Free magnesium concentration in isolated rabbit hearts subjected to high dose isoproterenol infusion: a ^{31}P NMR study

Isabelle Mottet, Jean-François Goudemant, Marc Francaux, Roger Demeure, and Xavier Sturbois

Abstract: The hypothesis of magnesium deficiency in isoproterenol (ISO) induced myocardial injury has been investigated by ^{31}P nuclear magnetic resonance spectroscopy. High energy phosphate concentrations, pH_i, and intracellular free magnesium concentration ($[Mg^{2+}]_i$) were measured in isolated rabbit hearts perfused at constant flow and subjected to 10^{-6} M isoproterenol during 30 min. Recent calibrations were used for $[Mg^{2+}]_i$ measurements, and uncertainties on $[Mg^{2+}]_i$ estimated values were calculated. During isoproterenol infusion, pH_i, [PCr], and [ATP] decreased, while $[P_i]$ increased. When it was stopped, [PCr] completely repleted, whereas only a partial restoration was observed for pH_i and $[P_i]$. A rise of end-diastolic pressure and perfusion pressure expressed a contracture, concomitant with a lack of [ATP] recovery, which remained at 59 ± 13% of the rest value. These results establish that 10^{-6} M isoproterenol caused severe myocardial injury. $[Mg^{2+}]_i$ increased from 0.70 mM at rest to 0.88 mM at the end of the isoproterenol period. Considering the estimated uncertainties on the $[Mg^{2+}]_i$ values, this increase was not significant. After isoproterenol infusion, $[Mg^{2+}]_i$ progressively decreased to reach 0.72 mM at 45 min recovery. It is concluded that isoproterenol myocardial toxicity may not be related to $[Mg^{2+}]_i$ deficiency.

Key words: magnesium, β-agonist, nuclear magnetic resonance.

Résumé : L'hypothèse d'une déficience en magnésium dans les lésions myocardiques induites par l'isoprotérénol a été étudiée par résonance magnétique nucléaire du ^{31}P. Des mesures de concentrations des phosphates riches en énergie, du pH_i et de la concentration intracellulaire en magnésium libre ($[Mg^{2+}]_i$) ont été réalisées sur des coeurs de lapins perfusés à un flux constant et soumis à 10^{-6} M d'isoprotérénol pendant 30 min. $[Mg^{2+}]_i$ a été mesurée grâce à des calibrations récentes et les incertitudes sur son estimation ont été calculées. Durant l'infusion d'isoprotérénol, pH_i, [PCr] et [ATP] diminuaient alors que la $[P_i]$ augmentait. Après interruption de l'infusion d'isoprotérénol, [PCr] revenait à des valeurs voisines de celles observées au repos, alors que pH_i et $[P_i]$ n'étaient que partiellement restaurés. Une élévation de la pression de fin de diastole et de la pression de perfusion exprimaient une contracture concomitante à l'absence de récupération de [ATP] qui restait à 59 ± 13% de sa valeur de repos. Ces résultats établissent que l'isoprotérénol 10^{-6} M a induit des lésions myocardiques sévères. $[Mg^{2+}]_i$ passait de 0,70 mM au repos à 0,88 mM à la fin de la période isoprotérénol. Considérant l'incertitude estimée sur la valeur de $[Mg^{2+}]_i$, cette augmentation n'était pas significative. Après l'infusion d'isoprotérénol, $[Mg^{2+}]_i$ redescendait progressivement jusqu'à 0,72 mM après 45 min de récupération. Il est conclu que la toxicité myocardique de l'isoprotérénol ne peut pas être liée à une déficience en $[Mg^{2+}]_i$.

Mots clés : magnesium, β-agoniste, résonance magnétique nucléaire.

Introduction

According to Rona et al. (1959), isoproterenol (ISO) causes multifocal infarct-like myocardial lesions. The relationship between the severity of these lesions and the doses of intravenously or subcutaneously administered ISO has been demonstrated (Rona et al. 1959). Since these lesions are similar to those observed in myocardial ischemia, they are used as a model for cardiac infarction (Rona 1985). The deleterious effects that may be caused to the heart by ISO administered for the treatment of asthma (Maguire et al. 1991) constitute another motivation to investigate ISO toxicity.

Several studies tried to determine the mechanism of ISO toxicity leading to myocardial cellular necrosis. Various hypotheses have been proposed, linked to a relative coronary insufficiency and hypoxia (Rona 1985), coronary microcirculation effects of catecholamines (Somani et al. 1970), calcium overload (Fleckenstein et al. 1975), magnesium leakage (Lehr 1969), increased activity of phospholipase (Kondo et al. 1987), and oxygen free radical production (Persoon-Rothert et al. 1989).

The hypothesis of magnesium deficiency was supported by measurements that did not distinguish between intra- and extra-cellular magnesium (Godfraind and Sturbois 1975; Lehr

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1969). With ^{31}P NMR spectroscopy, intracellular free magnesium concentration ($[Mg^{2+}]_i$) was previously measured in isolated hearts subjected to low doses of ISO (Headrick and Willis 1991; Nishimura et al. 1993). However, the accuracy of $[Mg^{2+}]_i$ measurements by ^{31}P NMR may be impaired by various sources of errors (Garfinkel and Garfinkel 1984; London 1991; Mosher et al. 1992; Mottet et al. 1994). Recently, new calibrations have been provided to improve the accuracy of this method (Williams et al. 1993).

The purpose of this study was to test the hypothesis of magnesium deficiency by applying ^{31}P NMR measurements to isolated perfused rabbit hearts subjected to a high dose (10^{-6} M) of ISO. In this study, we applied these more appropriate calibrations to the $[Mg^{2+}]_i$ measurements. Furthermore, this new approach allowed us to provide estimations of the uncertainty on the $[Mg^{2+}]_i$ measured values.

Concomitantly with $[Mg^{2+}]_i$ measurements, the values of high energy phosphate compound concentrations (phosphocreatine [PCr], adenosine triphosphate [ATP], inorganic phosphate [P_i]) and intracellular pH (pH_i) were calculated from the ^{31}P NMR spectra. The mechanical functioning of the hearts was also followed during the experiments.

Materials and methods

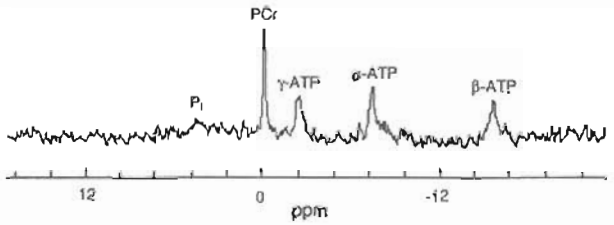
Animal preparations

New Zealand White rabbits, weighing 2.0 ± 0.08 kg (mean ± SEM), were cared for in accordance with the principles and guidelines of our institution and the Canadian Council on Animal Care. They were anesthetized with intramuscularly administered 10 mg·kg^{-1} fluanisone and 0.3 mg·kg^{-1} fentanyl citrate (HYPNORM; Janssen Pharmaceutica, Beerse, Belgium) supplemented with pentobarbital sodium (60 mg·kg^{-1}, i.v.). After heparinization (250 IU heparin·kg^{-1} i.v.), the heart was rapidly excised and cooled in ice-cold perfusate. After cannulation of the aorta, the heart was isovolumically perfused according to Langendorff's method with a constant flow (25 mL·min^{-1}) of modified Krebs–Henseleit solution without phosphate, containing insulin (70 mU·L^{-1}) and glucose (5.5 mmol·L^{-1}) as exogenous substrate, thermostated at 37°C, and saturated with gas containing 95% O_2 – 5% CO_2, resulting in a pH of 7.4. The flow from the besian veins was drained by a small cannula placed through the apex of the left ventricle. The left and right atria were closed by ligation of the caval and pulmonary veins, and the venous effluent was collected via the cannulated pulmonary artery. A small Latex balloon introduced in the left ventricle and connected to a Gould Statham P231D pressure transducer (Gould Instruments, Ballainvilliers, France) allowed continuous measurement of the heart rate (HR), the end-diastolic pressure (EDP), the systolic pressure (SP), and its maximal positive derivative (dP/dt). From these parameters the developed left ventricular pressure (LVP = SP – EDP) and the rate–pressure product (RPP = HR × LVP) were calculated. A second pressure transducer connected to the perfusion cannula ensured the measurement of the perfusion pressure (PP). Data were continuously recorded on a paper chart recorder (Gould Instruments ES1000).

NMR measurements

NMR measurements were performed with a 4.7 T Bruker (Bruker, Ettlingen, Germany) Biospec horizontal 40 cm bore magnet spectrometer. Hearts were placed in a Plexiglas perfusion chamber adapted to a 3-cm $^1H/^{31}P$ (200 MHz/81 MHz) loop gate resonator. This probe was placed at the center of the magnet, thermostated by a warm air flow. In the magnet, the perfusion solution was thermostated until the arrival to the perfusion chamber by a circulation of warm water around the perfusion tubing. After optimizing the magnetic field homogeneity by water proton shimming, the resulting line width was about 30 Hz. Spectra were obtained from 120 free induction decays (FIDs) accumulated with the following parameters: spectral width 6000 Hz, repetition time 1.5 s, and 60° pulses. The successive spectra were thus obtained every 3 min. Time-domain signals were filtered with a 20-Hz line broadening exponential function and Fourier transformed. A manual 0 and 1st order phase correction was applied to each spectrum. A 3-min spectrum acquired under control perfusion is displayed in Fig. 1. For metabolic and mechanical data recorded during the sequential 3-min periods, each data point has been represented at the time corresponding to the middle of the 3-min period.

Fig. 1. A representative ^{31}P spectrum acquired from a heart during the rest period. The spectrum was taken by accumulation of 120 FIDs over a period of 3 min. Chemical shifts are depicted in parts per million (ppm).



Estimation of metabolite concentrations, pH_i, and $[Mg^{2+}]_i$

The metabolite concentrations were calculated from the peak areas of phosphocreatine (PCr), inorganic phosphate (P_i), and the β-phosphate of ATP, compared with the signal of 10 µL of 50% methanephosphonic acid (MPA) (Merck, Overijse, Belgium) located in a capillary placed close to the heart. The standard routine for the spectrometer was used for the measurement of peak areas. Longitudinal relaxation times (T_1) of each compound were measured in preliminary experiments, with the progressive saturation technique. T_1 of MPA, PCr, P_i, and β-peak of ATP was 0.67 ± 0.04, 2.34 ± 0.15, 1.75 ± 0.28, and 0.70 ± 0.09 s, respectively. These values agree with previously reported values measured in isolated rabbit hearts at 4.2 T (Nakamura et al. 1993). Consequently calculated saturation factors (SFs) were used to correct peak areas for partial saturation (Tofts and Wray 1988). The calculated amounts of ATP, PCr, and P_i were divided by the measured dry weight of each heart to yield a concentration in units of micromoles per gram dry weight. The total phosphate content is defined as 3 × [ATP] + [PCr] + [P_i].

Intracellular pH was calculated from the chemical shift difference between intracellular P_i and PCr (δ_{Pi}) (Moon and Richards 1973), according to the relation

$$[1] \quad pH_i = 6.8 + \log [(\delta_{Pi} - 3.39)/(5.70 - \delta_{Pi})]$$

where the constants were determined by a previously realized titration curve.

The concentration of intracellular free magnesium ($[Mg^{2+}]_i$) was calculated from the separation of α- and β-peaks of ATP ($\delta_{\alpha\beta}$), according to the technique of Gupta and Moore (1980). The magnitude of $\delta_{\alpha\beta}$ changes is related to the degree of ATP complexation, following the equation

$$[2] \quad [MgATP]/[ATP_{free}] = (\delta_{\alpha\beta}{}^{ATP} - \delta_{\alpha\beta})/(\delta_{\alpha\beta} - \delta_{\alpha\beta}{}^{MgATP})$$

where the chemical shift limits $\delta_{\alpha\beta}{}^{ATP}$ and $\delta_{\alpha\beta}{}^{MgATP}$ are the $\delta_{\alpha\beta}$ measured in free and fully complexed ATP, respectively. It is then possible to calculate $[Mg^{2+}]_i$ using the equation

$$[3] \quad [Mg^{2+}]_i = K_D (\delta_{\alpha\beta}{}^{ATP} - \delta_{\alpha\beta}{}^{obs})/(\delta_{\alpha\beta}{}^{obs} - \delta_{\alpha\beta}{}^{MgATP})$$

where K_D is the apparent dissociation constant of MgATP. A value of 50 µM was taken for K_D (Gupta et al. 1978). Calculations of $[Mg^{2+}]_i$ were performed with the MAGPAC software, previously described

by its authors (Williams et al. 1993). Briefly, this method of $[Mg^{2+}]_i$ calculation based on $\delta_{\alpha\beta}$ measurements allowed us to avoid errors in the estimation of MgATP chemical shift limits (Mosher et al. 1992), to correct K_D for pH_i variations (Bock et al. 1985), and to estimate the errors due to uncertainties in $\delta_{\alpha\beta}$ and in pH_i measurements (Williams et al. 1993). For each heart, the $\delta_{\alpha\beta}$ values recorded during 15 min of NMR acquisition (5 spectra acquired in 3-min intervals) were averaged. A mean $\delta_{\alpha\beta}$ value was taken from averaging among hearts and the standard deviation was calculated. This mean value was introduced in the MAGPAC software for $[Mg^{2+}]_i$ calculation. The same averaging procedure was applied to pH_i values. $\delta_{\alpha\beta}$ and pH_i standard deviations were used for the computation of the uncertainties in $[Mg^{2+}]_i$ estimations, which were expressed by the lower and the upper limits of $[Mg^{2+}]_i$ values.

Experimental protocol

Preliminary experiments were performed to verify the stability of the preparation. Six hearts were perfused with a constant flow (25 mL·min^{-1}) under control conditions during 130 min. This flow leading to a PP of about 40–45 mmHg (1 mmHg = 133.3 Pa) allows the hemodynamics to be similar to those occurring in vivo while not inducing, in control conditions, any ischemia or hypoxia (Depré et al. 1995). [PCr], [ATP], $[P_i]$, pH_i, and mechanical parameters recorded at the end of the perfusion were not significantly different from the data recorded at the end of the equilibration period. The values of $[Mg^{2+}]_i$ did not vary more than 0.08 mM during the continuous control perfusion.

The effects of high dose ISO infusion were assessed on six hearts. They were allowed to equilibrate under control conditions during 42.5 min of perfusion. This period was called the rest period. When contracture evidence (EDP and PP rise) was observed during this period, the heart was eliminated from the study results. Isoproterenol, 10^{-6} M, was added to the perfusate during the following 30 min. Then the ISO infusion was stopped, and the hearts were allowed to recover during the last 45 min of perfusion. Hearts were removed from the perfusion setup, weighed, and placed for 24 h at 125°C for the measurement of dry weights.

Statistical analysis

Data are presented in figures as means ± SEM. Both for control and ISO groups, changes in variables of interest were assessed by an ANOVA for repeated measurements design. When ANOVA showed significant changes, contrast analysis was computed to compare each data point of the ISO and recovery periods with the five last points (15 min) of the rest period. Values of $p < 0.05$ were considered significant.

Results

At the end of the experiments, the weight of the hearts was 5.48 ± 0.65 g wet weight and 0.62 ± 0.03 g dry weight. The stimulation of the cardiac function induced by ISO infusion was expressed by a fast increase in RPP and dP/dt, which remained significantly higher, 165 ± 17 and 171 ± 21%, respectively, than their rest values until the end of ISO administration (Fig. 2). HR rose sharply (75 ± 25%) just after the beginning of ISO infusion and then decreased slightly (Fig. 2). SP showed a slow increase during the ISO period (Fig. 2). The changes of HR and SP were not statistically significant. Conversely, PP decreased slightly but significantly, and after 10 min of perfusion with ISO, PP dropped below 30 mmHg (Fig. 2). EDP was unchanged just after the beginning of ISO administration, and then it increased slowly after about 15 min, without reaching the significance threshold during the time of this experimentation (Fig. 2).

At the beginning of the ISO period, [PCr] decreased sharply and significantly to 60 ± 3% of the rest value (Fig. 3). Afterwards, a partial repletion of [PCr] was recorded before the ISO discontinuation. Consequently, at the end of the 30 min of ISO infusion, [PCr] was not significantly different than its rest value. In contrast, $[P_i]$ quickly significantly increased with ISO infusion and remained higher during the 30 min of ISO administration (331 ± 73% of the rest value at the end of the ISO period) (Fig. 3). ATP decreased slowly, continuously, and significantly, reaching 57 ± 9% of its rest value at the end of the ISO period (Fig. 3). During ISO administration, pH_i immediately significantly decreased to 6.83 ± 0.04 and stabilized at this value, compared with 7.00 ± 0.02 for the last point of the rest period (Fig. 3). On the other hand, the total phosphate content decreased slightly (89 ± 3% of the rest level), indicating a low but no significant leakage of phosphate during 30 min of 10^{-6} M ISO intoxication.

When ISO administration was stopped, RPP and dP/dt returned below their rest values. At the end of the experiment, these parameters were impaired: 70 ± 8% (statistically significant) and 75 ± 10% (not significant) of the rest values for RPP and dP/dt, respectively. HR continued its slow decrease during the recovery period, whereas SP decreased during the 5 min following the stopping of ISO perfusion and then rose progressively until the end of the experiment (Fig. 2). Changes of HR and SP were not statistically significant. On the other hand, the increase in EDP accelerated after the end of ISO infusion, reaching 27 ± 5 mmHg at the end of the experiment, significantly higher than the rest value (6 ± 1 mmHg). PP increased also progressively and significantly after ISO administration.

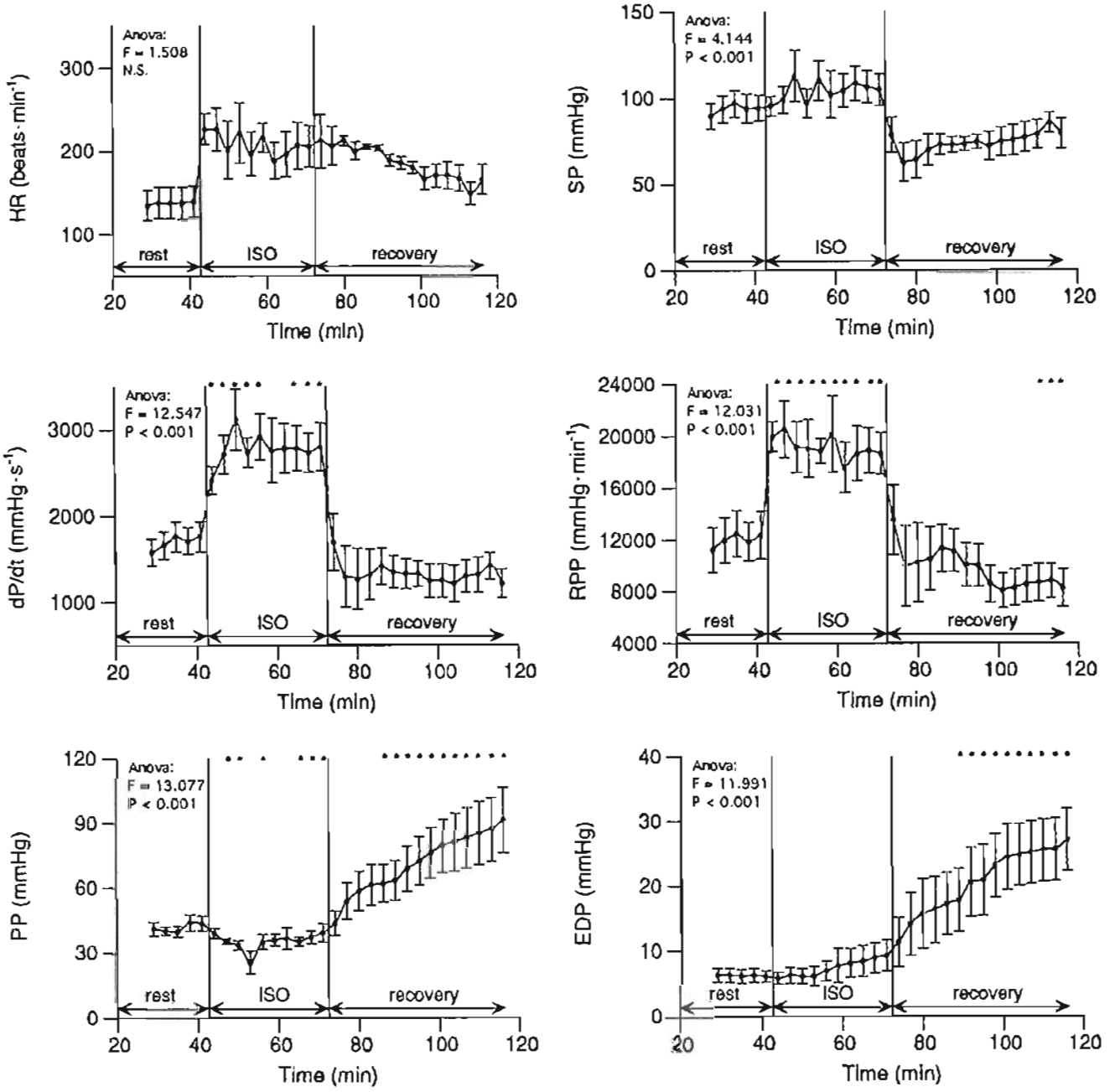
Although [PCr] was repleted completely after the end of the ISO period, [ATP] remained significantly depleted (59 ± 13% of the rest concentration) until the end of the experiment period. $[P_i]$ decreased sharply during ~10 min after the ISO discontinuation and then stabilized, remaining at 200 ± 32%, still significantly higher than its rest values. The total phosphate content did not change during the recovery period, whereas pH_i increased only to 6.93 ± 0.01, still significantly lower than the rest value of 7.00 ± 0.02.

Results of $[Mg^{2+}]_i$ are given in Table 1, with the estimations of upper limits and lower limits for the uncertainty on the results. The calculation of the uncertainty on $[Mg^{2+}]_i$ estimation is depicted in Fig. 4. A slight increase of $[Mg^{2+}]_i$ values was recorded during ISO administration (0.88 mM at the end of the ISO period compared with 0.70 mM during the rest period) and at the beginning of the recovery (0.84 mM). However, compared with the range of uncertainty of the values, these variations may not be considered significant. At 45 min after the end of ISO infusion, $[Mg^{2+}]_i$ was back to 0.72 mM.

Discussion

During ISO infusion, the β-adrenergic stimulation induced a 65 ± 17 and 71 ± 21% increase in RPP and dP/dt, respectively. With isolated perfused isovolumic rabbit hearts paced at 180 beats·min^{-1}, dose-dependent increases in LVP and dP/dt have been demonstrated with ISO administration ranging from 10^{-9} to 10^{-7} M (Aoyagi et al. 1991). At 10^{-7} M, LVP and dP/dt have been shown to increase by 24 and 28%, respectively. Our results with 10^{-6} M ISO are in agreement with this dose-dependent increase of the mechanical performances. The small

Fig. 2. Mechanical parameters recorded during the experiments: heart rate (HR), systolic pressure (SP), derivative of the systolic pressure (dP/dt), end-diastolic pressure (EDP), perfusion pressure (PP), and rate–pressure product (RPP). ISO, 10^{-6} M, was applied between 42.5 and 72.5 min. Data are presented as means ± SEM. *Significantly different from the last data of the equilibration period ($p < 0.05$).

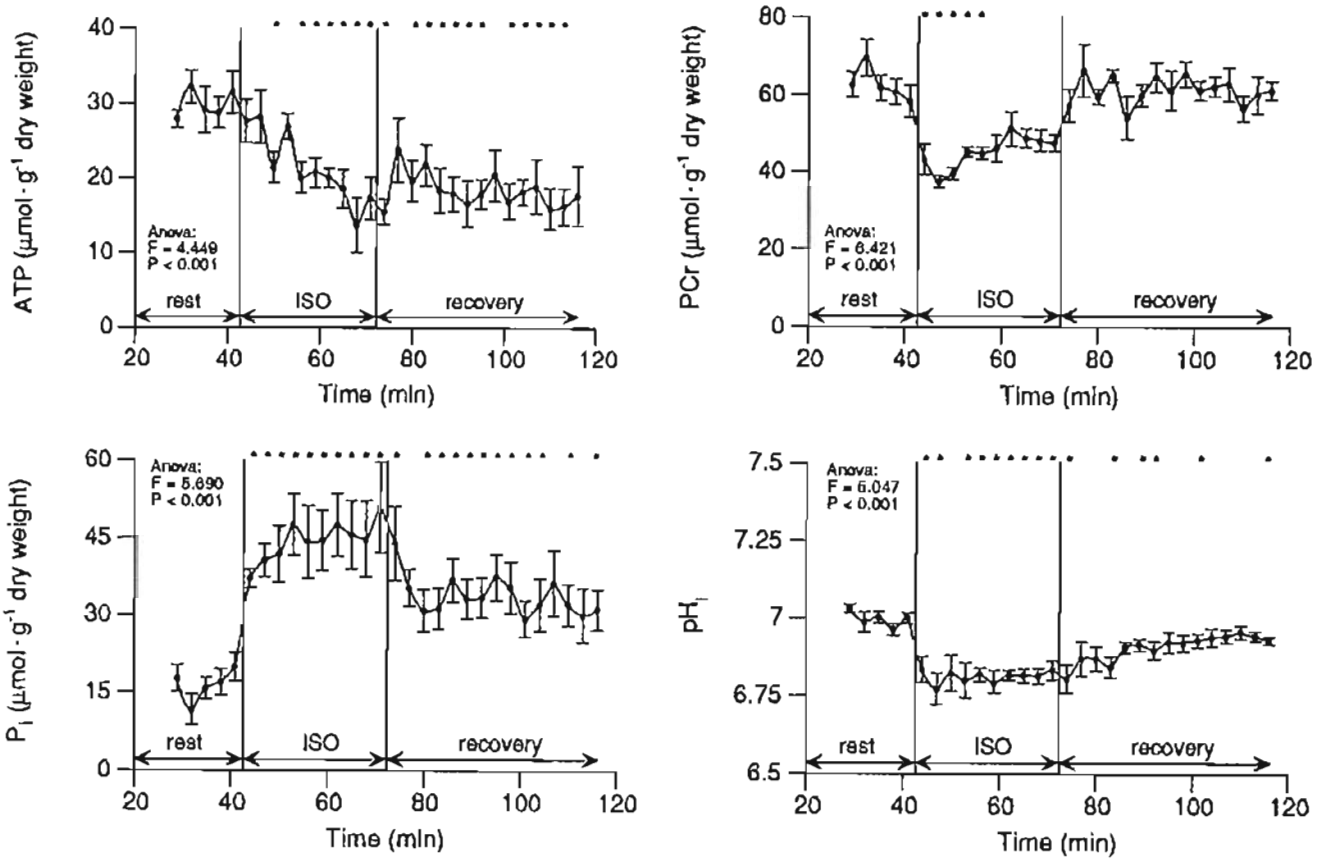


decrease of PP at the beginning of ISO infusion was the consequence of the previously reported vascular smooth muscle relaxation caused by β-agonists (Feigl 1983).

During ISO administration, a decrease in [ATP] and [PCr] and an increase in [P_i] were recorded. The same trends of variations of the high energy phosphate compounds have previously been observed in isolated rat hearts perfused with ISO concentrations ranging from 10^{-9} to 10^{-7} M (Headrick and Willis 1991; Kupriyanov et al. 1993). These variations have also been reported in isolated rabbit hearts submitted to 0.5×10^{-7} M ISO, excepted for [PCr], which did not significantly decrease (Nakamura et al. 1993). In our study, [PCr] decreased sharply in the early phase of ISO infusion, to 60 ± 3% of the rest value. A slow recovery of [PCr] began already during the continuation of ISO infusion. This observation is in agreement with a study of isolated rat hearts which reported a decrease in [PCr] during the first 25 min of 10^{-8} M ISO infusion, followed by a complete restoration of [PCr] already during the following 75 min of ISO administration (Nishimura et al. 1993).

EDP rose slowly during ISO administration and increased more sharply after the end of ISO administration. The rise in EDP during recovery occurred simultaneously with an increase in PP, probably caused by vascular compression. These increases in EDP and PP after ISO administration revealed a

Fig. 3. Metabolic parameters measured by ^{31}P NMR during the experiments: pH_i and concentrations of ATP, PCr, and P_i. ISO, 10^{-6} M, was applied between 42.5 and 72.5 min. Data are presented as means ± SEM. *Significantly different from the five last data of the equilibration period ($p < 0.05$).



contracture of the myocardium, which was concomitant with a small but significant loss in RPP and a persistent decrease of [ATP] (59 ± 13% of the rest value) and increase of [P_i] (200 ± 32% of the rest value). These results establish that the 30-min administration of 10^{-6} M ISO caused severe myocardial injury. In this model of constant infusion flow, the increase in cardiac work may have caused hypoxia. The respective parts of hypoxia and of other mechanisms in ISO-induced myocardial injuries cannot be determined by the present study.

The hypothesis of magnesium deficiency relied on the measurement of magnesium depletion in myocardial necrosis induced by subcutaneous injection of ISO (Lehr 1969). With the same model, an increase in magnesium was measured 1 h after ISO injection, followed by a progressive reduction of the myocardial magnesium content (Godfraind and Sturbois 1975). However, these measurements did not distinguish between extra- and intra-cellular magnesium. ^{31}P NMR allows estimation of the intracellular free magnesium concentration ($[Mg^{2+}]_i$) from the chemical shift between α-ATP and β-ATP (Gupta and Moore 1980). It must be mentioned that $[Mg^{2+}]_i$ measured by ^{31}P NMR spectroscopy most probably represents only the cytoplasmic pool of $[Mg^{2+}]_i$, because the NMR signals of ATP come from cytoplasmic ATP. The accuracy of these $[Mg^{2+}]_i$ measurements may be impaired by different sources of errors (Garfinkel and Garfinkel 1984; London 1991; Mosher et al. 1992; Mottet et al. 1994). Recently, new calibrations have been provided to improve the accuracy of the measurements (Williams et al. 1993) and calculations of the uncertainty on the $[Mg^{2+}]_i$ estimated values have been improved considering pH_i changes (see below). In this study, we applied these new calibrations to the $[Mg^{2+}]_i$ measurements.

It has been reported that β-adrenergic stimulation induces a large efflux of Mg^{2+} across the sarcolemma of heart myocytes, specifically through the increase in cyclic AMP (Romani and Scarpa 1990). This result could have implicated $[Mg^{2+}]_i$ changes that could have been responsible for impairments of several metabolic activities regulated by $[Mg^{2+}]_i$ (activities of kinases and ATPases, Ca^{2+} transport). This idea was not corroborated by ^{31}P NMR measurement of $[Mg^{2+}]_i$ in hearts submitted to β-adrenergic stimulation. An increase in $[Mg^{2+}]_i$ has been previously reported for isolated rat hearts exposed for 20 min to ISO concentrations ranging from 0.4 to 75×10^{-9} M (Headrick and Willis 1991), attributed to the decrease in [ATP] and the subsequent dissociation of Mg^{2+} from ATP. In isolated rat hearts submitted for 100 min to 10^{-8} M ISO, an increase in $[Mg^{2+}]_i$ was reported during the first 50 min, but it was followed by a slight $[Mg^{2+}]_i$ decrease during the latter half of ISO infusion. This decrease was attributed by the authors to the specific control through β-adrenoceptor stimulation. The present study demonstrates that this delayed $[Mg^{2+}]_i$ decrease may not be responsible for ISO toxicity, because 10^{-6} M ISO infused for 30 min induced [ATP] loss and contracture without decreasing $[Mg^{2+}]_i$.

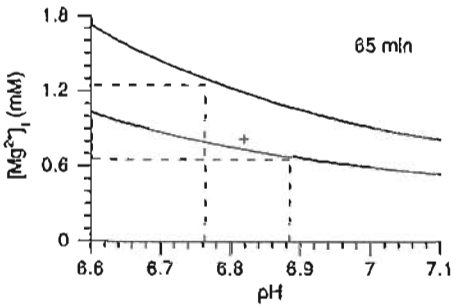
During the 10^{-6} M ISO infusion, [ATP] decreased from

Table 1. Calculation of the intracellular free magnesium concentration ($[Mg^{2+}]_i$) based on the separation between α- and β-peaks of ATP ($\delta_{\alpha\beta}$).

	Time (min)	$\delta_{\alpha\beta}$ (ppm)	pH (mM)	$[Mg^{2+}]_i$ (mM)	Uncertainty in $[Mg^{2+}]_i$ Upper limit (mM)	Lower limit (mM)
Rest period	35	8.45±0.02	6.99±0.03	0.70	0.80	0.60
Isoproterenol	50	8.45±0.03	6.81±0.08	0.83	1.14	0.62
	65	8.44±0.04	6.82±0.06	0.88	1.24	0.66
Recovery	80	8.44±0.06	6.87±0.07	0.84	1.49	0.56
	95	8.45±0.02	6.92±0.06	0.73	0.88	0.61
	110	8.45±0.05	6.94±0.04	0.72	1.01	0.54

Note: For the computation of each $[Mg^{2+}]_i$ value, the MAGPAC software used averaged $\delta_{\alpha\beta}$ values corresponding to 15 min experiment, calibrated MgATP chemical shift limits, and corrected the MgATP dissociation constant as a function of pH. $\delta_{\alpha\beta}$ and pH_i standard deviations among hearts (SD) were used for the computation of the upper and lower limits of $[Mg^{2+}]_i$ values.

Fig. 4. Error analysis illustrated for the 71 min $[Mg^{2+}]_i$ measurement (+). The continuous lines delimitate the range of calculated $[Mg^{2+}]_i$ for mean $\delta_{\alpha\beta}$ ± variation among hearts (standard deviation) (8.44 ± 0.04). The broken lines indicate the uncertainty in $[Mg^{2+}]_i$ due to the uncertainty in pH (6.82 ± 0.06).



32 ± 2 to 17 ± 3 μm/g dry weight. Considering that the intracellular water contained in 1 g tissue is estimated as 0.6 mL (Clarke and Willis 1987) and that drying reduced the weight of the hearts by a factor of 8, the calculated decrease in [ATP] may be evaluated to 3 mM, whereas no significant change of $[Mg^{2+}]_i$ was recorded. The major proportion of Mg^{2+}_i dissociated from ATP must thus have been lost to extracellular space or bound to other intracellular sites. From our point of view, the first hypothesis is the most plausible because the specific β-adrenergic stimulation of Mg^{2+} efflux has been previously demonstrated and because this stimulation has been shown to be dose dependent (Romani and Scarpa 1990). This may explain the absence of significant $[Mg^{2+}]_i$ increase during the [ATP] decrease caused by 10^{-6} M ISO infusion, in contradiction with the previous ^{31}P NMR studies of hearts submitted to ISO (Headrick and Willis 1991; Nishimura et al. 1993).

This contradiction may also be due to differences in the $[Mg^{2+}]_i$ calculation method. The method used here considered more sources of errors and yielded larger uncertainties on the calculated $[Mg^{2+}]_i$ values, but was probably more accurate than the previous ^{31}P NMR $[Mg^{2+}]_i$ measurements. Accurate determination of $[Mg^{2+}]_i$ by ^{31}P NMR depends on the precision in the estimation of K_D and of $[MgATP]/[ATP_{free}]$. K_D changes with temperature, pH, and ionic strength variations (Garfinkel and Garfinkel 1984; London 1991; Mottet et al. 1994). In the present study, temperature did not change during the experiment. If the ionic strength did not undergo important variations, $[Mg^{2+}]_i$ changes were accurately evaluated by correcting K_D as a function of the pH_i variations that were recorded. The commonly used value of 50 μM was chosen for K_D corresponding to pH 7.2. This value has not yet been firmly established, but an error in this choice would only induce linear scaling of the absolute $[Mg^{2+}]_i$ values. As the precision in the estimation of $[MgATP]/[ATP_{free}]$ is concerned, Mosher et al. (1992) recently showed that the use of a $\delta_{\alpha\beta}{}^{MgATP}$ determined with too high Mg_{total}/ATP_{total} ratios would lead to overestimations of $[Mg^{2+}]_i$, which may be greater than 300% for $[Mg^{2+}]_i$ greater than 0.6 mM. In the present study, this overestimation of $[Mg^{2+}]_i$ has been avoided by using the correct calibrated shift limits implemented in the MAGPAC software. The ignorance of this potential source of error in the previous ^{31}P NMR $[Mg^{2+}]_i$ measurements may explain the larger $[Mg^{2+}]_i$ increases reported during ISO administration.

In conclusion, simultaneous measurements of mechanical and metabolic parameters in isolated rabbit hearts established the myocardial injury caused by a 30-min exposure to 10^{-6} M ISO. This injury was not related to $[Mg^{2+}]_i$ deficiency, in contradiction with the previous hypothesis of magnesium depletion consecutive to ISO administration.

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References

Aoyagi, T., Momomura, S.I., Serizawa, T., Tizuka, M., Ohya, T., and Sugimoto, T. 1991. α-Adrenoceptor-mediated inotropism and β-adrenoceptor-mediated inotropism in isolated rabbit ventricles: a comparison in mechanical effects and energetic efficiency. J. Cardiol. Pharmacol. 17: 647–655.

Bock, J.L., Wenz, B., and Gupta, R.K. 1985. Changes in intracellular Mg adenosine triphosphate and ionized Mg^{2+} during blood storage: detection by ^{31}P nuclear magnetic resonance spectroscopy. Blood, 65: 1526–1530.

Clarke, K., and Willis, R.J. 1987. Energy metabolism and contractile function in rat heart during graded, isovolumic perfusion using ^{31}P NMR spectroscopy. J. Mol. Cell. Cardiol. 19: 1153–1160.

Depré, C., Vanoverschelde, J.-L., Goudemant, J.-F., Mottet, I., and Hue, L. 1995. Protection of the perfused rabbit heart against ischemic injury by non-vasoactive concentrations of NO synthase inhibitors. Circulation, 92: 1911–1918.

Feigl, E.O. 1983. Coronary physiology. Physiol. Rev. 63: 1–205.

Fleckenstein, A., Janke, J., Döring, H.J., and Leder, O. 1975. The key

role of Ca in the production of noncoronarogenic myocardial necroses. *In* Pathophysiology and morphology of myocardial cell alteration. Recent advances in studies on cardiac structure and metabolism. Vol. 6. *Edited by* A. Fleckenstein and G. Rona. University Park Press, Baltimore. pp. 21–32.

Garfinkel, L., and Garfinkel, D. 1984. Calculation of free Mg^{2+} concentration in adenosine 5′-triphosphate containing solutions in vitro and in vivo. Biochemistry, **23**: 3547–3552.

Godfraind, T., and Sturbois, X. 1975. Inhibition by cinnarizine of heart ionic changes induced by isoprenaline. *In* Pathophysiology and morphology of myocardial cell alteration. Recent advances in studies on cardiac structure and metabolism. Vol. 6. *Edited by* A. Fleckenstein and G. Rona. University Park Press, Baltimore. pp. 127–134.

Gupta, R.K., and Moore, R.D. 1980. ^{31}P NMR studies of intracellular free Mg^{2+} in intact frog skeletal muscle. J. Biol. Chem. **255**: 3987–3993.

Gupta, R.K., Benovic, J.L., and Rose, Z.B. 1978. Magnetic resonance studies of the binding of ATP and cations to human hemoglobin. J. Biol. Chem. **253**: 6165–6171.

Headrick, J.P., and Willis, R.J. 1991. Cytosolic free magnesium in stimulated, hypoxic, and underperfused rat heart. J. Mol. Cell. Cardiol. **23**: 991–999.

Kondo, T., Ogawa, Y., Sugiyama, S., Ito, T., Satake, T., and Ozawa, T. 1987. Mechanism of isoproterenol induced myocardial damage. Cardiovasc. Res. **21**: 248–254.

Kupriyanov, V.V., Korchazhkina, O.V., and Lakomkin, V.L. 1993. Regulation of cardiac energy turnover by coronary flow: a ^{31}P-NMR study. J. Mol. Cell. Cardiol. **25**: 1235–1247.

Lehr, D. 1969. Tissue electrolyte alteration in disseminated myocardial necrosis. Ann. N.Y. Acad. Sci. **156**: 344–378.

London, R.E. 1991. Methods for measurement of intracellular magnesium: NMR and fluorescence. Annu. Rev. Physiol. **53**: 241–258.

Maguire, J.F., O'Rourke, P.P., Colan, S.D., Geha, R.S., and Crone, R. 1991. Cardiotoxicity during the treatment of severe childhood asthma. Pediatrics, **88**: 1180–1186.

Moon, R.B., and Richards, J.H. 1973. Determination of intracellular pH by ^{31}P magnetic resonance. J. Biol. Chem. **248**: 7276–7278.

Mosher, T.J., Williams, G.D., Doumen, C., LaNoue, K.F., and Smith, M.B. 1992. Error in the calibration of the MgATP chemical-shift limit: effects on the determination of free magnesium by ^{31}P NMR spectroscopy. Magn. Reson. Med. **24**: 163–169.

Mottet, I., Demeure, R., Gallez, B., Grandin, C., Van Beers, B.E., and Pringot, J. 1994. Experimental ^{31}P NMR study of the influence of ionic strength on the apparent dissociation constant of MgATP. Magn. Reson. Mater. Phys. Biol. Med. **2**: 101–107.

Nakamura, K., Kusuoka, H., Ambrosio, G., and Becker, L.C. 1993. Glycolysis is necessary to preserve myocardial Ca^{2+} homeostasis during β-adrenergic stimulation. Am. J. Physiol. **264**: H670–H678.

Nishimura, H., Matsubara, T., Ikoma, Y., Nakayama, S., and Sakamoto, N. 1993. Effects of prolonged application of isoprenaline on intracellular free magnesium concentration in isolated heart of rat. Br. J. Pharmacol. **109**: 443–448.

Persoon-Rothert, M., van der Valk-Kokshoorn, E.J.M., Egas-Kenniphaas, M., Mauve, J., and van der Laarse, A. 1989. Isoproterenol-induced cytotoxicity in neonatal rat heart cell cultures is mediated by free radical formation. J. Mol. Cell. Cardiol. **21**: 1285–1291.

Romani, A., and Scarpa, A. 1990. Hormonal control of Mg^{2+} transport in the heart. Nature (London), **346**: 841–844.

Rona, G. 1985. Catecholamine cardiotoxicity. J. Mol. Cell. Cardiol. **17**: 291–306.

Rona, G., Chappel, C.I., Balazs, T., and Gaudry, R. 1959. An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. Arch. Pathol. **67**: 443–455.

Somani, P., Laddu, A.R., and Hardman, H.F. 1970. Nutritional circulation in the heart. III. Effect of isoproterenol and beta adrenergic blockade on myocardial hemodynamics and rubidium-86 extraction in the isolated supported heart preparation. J. Pharmacol. Exp. Ther. **175**: 577–592.

Tofts, P.S., and Wray, S. 1988. A critical assessment of methods of measuring metabolite concentrations by NMR spectroscopy. NMR in Biomedicine, **1**: 1–10.

Williams, G.D., Mosher, T.J., and Smith, M.B. 1993. Simultaneous determination of intracellular magnesium and pH from the three ^{31}P NMR chemical shifts of ATP. Anal. Biochem. **214**: 458–467.