Magnesium in cardioplegic solutions as assessed by ^{31}P NMR spectroscopy

An experimental study in the isolated rat heart

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Summary

A number of cardioplegic solutions contain magnesium as a potential protective agent and most commonly as an additive to an elevation of potassium. In the present study the efficacy of potassium-based (20 mM) cardioplegic solutions in the abscence (Mg 0 mM) and in the presence (Mg 15 mM) of magnesium were assessed for in the isolated rat heart. It was found by ^{31}P NMR examination that in Mg 15 mM hearts the decline in high energy phosphate compounds and in intracellular pH during 32 min of ischemia at 33°C was delayed in comparison with Mg 0 mM hearts. During postischemic reperfusion ^{31}P NMR spectroscopy, physiological examination and biochemical assessment of tissue specimens revealed only minor differences in favour of the Mg 15 mM hearts. It is concluded that Mg may be one among a wide number of potentially protective components of cardioplegic solutions.

Résumé

Un nombre de solutions cardioplegiques contiennent du magnesium comme une substance protective, et plus habituellement comme un additive à l'évélation de potassium. Concernant l'étude present, l'efficacité des solutions cardioplégiques fondé sur potassium dans l'absence de (Mg 0 mM) et dans la présence de (Mg 15 mM) magnesium à été examiné sur coeurs de rats isolés. Pendant l'examination par ^{31}P NMR on avait trouvé que pour des coeurs de Mg 15 mM un abaissement en CrP et ATP, et en pH intracellulaire pendant 32 min de l'ischemie à 33°C, à été rétardé en rapport avec coeurs de Mg 0 mM. Pendant reperfusion postischemique par ^{31}P NMR spectroscopie, et par des examinations physiologiques et biochimiques d'épreuves du tissu, on avait trouvé uniquement des petites differences en faveur des coeurs de Mg 15 mM. C'est à conclure que magnesium peut étre l'un de plusieurs ingrédients protectives des solutions cardioplegiques.

Zusammenfassung

Magnesium ist eine übliche Komponente in Kardioplegie-Lösungen, und dient meistens als ein protektiver Zusatz zum Kalium-induzierten Herzstillstand. Isolierte Rattenherzen und ^{31}P NMR-Spektroskopie wurden in einer Untersuchung von Kalium-basierten (20 mM) Kardioplegie-Lösungen, ohne (Mg 0 mM) oder mit (Mg 15 mM) Zusatz von Magnesium, benutzt. Die Ergebnisse sind wie folgt: Der, durch Ischämie (32 min, 33°C) ausgelöste, Abbau der energiereichen Phosphat-Verbindungen und die Abnahme von pH sind in den Mg 15 mM-Herzen etwas verzögert worden. Nach Reperfusion erscheint in den Mg 15 mM-Herzen, im Vergleich zu den Mg 0 mM-Herzen, nur eine teilweise Verbesserung der ^{31}P NMR spektroskopischen und der physiologischen und biochemischen Meßwerte. Diese Arbeit zeigt, daß Magnesium eine von mehreren wertvollen und protektiven Komponenten der Kardioplegie-Lösungen sei.

Introduction

Cold chemical cardioplegia, or briefly named cardioplegia, has over the last 10 years become the standard procedure for cardiac arrest and myocardial protection during open heart surgery [3—5, 9, 11, 18, 29, 36, 46]. The specially designed cardioplegic solutions are applied cold (4—10°C) and are administered initially or intermittently into the aortic root or directly into the coronary arteries during aortic occlusion. In this period the myocardium is left in a globally ischemic state and may suffer from ischemic cell injury in the absence of appropriate protective measures [10, 18]. The repeated coronary perfusion with cold cardioplegic solutions is complemented with external (topical) cooling of the heart using a chemically indifferent solution in order to improve and maintain hypothermia. Mechanisms behind myocardial protection with cardioplegia are complex involving at least [18] three essential elements: chemical arrest, which may conserve cellular energy; hypothermia, which may slow the rate of energy-consuming and degenerative processes; and chemical protection, whereby specific protective agents may combat various ischemia-induced cellular reactions. Also the composition of cardioplegic perfusates may be rather complex and include such widely differing components as: ions; drugs acting primarily through ionic effects; substrates and hormones; buffering, osmolal and oncotic agents; oxygen and oxygen-carrying vehicles; free oxygen radical scavengers; high energy phosphates and related compounds; and a number of other, well or less well characterized, substances [4, 6, 8, 11, 16—20, 22—24, 29, 35, 36, 38, 44—46]. It has, however, been recognized that the main principle behind the successful formulation of cardioplegic solutions lies in the creation of an ionic environment optimal for cellular calcium control [18, 22, 24]. On this basis the solution content of and balance between the four major cations calcium, sodium, potassium and magnesium would appear as essential. The rationale behind including

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magnesium in cardioplegic solutions may be sought for in a wide number of factors. Of particular interest in terms of obtaining calcium control and ischemic protection are: the improvement upon other arresting principles such as potassium elevation [17, 18, 24] or calcium and sodium withdrawal [35]; the direct or indirect use as a calcium antagonist during ischemia and early reperfusion [12, 13]; the prevention of cellular potassium [41, 42] and magnesium [30, 40] loss; and the potential improvement in cellular energy state and enzyme function during the early reperfusion period.

The practice concerning inclusion of magnesium in clinical cardioplegic solutions varies widely. Thus, some solutions are devoid of magnesium [9, 29] while others contain magnesium near to the normal extracellular level [4, 36, 46]. An elevated magnesium has been held as an essential or contributary factor behind the efficacy of the extracellularly formulated St. Thomas' Hospital solution (16.0—20.0 mM) [5, 17, 18, 23, 24] and the intracellularlike *Bretschneider* solution (8.0 mM) [35]. With the St. Thomas' Hospital solution the 16 mM of magnesium is thought mainly to improve tissue protection above what is obtained through the rapid chemical arrest by 16 or 20 mM of potassium.

The ability of magnesium elevation to improve upon the efficacy of potassium-based (16 mM) and extracellularly formulated cardioplegic solutions has been well documented in isolated rat heart studies [17, 18, 24]. In these experiments myocardial protection during ischemia has been assessed by physiological indices, conventional metabolic indices and by enzymatic indices. The main intention behind the present study was to examine in the isolated rat heart the potential additive effects of potassium arrest (20 mM) and magnesium supplementation (15 mM) by use of ^{31}P NMR spectroscopy. Of particular interest beside the continuous evaluation of cardiac energy metabolism was to follow changes in intracellular pH during ischemia and reperfusion.

Materials and methods

Isolated heart perfusion

Male rats (280—320 g body wt) of the Wistar strain were anesthetized with diethyl ether. Following intravenous heparin injection (200 IU), the heart was rapidly excised and transiently placed in ice-cold perfusion medium prior to cannulation of the aorta. Thereafter retrograde aortic perfusion was established. The perfusion apparatus was a modified *Langendorff* [27] system with reservoirs attached to a 90 cm central perfusion tube of 25 mm external diameter. The system was designed such that during the experiment the heart was perfused within a vertically positioned, narrow-bore NMR spectrometer and contained within a NMR tube of 13 mm internal diameter. The coronary effluent was continuously removed from the bottom of the NMR tube by use of a vacuum pump. A fluid filled latex balloon (volume 0.2 ml) was introduced into the left ventricle via the mitral valve and connected to a pressure transducer. All parts of the perfusion system were waterjacketed for temperature control. Also a constant temperature of the heart was secured by use of the ventilation-based temperature control unit of the NMR spectrometer.

Perfusates

The normal perfusion medium was the glucose-containing (11.1 mM) and oxygenated (95% O_2 and 5% CO_2) *Krebs-Henseleits* bicarbonate buffer [26] with a calcium concentration of 1.2 mM and a pH of 7.4. The two cardioplegic perfusates in study represented, as seen in Tab. 1, modified *Krebs-Henseleits* buffers containing 20 mM of potassium and either 0 or 15 mM of magnesium, and were oxygenated (95% O_2 and 5% CO_2) and maintained at a pH of 7.4. No corrections were made for osmolal differences between the cardioplegic solutions. All perfusates were filtered through a cellulose acetate filter (pore size 0.8 um) prior to use.

Tab. 1: The composition of cardioplegic solutions. The concentrations of solution components are expressed in mmoles/liter. The osmolality of cardioplegic solutions as measured by a depression of freezing point method and the solution pH are also included

Components	mmoles/liter
NaCl	91.6
$NaHCO_3$	25.0
KCl	18.8
KH_2PO_4	1.2
$MgCl_2 \cdot 6\ H_2O$	0 or 15.0
$CaCl \cdot 2\ H_2O$	1.2

pH: 7.4
Osmolality: 304 mOsm/kg H_2O (Mg 15)
285 mOsm/kg H_2O (Mg 0)

Experimental conditions and time course

The experiments were conducted under light hypothermia with a temperature in perfusates and in hearts of 33°C. The coronary perfusion pressure during delivery of normal medium or of cardioplegic solutions was set to 75 mmHg. The experimental time course was as follows: 20 min control perfusion (0.—20. min); 4 min cardioplegic perfusion (20.—24. min); 32 min total global ischemia (24.—56. min); and 20 min reperfusion (56.—76. min).

^{31}P NMR spectroscopy

Phosphorus nuclear magnetic resonance spectroscopy was performed on a Bruker WM 400 9.4T narrow-bore and vertically positioned spectrometer operating at a phosphorus frequency of 161.9 MHz. Free induction decay signals (FIDs) were obtained using a 2 K data table, 30° radiofrequency pulses, and aquisition and delay times of respectively 180 and 820 msec. 224 FIDs obtained over 4 min periods were averaged, and the accumulated FID was then Fourier transformed to produce a spectrum where the various compounds were displayed according to their chemical shifts. These phosphorus compounds were observed: inorganic phosphate, P_i; creatine phosphate, CrP; adenosine triphos-

phate (3 peaks), γ ATP, α ATP and β ATP. After the experiment the data were analyzed, and integration of peak areas was used for the assessment of relative concentrations of the various substances. The β ATP peak was employed for measurement of ATP [14]. Differences in chemical shift between the pH-sensitive P_i peak and the pH-insensitive CrP peak formed the basis for calculation of pH [14, 21]. Also intracellular pH, pH_i, was recognized under normal perfusion conditions by hyperfine splitting of the main P_i peak [14].

The results for relative concentrations of P_i, CrP and ATP are expressed in per cent of values obtained during the preischemic control perfusion period. The values for pH_i are expressed in pH units. Each data represents the value for a 4 min total sampling period.

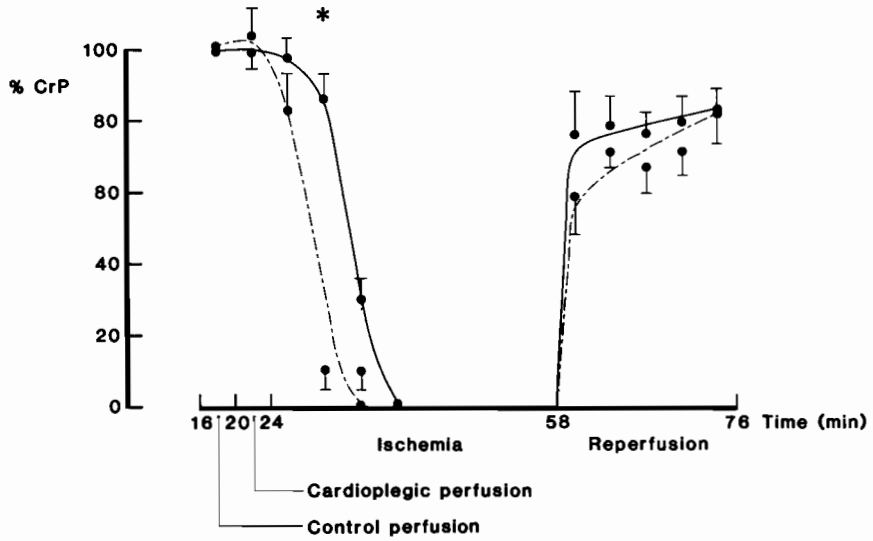

Fig. 1: Changes in myocardial creatine phosphate (CrP). The data are derived from ^{31}P NMR spectra obtained over 4 min periods and are expressed in per cent of preischemic (0—20 min) control values. The continuous and stippled lines represent the results from hearts being perfused (20—24 min) with cardioplegic solutions containing respectively 15 mM and 0 mM of magnesium. Observe that 0% indicates the ^{31}P NMR detection level for CrP. The asterix indicates significant intergroup differences

Physiological indices

The left ventricular developed pressure (LVDP) and the heart rate (HR) were continuously monitored on a Gould 2400 recorder and allowed for calculation of the left ventricular pressure-rate product (LVDP x HR). The coronary flow rate (CFR) was measured from minute-wise collection of the coronary effluent. During reperfusion the postischemic recovery of LVDP, HR, LVDP x HR and CFR was expressed in per cent of the values obtained during the initial control period.

Biochemical indices at end of the experiment

At the end of the experiment the perfusion apparatus was removed from the NMR spectroscope. Approximately 2 min later the heart was, while still being perfused, freeze-clamped between stainless steel tongues precooled in liquid nitrogen. Thereafter the atria of the frozen heart were discarded and the ventricular (right plus left) portions were freeze-dried. Following extraction in perchloric acid the myocardial content (μmoles/g dry wt) of high energy phosphate compounds was measured by high performance liquid chromatography (HPLC) technique [39]. The myocardial content (μmoles/100 g dry wt) of calcium and magnesium was measured by atomic absorption spectrophotometry [1] in HNO_3 prepared extracts of the ventricular tissue.

Expression of results

4 hearts were included in each experimental group. All results were expressed as the mean ± standard error of the mean. Comparison between groups was by the student's t-test. Significant differences between group means were inferred for p-values less than 0.05.

Results

Since the main intention behind the study was to employ ^{31}P NMR for the examination of magnesium as a cardioplegic additive, the spectroscopic data are presented prior to the obtained data on recovery of cardiac physiology and of end point biochemical analysis of ventricular tissue. The two experimental groups are referred to as Mg 0 mM or Mg 15 mM according to the content of magnesium in the two cardioplegic solutions.

Changes in myocardial creatine phosphate (CrP)

Fig. 1 presents the time-based and per cent changes in CrP from the end of the preischemic control period to the end of the postischemic reperfusion period. With CrP there was a rapid fall during the initial period of ischemia with peak loss occurring between the 2. and the 10. minute after the end of cardioplegic perfusion. In the Mg 15 mM group of hearts there was a significant, approximately 4 min, delay in the CrP breakdown. After 10—14 min of ischemia CrP fell below detection level in both groups. On reperfusion there was a rapid and much similar rebuilding of CrP in both groups of hearts which by the end of the experiment had recovered to about 90% of the preischemic level.

Changes in myocardial adenosine triphosphate (ATP)

With ATP there was, as seen from Fig. 2, a more gradual and moderate (30%) decline during the first half of the

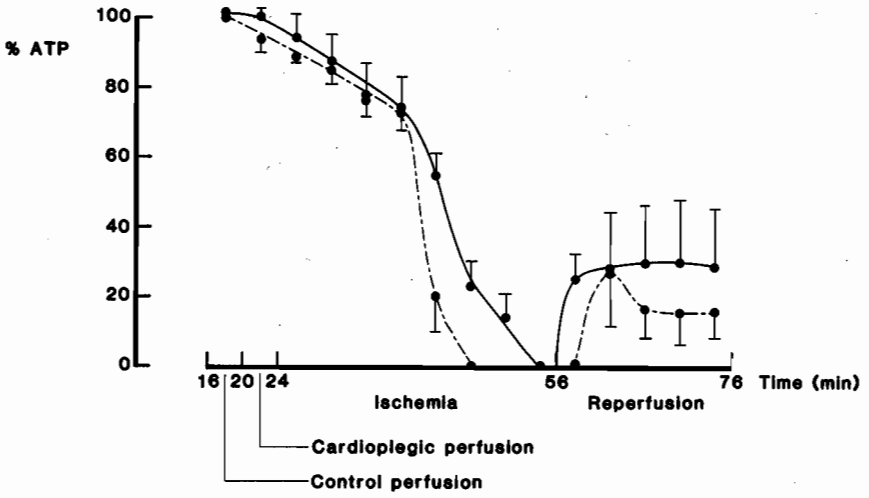

Fig. 2: Changes in myocardial adenine triphosphate (ATP). The data are derived from ^{31}P NMR spectra obtained over 4 min periods and are expressed in per cent of preischemic (0—20 min) control values. The continuous and stippled lines represent the results from hearts being perfused (20—24 min) with cardioplegic solutions containing respectively 15 mM and 0 mM of magnesium. Observe that 0% indicates the ^{31}P NMR detection level for ATP. The asterix indicates significant intergroup differences

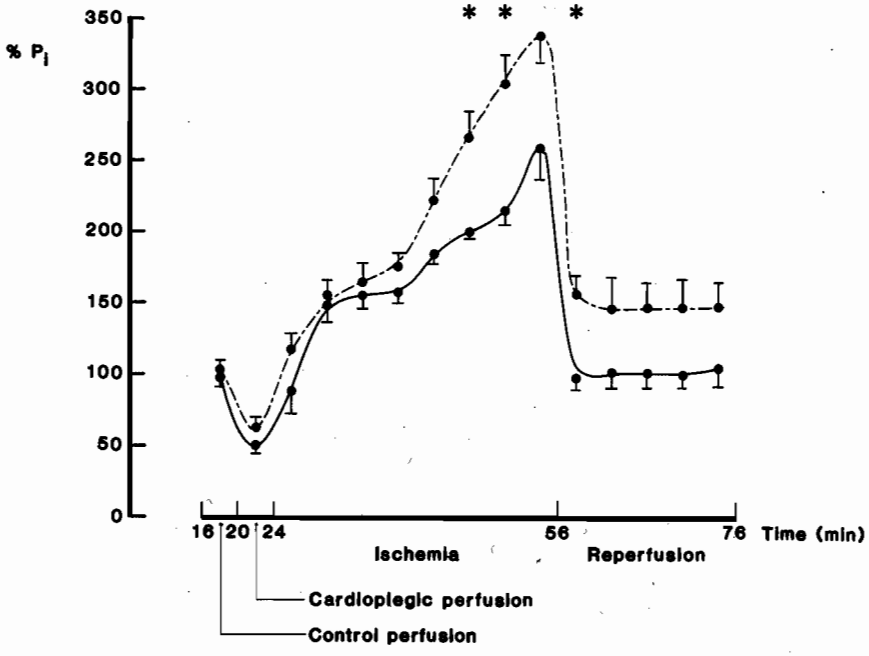

Fig. 3: Changes in myocardial inorganic phosphate (P_i). The data are derived from ^{31}P NMR spectra obtained over 4 min periods and are expressed in per cent of preischemic (0—20 min) control values. The continuous and stippled lines represent the results from hearts being perfused (20—24 min) with cardioplegic solutions containing respectively 15 mM and 0 mM of magnesium. Observe that 0% indicates the ^{31}P NMR detection level for P_i). The asterix indicates significant intergroup differences

ischemic period in both groups of hearts. Thereafter the ATP broke down more rapidly and finally fell below detection level. On reperfusion the recovery of ATP was greatly limited in both groups of hearts with a final recovery in the 15—30% range. Although the Mg 0 mM group of hearts presented lower mean values of ATP during ischemia and reperfusion than the Mg 15 mM group of hearts, these differences were not significant.

Changes in myocardial inorganic phosphate (P_i)

Fig. 3 presents the time-based changes in P_i from the preischemic control period to the end of the postischemic reperfusion period. After an initial fall in P_i during cardioplegic perfusion, probably caused by reduced energy consumption, P_i rose steadily throughout the ischemic period. In the Mg 0 mM group of hearts the P_i elevation was significantly greater than in the Mg 15 mM groups of hearts. P_i at end of ischemia was about 3.4 and 2.6 times the control level in respectively the Mg 0 mM and the Mg 15 mM group of hearts. On reperfusion the P_i values fell to the preischemic control level in the Mg 15 mM group of hearts and to a 50% higher level in the Mg 0 mM group of hearts. The intergroup difference in P_i values was significant during the initial part of the reperfusion period.

Changes in myocardial intracellular pH (pH_i)

Myocardial pH_i remained, as seen from Fig. 4, close to 7.10 during the control period and during cardioplegic perfusion. During ischemia the initial pH_i decline was significantly greater in the Mg 0 mM than in the Mg 15 mM group of hearts. At the end of ischemia, however, pH_i had fallen to about 6.50 in both groups of hearts. On reperfusion there was a continuous rise in pH_i towards the preischemic control level. At the end of the experiment the mean pH_i values of the Mg 0 mM and the Mg 15 mM groups of hearts had recovered to respectively 7.01 and 7.11, the observed difference being significant.

Changes in indices of cardiac function

All hearts in both groups were rapidly arrested in the diastolic state during cardioplegic perfusion. During ischemia the left ventricular pressure record-

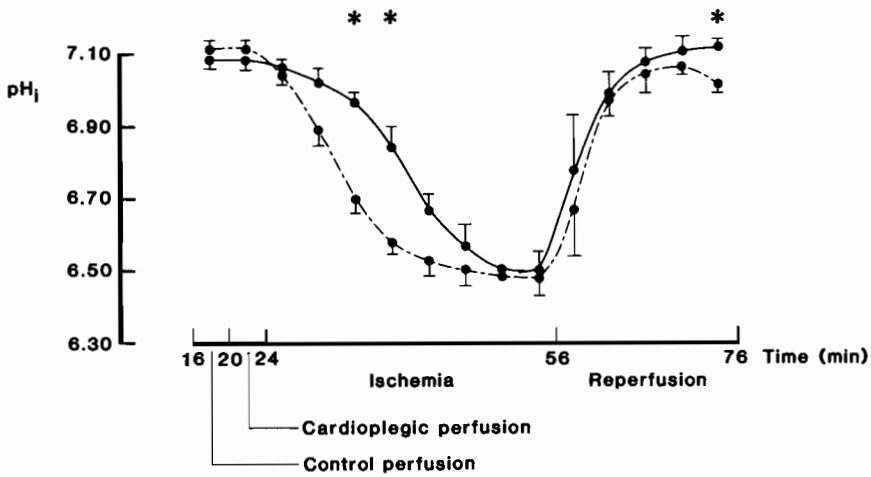

Fig. 4: Changes myocardial intracellular pH (pH_i). The data are derived from ^{31}P NMR spectra obtained over 4 min sampling periods and are expressed in pH units. The continuous and stippled lines represent the results from hearts being perfused (20—24 min) with cardioplegic solutions containing respectively 15 mM and 0 mM of magnesium. The asterix indicates significant intergroup differences

ings revealed a slightly longer delay before the onset of a rather marked rise in resting tension (contracture) in Mg 15 mM hearts compared to Mg 0 mM hearts. During reperfusion short episodes or longer periods of ventricular fibrillation (VF), as judged from the LVDP curves, were more frequently encountered in Mg 0 mM hearts than in Mg 15 mM hearts. The accumulated VF duration was 38 min and 15 min in respectively Mg 0 mM hearts and Mg 15 mM hearts.

The postischemic recovery of various indices of cardiac function expressed in per cent of the preischemic control values is presented in Tab. 2. A general trend, from the physiological results obtained after 10 min and 20 min of reperfusion, of a superior recovery of the hearts in the Mg 15 mM group is observed. Thus the values for LVDP, HR, LVDP x HR and CFR were considerably higher. These apparent differences between Mg 15 mM and Mg 0 mM hearts were not significant, except for the HR recording following 10 min of reperfusion.

Biochemical analysis of ventricular myocardium at the end of the experiment

Tab. 3 presents the values for the content of high energy phosphates and of magnesium plus calcium in frozen and freeze-dried ventricular specimens. The mean values for CrP, ATP and adenine nucleotide sum are higher in the Mg 15 mM than in the Mg 0 mM group of hearts, but the apparent differences were not significant. A comparison of NMR and HPLC derived data for ATP in individual hearts of both groups revealed that the detection level for ATP by NMR spectroscopy corresponded to a tissue content at or slightly above 8 µmole/g dry wt. This value represents about one third of the ATP content in normal hearts as determined by the employed HPLC method [39].

The myocardial content of magnesium was almost identical for the two groups of hearts, indicating that the omission of magnesium from the cardioplegic solution did not result in any exaggerated magnesium loss in Mg 0 mM hearts. Although the mean value for tissue calcium was about 25% higher in the Mg 0 mM than in the Mg 15 mM group of hearts, the apparent difference did not achieve statistical significance.

Discussion

The main results from the present study underline to a certain extent the potentially important role of magnesium as a component of cardioplegic solutions. Thus it was found by ^{31}P NMR spectroscopy that the addition of 15 mM of

Tab. 2: The postischemic recovery of various indices of cardiac function. The data are recorded following 10 min and 20 min of postischemic reperfusion and are expressed in per cent of values obtained during the preischemic contro period

Cardioplegic solution	Postischemic recovery of cardiac function in % of preischemic control values							
	LVDP		HR		LVDP × HR		CFR	
	10 min	20 min	10 min	20 min	10 min	20 min	10 min	20 min
Mg 0 mM	17.5 ± 17.5	30.9 ± 18.3	21.0 ± 21.0	57.2 ± 19.7	14.7 ± 14.7	25.8 ± 16.5	68.4 ± 9.9	66.5 ± 9.8
Mg 15 mM	47.6 ± 12.0	54.5 ± 11.6	99.0* ± 2.1	97.4 ± 2.5	47.8 ± 12.4	53.5 ± 12.5	85.7 ± 6.4	82.5 ± 3.1

* Intergroup difference significant ($p < 0.05$)

Tab. 3: The myocardial content of high energy phosphate compounds and of magnesium and calcium measured in extracts from hearts freeze-clamped at the end of the experiment

Cardioplegic solution	CrP	ATP	ADP	AMP	Mg	Ca
	µmoles/g dry wt				µmoles/100 g dry wt	
Mg 0 mM	20.7 ± 4.2	8.5 ± 1.3	2.8 ± 0.4	1.1 ± 0.4	3.93 ± 0.17	2.64 ± 0.25
Mg 15 mM	31.5 ± 4.2	11.5 ± 1.6	2.8 ± 0.3	0.7 ± 0.2	4.03 ± 0.09	2.16 ± 0.11

magnesium to a potassium-based (20 mM) cardioplegic solution caused a transient delay in various ischemia induced reactions such as energy breakdown (CrP), phosphate accumulation (P_i) and cellular acidosis (pH_i). These findings of additive protection by magnesium were only partly paralleled by physiological recovery rates during the reperfusion period and by biochemical analysis of ventricular specimens at the end of the experiment.

The results from the present study with a certain but limited potential of magnesium as a protective agent requires at least three qualifications to be made. Firstly, the number of observations at each time point is small rendering it difficult to obtain statistically significant results in the presence of widely scattered data. However, the impression from NMR spectroscopy (Fig. 1—4) is not wide data scattering but the rather close time course during ischemia and reperfusion of changes in CrP, ATP, P_i and pH_i for the two experimental groups. Thus the overall conclusion from the spectroscopic examination would be that Mg 15 mM conferred only a moderate additive protection. Secondly, it could be argued that the ischemic period was too long for an effective postischemic evaluation of magnesium effects. Such an argument finds support in the rather poor recovery both of physiological indices and of myocardial ATP in the Mg 15 mM group of hearts. Also the NMR spectroscopic data indicate that an optimal duration of ischemia for evaluation of magnesium effects would be close to 20 min as the differences between the experimental groups were maximal at this particular time. A third point to be considered in the experimental protocol would be the rather modest use of hypothermia and the lack of repeated cardioplegic perfusions during the total ischemic period. It is difficult, however, to see how these deviations from clinical application of cardioplegia might have reduced the validity of the main observations. A further objection to the present protocol would be that no osmolal corrections were made for the Mg 0 mM cardioplegic solution. However, from the previous demonstration of some sensitivity of the isolated rat heart to hypertonic solutions [16] this would indicate slightly more favourable conditions for the evaluation of the isotonic Mg 0 mM solution than for the moderately hypertonic Mg 15 mM solution.

The ^{31}P NMR examination was based on 4 min signal sampling behind each spectrum and would therefore not reveal very rapid changes in energy metabolism or pH. In the present study the method was able to give an overall impression of major changes in intracellular phosphates and pH. The parallel measurement of tissue ATP by HPLC at the end of the experiment confirmed as has been found by others [21, 28] that the NMR spectroscopic detection level did not allow for recognition of a very low tissue ATP. Also it is to be remembered that NMR requires a mobile molecular environment of the nucleus in study and that some ATP tightly bound to macromolecules or membrane-bound enzymes may escape detection [14].

A particularly interesting aspect of ^{31}P NMR spectroscopy was to observe in parallel the ischemia induced changes in pH_i and ATP for the two experimental groups. Thus a marked fall in pH_i occured early in the Mg 0 mM hearts, while the initial ATP reduction was only moderate and much similar for the two groups of hearts. Since hydrolysis of ATP [15] and glycogenolysis [33] are major sources of hydrogen ions in ischemia induced cellular acidosis, the observations indicate that glycogen breakdown may have been a more rapid and early event in Mg 0 mM hearts than in Mg 15 mM hearts.

The results from the present study of only a moderate additive protective effect of magnesium supplemented to a potassium-based cardioplegic solution are somewhat in contrast to what has been found in other rat heart studies [17, 24]. Thus *Hearse* and *Stewart* in their dose-response study [17] of magnesium elevation in cardioplegic solutions containing 16 mM of potassium for rapid induction of cardiac arrest, demonstrated a marked additive tissue protection with increasing concentrations of magnesium up to about 15—20 mM. Above this concentration tissue protection was reduced. In this study the postischemic recovery of aortic flow rate in the working rat heart preparation formed the basis for assessment of magnesium effects under both normothermic and hypothermic ischemia. In another rat heart study [24] *Jynge* examined the importance of sodium-calcium relationships with cardioplegic solutions (variable sodium 30—150 mM, constant calcium 1.2 mM) containing either normal or elevated concentrations of potassium (5.9 or 16.0 mM) and of magnesium (1.2 or 16.0 mM). In these experiments tissue protection during and following normothermic ischemia was assessed by measurement of creatine kinase leakage during the postischemic reperfusion period. The main results were that magnesium elevation afforded a very effective tissue protection on its own and that magnesium elevation improved considerably upon the otherwise moderate protective effects of elevated potassium. In a third study [34] using a working rat heart preparation and ^{31}P NMR spectroscopy *Pernot* and *Ingwall* made much similar observations as in the present experiments.

Thus the elevation of magnesium from 0 mM to 13.0 mM in a potassium-based (30.0 mM) and calcium-poor (0.25 mM) cardioplegic solution conferred little additive protection during and following 60 min of ischemia at a temperature level of about 15°C. In these experiments it appears, however, that the duration of ischemia was probably to short in order to allow for an effective evaluation of potassium-based solutions with or without magnesium present. Another interesting observation from the same study was that magnesium elevation (13.0 mM) in the presence of normal potassium (4.0 mM), near to normal sodium (100.0 mM) and low calcium (0.25 mM) proved to be the optimal formulation of the cardioplegic solutions examined.

From the above paragraph the overall impressions of isolated rat heart studies are that elevated magnesium may be ef-

fective on its own in the absence of potassium elevation [24, 34] and that magnesium may overcome, more [17] or less [34] (present study) convincingly, some of the shortcomings of elevated potassium in cardioplegic solutions. However, it is also to be noted that in a recent cardioplegia study [2] *Bersohn* and *Shine*, using an isolated rabbit septal preparation, did not find any effective protection by magnesium elevation (16 mM) alone, and that in a dog cardiopulmonary bypass study [32] from 1970 *Mundth* and *Sokol* demonstrated a very marked protective effect during hypothermic ischemia of elevated magnesium (30 mM) alone. Clearly, the cited magnesium cardioplegia studies from the rat, rabbit and dog underline the role of species differences in ionic regulation. Accordingly, the extracellular calcium-magnesium antagonism is strong in the rat heart [41] while being nearby absent in the rabbit [42] and of intermediate strength in the dog [7] myocardium. Until now little is known about magnesium-calcium antagonism in the human heart. Also magnesium-potassium interactions as expressed by the ability of extracellular magnesium to prevent cellular potassium loss are more prominent in the rat [41] than in the rabbit [42] myocardium. Concerning magnesium-sodium interactions [25, 43] species differences are at present less well defined.

The present study has covered only one, however essential, aspect of magnesium in relation to cardiac sugery. Of much recent interest has been studies [12, 31] devoted to ionic manipulation at the onset of postischemic reperfusion and which seem to indicate that elevation of extracellular magnesium may be one among other promising interventions in the prevention of an excessive reperfusion injury. Of other forthcoming research areas it is appropriate to mention protection of the coronary vascular endothelium [37], the optimization of myocardial energy reserves in the preoperative period [3], and the general use of magnesium as an agent on its own or as an additive in the prevention or treatment of coronary artery spasm and arrhythmias. In conclusion to the present study the authors would like to stress that magnesium may be of considerable importance in the overall context of myocardial protection, that mechanisms behind magnesium protection may be complex and are still poorly understood, and that more basic research on magnesium is needed.

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