.3×10^{-7}, 1.4×10^{-3}, -1.0×10^{-1} (standard deviation of concentration 0.06). The concentration of monoesters rises since the start of measurement and the increase is faster in HTK-B till 400 min. After 600–700 min the relative concentration of monoesters falls in both solutions. Our results are in good agreement with biochemical analysis. It was found that the depletion of glycogen is faster till 180 min from the beginning of ischaemia in HTK-B, and thus a greater amount of monoesters, intermediates of glycogen utilization, is formed.

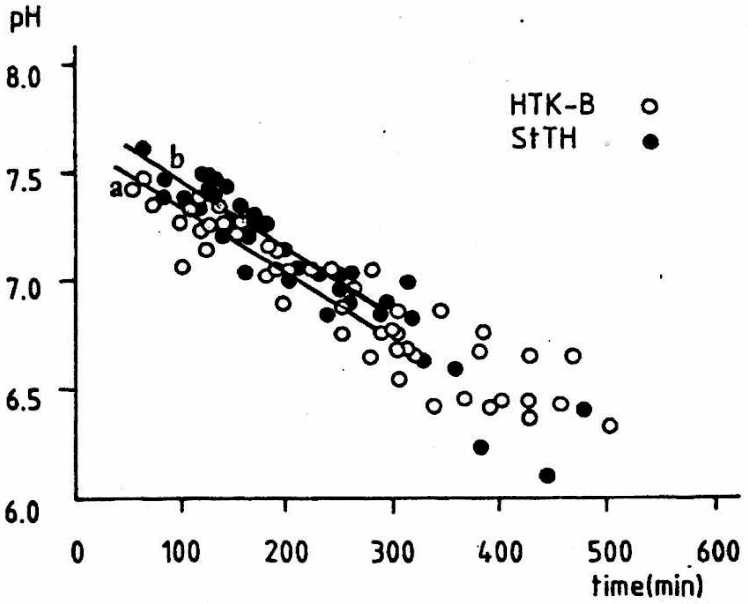

Fig. 4
Time course of intracellular myocardial pH. Following equations were calculated for pH decrease till 300 min since the onset of ischaemia:
(HTK-B group, line a) pH = -2.9×10^{-3} t + 7.6 (r = 0.91),
(StTH group, line b) pH = -3.0×10^{-3} t + 7.7 (r = 0.93).

Biochemical analyses revealed that, at 180 min of ischaemia, glycogen decreased from 225.1 ± 25.7 μmol glucose/g d.w. by 80.0 ± 5.0 μmol glucose/g d.w. in the HTK-B group, and from 225.5 ± 9.2 μmol glucose/g d.w. by as little as 30.2 ± 3.8 μmol glucose/g d.w. in the StTH group. The different rate of glycogen

degradation is also documented by lactate increments in the heart. While, in the HTK-B group, the initial lactate content of 19.1 ± 3.5 μmol/g d.w. rose by 61.0 ± 5.8 μmol/g d.w. within the 180 min of ischaemia, the increase was as small as 35.7 ± 5.8 μmol/g d.w. in the StTH group. The changes in the content of these metabolites were significantly different between both groups.

The time course of pH changes is depicted in Fig. 4. The dependence is linear with nearly equal slopes for both groups (HTK-B group: $-2.9 \times 10^{-3} \pm 2.4 \times 10^{-4}$, r = 0.91; StTH group: $-3.0 \times 10^{-3} \pm 2.0 \times 10^{-4}$, r = 0.93) till 300 min from the beginning of ischaemia. Surprisingly higher average pH values were found in the StTH solution in spite of the high concentration of the histidine buffer in HTK-B (StTH is nonbuffered). The difference between the intercepts is statistically significant (for t = 0 $pH_{StTH} = 7.7$, $pH_{HTK-B} = 7.6$). The difference in intracellular pH between HTK-B and StTH groups may, however, be within the error of determination of intracellular pH (0.1 pH units) (Roberts *et al.* 1981, Wilkie *et al.* 1984) by ^{31}P NMR.

Discussion

Although a correlation of ATP concentration in the ischaemic myocardium with functional recovery has not been established yet, the limit of revival of the heart is practically represented by the critical ATP concentration. Some authors have experimentally determined the critical ATP concentration as that ATP value below which the content of ATP must not fall during ischaemia, if the heart function revival during reperfusion is to occur (Hearse *et al.* 1977, Preusse *et al.* 1982). This means that hearts with this ATP content by the end of ischaemia are capable to constantly maintain at least this critical ATP value during reperfusion. They must therefore be able to carry on the turnover rate of ATP at a sufficient level. Methods upholding the ATP levels in the heart during ischaemia as high as possible are evaluated as perspective for maintaining good functioning of the heart for transplantation. Bretschneider determined as critical that ATP value which is 60 % of initial ATP levels (Preusse *et al.* 1982). In their experiments with canine hearts in HTK-B during ischaemia at 15 °C this point was reached at about 600 min. In our experiments the decrease in ATP concentration under 60 % of the initial concentration was reached 2 hours earlier (although at lower temperature, 9 °C) – at 340 min in StTH and at 450 min in HTK-B. It is interesting that both values correspond to those of total phosphocreatine depletion (370 min in StTH and 470 min in HTK-B).

English *et al.* (1988) compared rabbit heart preservation at 0 °C in StTH and Bretschneider HTP solutions as well and found that the rate of ATP loss was higher in StTH cardioplegic solution than in Bretschneider HTP. Van Echteld *et al.* (1989) also reported that ATP in human hearts was maintained at higher concentrations in HTK-B than in StTH at 0 °C. Our results confirmed that high-energy phosphates in the dog myocardium during global ischaemia at 9 °C are better preserved in HTK-B than in StTH. Improved provision of ATP in HTK-B can be explained by enhanced glycogen degradation and gain of ATP by anaerobic glycolysis which may be influenced by the buffering capacity of the cardioplegic solution (Gebhard *et al.* 1987, Lareau *et al.* 1989). Dennis *et al.* (1986) found that the higher is the concentration of the extracellular buffer, the more intense is the lactate elimination

from the heart at low-flow ischaemia (5 % of the initial flow rate). A more rapid rate of elimination of the product of anaerobic glycolysis from the intracellular space can thus have an effect even on the rate of glycogenolysis. Really faster depletion of glycogen and a higher lactate concentration in the hearts of the HTK-B group was found by biochemical analysis at the beginning of ischaemia. Increased depletion of glycogen resulted in a higher relative concentration of monoesters in HTK-B found by ^{31}P NMR.

In our experiments, we noted, in the time intervals studied, a lower intracellular pH in hearts perfused with HTK-B compared with those perfused with StTH. Considering the higher buffer capacity of HTK-B, just opposite results were anticipated. The value of intracellular pH obtained may be the result of the different ion composition of the cardioplegic solution employed, especially the difference in sodium content. In their experiments with Purkinje fibres of ovine hearts, Stinner *et al.* (1987) found that intracellular pH of fibres incubated under normoxic conditions in HTK-B was 7.01, and 7.25 in fibres incubated in StTH. At the same time, the intracellular contents of sodium (4.2 mmol/l Na^+ in HTK-B and 110 mmol/l Na^+ in StTH) reflected the sodium contents in the extracellular environment (15 mmol/l Na^+ in HTK-B and 110 mmol/l Na^+ in StTH). These findings suggest that intracellular pH is influenced by the extracellular concentration of sodium probably *via* Na^+-H^+ exchange (Mahnensmith *et al.* 1985). As Na^+-H^+ exchange occurs without requiring energy, it is probable that the process affecting intracellular pH is also maintained during ischaemia. The steady decline in pH found in both experimental groups during ischaemia may be due to the additive effect of ischaemic acidification of intracellular space by products of anaerobic glycolysis.

Van Echteld *et al.* (1989) also compared the effect of cardioplegic solutions used by us on the changes of intracellular pH using measurement of NMR in the human heart. Using HTK-B, the values of intracellular pH after 12 hours of ischaemia was 6.98, and 6.04 when using StTH. The difference may be attributable to the fact that the heart was kept at 0 °C during ischaemia. Under these conditions, it is possible to assume that changes in membrane transport mechanisms, and that ion and proton balance may be governed by different principles.

It has been confirmed that NMR spectroscopy is a suitable method for following heart preservation in cardioplegic solutions. Use of a whole-body magnet made it possible to measure NMR spectra of organs in a transport box whose design can be adapted to the clinical needs of non-invasive measurements.

In agreement with biochemical analysis, use of HTK-B during preservation results in slower rates of depletion of high-energy phosphates in the heart compared with the situation in the StTH group. The rise in monoester content within the first hours of ischaemia in the hearts protected with HTK-B was consistent with the biochemically documented increase of glycogen degradation. Contrary to our anticipation, an intracellular pH lower than that in StTH was found in the same group of hearts, presumably as the result of a low concentration of Na^+ ions.

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