Effects of prolonged application of isoprenaline on intracellular free magnesium concentration in isolated heart of rat

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1 The effect of prolonged application of isoprenaline on intracellular free-Mg²⁺ concentration ([Mg²⁺]_i) was examined by use of ³¹P-nuclear magnetic resonance (³¹P-n.m.r.) in rat isolated hearts. Left ventricular pressure (LVP) was simultaneously measured.

2 [Mg²⁺]_i was estimated from the separation of the α - and β -ATP peaks, using the dissociation constant of MgATP 38 μ M (established previously). In normal (phosphate-free, Krebs-Henseleit) solution, [Mg²⁺]_i was approximately 0.4 mM.

3 When isoprenaline was applied for 100 min, a transient increase in [Mg²⁺]_i was observed during the initial 25 min, whilst concentrations of ATP ([ATP]) and phosphocreatine ([PCr]) decreased and [P_i] correspondingly increased. During the subsequent 75 min of isoprenaline application, [Mg²⁺]_i decreased below its resting levels. Washout of isoprenaline restored [Mg²⁺]_i and [PCr], but [ATP] remained low. These changes elicited by isoprenaline were not observed in the presence of propranolol, a typical α -adrenoceptor blocker.

4 Isoprenaline increased both LVP and heart rate. The increased LVP and heart rate slowly returned to lower values during prolonged application of isoprenaline, but remained higher than those before application.

5 The transient rise in [Mg²⁺]_i elicited by isoprenaline could be attributed to the decrease in [ATP] resulting in a release of Mg²⁺. The subsequent decrease in [Mg²⁺]_i during the prolonged applications suggests that β -adrenoceptor stimulation itself facilitates Mg²⁺-extruding mechanism(s).

Keywords: Rat heart; Mg²⁺; nuclear magnetic resonance; isoprenaline; β -adrenoceptors; phosphorous compounds

Introduction

It is generally believed that changes in intracellular magnesium can modify cardiac function. By use of material isolated from heart, e.g. skinned fibres (Kusuoka & Marban, 1992) and single cells and cell membranes in patch clamp recordings (White & Hartzell, 1989; Matsuda, 1991; Agus & Morad, 1991), possible effects of magnesium ions on contractility have been assessed.

In diseased hearts, the importance of the effects of intracellular magnesium on cardiac function has been emphasized: epidemiological studies (Stitt *et al.*, 1973; Anderson *et al.*, 1975) have indicated that in soft water areas, relatively high mortality from cardiovascular disease may be due to magnesium deficiency. On the other hand, it has been suggested that Mg²⁺ deficiency in cardiovascular disease is due to the action of catecholamines (Ebel & Günther, 1983).

Recently, conflicting results have been published on the regulation of intracellular free Mg²⁺ concentration ([Mg²⁺]_i) in rat isolated hearts. Romani & Scarpa (1990) found that Mg²⁺ efflux was increased by a short (approx. 10 min) application of noradrenaline and the increase was not observed in the presence of propranolol, suggesting a decrease in [Mg²⁺]_i by β -adrenoceptor stimulation. On the other hand, using ³¹P-nuclear magnetic resonance (³¹P-n.m.r.), Headrick & Willis (1989) estimated that [Mg²⁺]_i was increased by application of isoprenaline for a similar short duration (20 min). In the present study, we measured [Mg²⁺]_i during prolonged (100 min) applications of isoprenaline using the same n.m.r. techniques, and obtained results which can explain the discrepancy between these two studies.

Methods

Preparation and solution

Male Wistar rats weighing 350–400 g were anaesthetized with sodium pentobarbitone. Hearts were rapidly excised and arrested in ice-cold saline. The aorta was cannulated for retrograde perfusion, and a water-filled balloon was placed in the left ventricle for monitoring developed pressure. The heart preparation was mounted in an n.m.r. tube of 25 mm diameter, and perfused at a constant flow rate of 15–16 ml min⁻¹ with a peristaltic pump. The perfusate was a phosphate-free, Krebs-Henseleit solution (KH) of the following composition (mM): NaCl 118, KCl 5.9, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25, glucose 12, Na₂EDTA 0.5 and was oxygenated with a mixture of 95% O₂ and 5% CO₂. The temperature in the sample tube was 37°C. The perfusing set-up was approximately 3 m from the main magnet. The temperature and oxygen tension were maintained using a triple-walled vinyl tube (Nakayama *et al.*, 1988). The inner tube was used for perfusing solution pre-warmed to 40°C, the middle and outer tubes were for circulation of the gas mixture and warmed water (40°C), respectively. After inserting the sample into the bore, the proton signal from water was used to improve the homogeneity of the magnetic field.

³¹P-n.m.r.

The methods employed for measurements of ³¹P-n.m.r. spectra and estimation of [Mg²⁺]_i were essentially the same as those described previously (Nakayama & Tomita, 1990). An n.m.r. spectrometer (JEOL GSX270W) was operated at 109.4 MHz. Radiofrequency pulses corresponding to a 30° flip angle were repeated at 0.6 s intervals. Spectra were usually obtained by accumulating 2500 signals over 25 min. In some experiments, a 5 min accumulation period was tested, but was judged to be inappropriate because of large

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Figure 1 Time table of experiment. Perfusion of the heart preparation starts at -30 min. ^{31}P -n.m.r. measurement starts at 0 min and lasts for 200 min. Spectra are obtained by 25 min accumulation periods of n.m.r. signals. Drugs are applied from 50 to 150 min.

variation in the spectral peak analysis. Before Fourier transformation, a line broadening factor of 15 Hz was applied to enhance the signal-to-noise ratio. Spectral peak resonances were measured relative to that of PCr (phosphocreatine) in parts per million (p.p.m.). Digital resolution of the spectrum was set to be approximately 0.005 p.p.m. by zero-filling to free induction decay.

Definition of the experimental periods

The experimental protocol is shown in Figure 1. ^{31}P -n.m.r. measurements were performed for 200 min after the heart preparation has been equilibrated by normal (KH) solution for approx. 30 min. Each spectrum required a 25 min accumulation of n.m.r. signals, thus afforded eight during the 200 min measurement. After two spectra had been obtained in normal (KH) solution (50 min total accumulation), drugs (isoprenaline and/or propranolol) were applied for 100 min. Thus, four spectra were obtained during the application of drugs. Recovery was assessed by observing the two spectra (50 min total accumulation) during the subsequent washout of the drugs.

In some heart preparations, ^{31}P -n.m.r. measurements were performed during continuous perfusion of normal solution (200 min, 8 spectra) without any drugs, to assess the stability of the preparation.

Estimation of intracellular Mg^{2+} and pH

$[\text{Mg}^{2+}]_i$ was estimated from the separation of the α - and β -peaks of ATP (δ_x), using the following equations (Gupta & Moore, 1980; Gupta *et al.*, 1984; Nakayama & Tomita, 1990):

$$[\text{Mg}^{2+}]_i = K_D (\Phi^{-1} - 1), \quad (1)$$

$$\Phi = [\text{ATP}]_{\text{free}}/[\text{ATP}]_{\text{total}} = (\delta_x - \delta_1)/(\delta_2 - \delta_1), \quad (2)$$

where K_D is the dissociation constant of MgATP, and δ_1 and δ_2 are the resonance separations of the α - and β -peaks in MgATP and free ATP, respectively. We used the values of 8.35 and 10.85 p.p.m. for δ_1 and δ_2 , respectively, and 38 μM for K_D (Gupta & Moore, 1980).

Intracellular pH (pH_i) was estimated from the chemical shift of inorganic phosphate (P_i) (Moon & Richards, 1973). The pK_a value for H_2PO_4^- used in the estimation was 6.65, and the chemical shifts used of H_2PO_4^- and HPO_4^{2-} were 3.14 and 5.61 p.p.m., respectively (Eisner *et al.*, 1987).

Statistics

Numerical data are expressed as means $\pm$ standard deviation (s.d.). Differences between means were evaluated by paired t tests, and a test value of less than 0.05 was taken as a statistically significant difference.

Drugs

Isoprenaline hydrochloride and propranolol hydrochloride were purchased from Sigma. Stock solutions of the drugs,

Figure 2 Effects of isoprenaline on ^{31}P -n.m.r. spectra. (a) After obtaining the second spectrum ((i); $t = 25$ –50 min) in normal (KH) solution, isoprenaline (10 nM) was added for 100 min, then washed out for 50 min. The spectra (ii) and (iii) were obtained during 0–25 and 75–100 min for isoprenaline application, respectively, and (iv) was obtained during 25–50 min washout. In (a), the whole spectra are shown. Each spectrum was taken by accumulation of 2500 signals (25 min). Spectral peaks were assigned as P_i , PCr and three ATP peaks (i). In (b), the P_i and β -ATP peaks are shown expanded. Vertical lines indicate the initial values of each peak.

Figure 3 Changes in $[Mg^{2+}]_i$ (a) and pH_i (b). After two spectra were obtained over 50 min in KH solution, isoprenaline was applied for 100 min (●, ○). Each point represents averaged estimates from spectra obtained by accumulation over 25 min. The values of $[Mg^{2+}]_i$ and pH_i obtained by continuous perfusion (200 min) of KH solution (■, □) are shown for comparison. (i)–(iv) correspond to those in Figure 1. Vertical bars represent s.d. values ($n = 6$, isoprenaline; $n = 5$, control); for clarity, some of the s.d. values were omitted. $[Mg^{2+}]_i$ during exposure of isoprenaline, was statistically significantly different (larger or smaller) from that in (i) ($P < 0.05$). pH_i significantly decreased during the first 25 min application.

which were prepared on the same day, were kept cold and protected from the light, before use.

Results

³¹P-n.m.r. spectra from rat isolated hearts

Acquisitions of ³¹P-n.m.r. started after the heart preparations had been equilibrated by perfusing with a KH solution for approximately 30 min. Spectra were subsequently taken every 25 min. In normal (KH) solutions, five major peaks were observed (P_i , PCr and γ -, α - and β -ATP: Figure 2a(i)). In the spectra obtained during the 25–50 min acquisition period, the chemical shifts of P_i , α - and β -ATP peaks were 4.98 ± 0.03 , -7.53 ± 0.03 and -16.12 ± 0.02 p.p.m. ($n = 16$), respectively. The pH_i and $[Mg^{2+}]_i$ estimated from these chemical shifts were 7.12 ± 0.03 and 0.38 ± 0.06 mM, respectively. To assess the stability of this preparation, five hearts were continuously perfused with KH solution for 200 min in five independent experiments. The mean values ($n = 5$) of pH_i and $[Mg^{2+}]_i$ corresponded to within 0.05 pH units and 0.07 mM, respectively, during the continuous perfusion of KH solution (Figure 3). [PCr] and [ATP] gradually decreased by 15 and 10%, respectively, over 200 min.

Effects of isoprenaline on $[Mg^{2+}]_i$ and phosphorous compounds

In Figure 2, changes in ³¹P-n.m.r. spectra on application and washout of isoprenaline (10 nM) are shown. As changes in

pH_i and $[Mg^{2+}]_i$ are most strongly expressed in the chemical shifts of the P_i and β -ATP peaks, respectively, these peaks are expanded in Figure 2b. After the two spectra had been obtained during the 50 min perfusion with KH solution alone, isoprenaline was added for 100 min. During the first 25 min of application of isoprenaline, the β -ATP peak was shifted to the higher p.p.m. (leftwards) by 0.08 ± 0.03 p.p.m. ($n = 6$). However, the β -ATP peak shifted in the opposite direction during the subsequent 75 min in the presence of isoprenaline, and reached lower frequencies than the chemical shift of the β -ATP peak observed before the application. The chemical shift of the β -ATP peak was -16.22 ± 0.01 p.p.m. in the last (4th) spectra obtained during application of isoprenaline (during 75–100 min of the application). The subsequent washout of isoprenaline restored the chemical shift of the β -ATP peak to its normal values. These changes in the chemical shift of the β -ATP peak revealed that $[Mg^{2+}]_i$ transiently increased from 0.37 ± 0.05 to 0.63 ± 0.09 mM during the initial 25 min application of isoprenaline and then gradually decreased to 0.25 ± 0.02 mM during the subsequent 75 min in the presence of isoprenaline (Figure 3a). The subsequent washout of isoprenaline recovered $[Mg^{2+}]_i$ to its normal concentrations in 25 min.

The P_i peak was shifted to lower p.p.m. (rightwards) by 0.09 ± 0.02 p.p.m. in the first spectra of the four obtained during application of isoprenaline, and gradually returned towards its normal chemical shift value during continued application (Figure 2b). Thus, the largest intracellular acidosis (pH_i 7.03 ± 0.02) was observed during the first 25 min application of isoprenaline, whence the estimated pH_i was gradually restored in the continued presence of isoprenaline (Figure 3b).

The concentrations of phosphorus compounds correspond to the integrals of the peaks. The intracellular concentration of ATP ([ATP]) is estimated from the integral of the β -ATP peak, because the γ - and α -ATP peaks have a contribution from ADP. When isoprenaline (10 nM) was applied, during the first 25 min, [PCr] and [ATP] significantly decreased to 56 ± 6 and $57 \pm 4\%$ of each control value (estimated from the 2nd of the initial spectra before the application), respectively, while [P_i] correspondingly increased. During the subsequent 75 min application of isoprenaline, [PCr] recovered to $95 \pm 18\%$, whereas [ATP] was further decreased to $49 \pm 9\%$ (Table 1). Like the β -ATP peak, the areas of the γ - and α -peaks decreased simultaneously during application of isoprenaline (Figure 2a), indicating further degradation of ADP. When isoprenaline was washed out, [ATP] was not restored. The lack of restoration in [ATP] is presumably due to deamination of adenosine and adenylate (Jennings & Steenbergen, 1985), and/or release of adenosine (Headrick & Willis, 1990).

Effects of propranolol

In five hearts, after observing two spectra during 50 min perfusion of normal (KH) solutions, 1 μ M propranolol was simultaneously applied with isoprenaline (10 nM) for 100 min. The simultaneous application of propranolol eliminated both

Table 1 Effects of isoprenaline on phosphorus compounds ($n = 6$)

	Normal (KH) 25–50	Isoprenaline (10 nM) 0–25	Washout 75–100	Washout 25–50 min
[PCr]	100	$56 \pm 6^*$	95 ± 18	$115 \pm 8^*$
[ATP]	100	$57 \pm 4^*$	$49 \pm 9^*$	$46 \pm 8^*$
[P _i]	100	$274 \pm 105^*$	$228 \pm 108^*$	117 ± 60

The concentrations of phosphorus compounds are expressed as percentages of respective initial concentrations in normal solution.

*Significantly different from the concentrations in normal solutions (25–50 min).

Table 2 Effects of isoprenaline and propranolol on developed pressure (A) and heart rate (B)

	Normal (KH) 25–50	Drug application		Washout 25–50 min
		0–25	75–100	
A Developed pressure (mmHg)				
Control (<i>n</i> = 5)	121 ± 9	115 ± 7	115 ± 9	119 ± 4
Isoprenaline (10 nM) (<i>n</i> = 6)	114 ± 6	207 ± 18*	140 ± 11*	118 ± 15
Isoprenaline (10 nM) + propranolol (1 µM) (<i>n</i> = 5)	114 ± 9	130 ± 14	110 ± 9	117 ± 9
B Heart rate (beats min⁻¹)				
Control (<i>n</i> = 5)	219 ± 7	214 ± 9	216 ± 7	215 ± 8
Isoprenaline (10 nM) (<i>n</i> = 6)	229 ± 12	367 ± 18*	285 ± 10*	220 ± 19
Isoprenaline (10 nM) + propranolol (1 µM) (<i>n</i> = 5)	232 ± 32	278 ± 16*	241 ± 8	219 ± 10

Contractility was simultaneously monitored during ³¹P-n.m.r. measurements for 200 min. After 50 min perfusion of normal (KH) solutions, isoprenaline alone, or isoprenaline and propranolol were applied for 100 min. In control experiments, no drug was applied during these periods.

*Significantly different from the values in normal solution (25–50 min).

of the changes in $[Mg^{2+}]_i$ and pH_i observed during applications of isoprenaline alone (Figure 4a and b). The concentrations of high energy phosphates, however, changed little throughout the experiments.

When propranolol (1 µM) alone was applied for 100 min, no significant changes were observed in $[Mg^{2+}]_i$, pH_i and phosphate concentrations.

Contractility

Contractility was simultaneously monitored during the ³¹P-n.m.r. measurements, by a water-filled balloon in the left ventricle. Changes in developed pressure and heart rate are summarised in Table 2A and 2B, respectively. During 200 min continuous perfusion of KH solution neither developed pressure, nor heart rate changed significantly (*n* = 5). When isoprenaline (10 nM, 100 min) was applied, both developed pressure and heart rate increased by 82 and 60% (*n* = 6), respectively, during the first 25 min of drug application, and gradually decreased during the subsequent 75 min. Both of the values towards the end of isoprenaline

application were still higher than those before application. Propranolol (1 µM) inhibited both the inotropic and chronotropic effects of isoprenaline. In the presence of propranolol, the contractility towards the end of isoprenaline application was nearly the same as in KH solution (control values).

Discussion

In heart muscle, reported values of $[Mg^{2+}]_i$ vary considerably (0.4–3 mM), however, recent estimated values are mostly less than 1 mM (reviewed by McGuigan *et al.*, 1991). Our estimation of $[Mg^{2+}]_i$ (approx. 0.4 mM) using ³¹P-n.m.r. in normal (KH) solutions agrees with these values. In the present study, we measured changes in $[Mg^{2+}]_i$ during prolonged applications of isoprenaline (over 100 min). $[Mg^{2+}]_i$ increased transiently (0.63 mM), but significant decreases in $[Mg^{2+}]_i$ (0.25 mM) were observed during the subsequent 75 min of drug applications (Figure 3a). These changes in $[Mg^{2+}]_i$ were markedly diminished by propranolol (Figure 4a), suggesting a hormonal regulation of $[Mg^{2+}]_i$ through a β-adrenoceptor effect.

We also measured changes in pH_i during application of isoprenaline. The pH_i estimated was approximately 7.1 in normal solutions, and transiently decreased by 0.1 units during the first 25 min application of isoprenaline (Figure 3b). Changes in pH_i are known to perturb the estimation of $[Mg^{2+}]_i$ (London, 1991). According to an equation described by Bock *et al.* (1985), the decrease in pH_i by 0.1 units from 7.1 leads to an increase in the estimation of $[Mg^{2+}]_i$ by less than 5%. Thus, in the present study, estimation of $[Mg^{2+}]_i$ does not seem to be perturbed significantly. Recently, Freudenrich *et al.* (1992) have reported that intracellular pH_i modulates $[Mg^{2+}]_i$ probably by affecting cytosolic Mg^{2+} -binding and/or the transport of Mg^{2+} across subcellular organelles. In cultured chicken heart cells, acidification of 0.9 pH units caused an increase in $[Mg^{2+}]_i$ of 0.1 mM. This modulation by pH_i of $[Mg^{2+}]_i$ is too small to explain the transient rise in $[Mg^{2+}]_i$ by applications of isoprenaline.

Adenosine triphosphate (ATP) is known as an important intracellular Mg^{2+} buffer. Headrick & Willis (1989) reported that inotropic stimulation using isoprenaline or ouabain results in a decrease in [ATP] and subsequently in an increase in $[Mg^{2+}]_i$ caused by dissociation of Mg^{2+} from ATP. The authors also showed an inverse relationship between $[Mg^{2+}]_i$ and [ATP]. Our results, a transient rise in $[Mg^{2+}]_i$ observed during the initial 25 min of isoprenaline application, accompanied by a marked decrease in [ATP], support their notion on regulation of $[Mg^{2+}]_i$ through a Mg^{2+} -buffering effect on ATP. However, in the present study, $[Mg^{2+}]_i$ decreased below its normal concentrations during the prolonged application of isoprenaline (over 100 min), while [ATP] decreased fur-

Figure 4 Effects of propranolol on $[Mg^{2+}]_i$ (a) and pH_i (b). After observing spectra in normal solutions for 50 min, propranolol (1 µM) was applied simultaneously with isoprenaline (10 nM) for 100 min. Vertical bars represent s.d. values (*n* = 5).

ther. If there were no other regulation except Mg^{2+} buffering by ATP, $[Mg^{2+}]_i$ would be expected to remain at the high concentration which was observed during the first 25 min of isoprenaline application, so long as [ATP] is not restored. On the other hand, Romani & Scarpa (1990) showed that 20% of total cellular Mg^{2+} was extruded by noradrenaline within a few minutes, and that this very large efflux of Mg^{2+} was inhibited by the preapplication of propranolol. They proposed a hormonal control of $[Mg^{2+}]_i$ and that β-adrenoceptor stimulation decreases $[Mg^{2+}]_i$. This β-adrenoceptor effect on Mg^{2+} efflux is in accordance with our observations of decreases in $[Mg^{2+}]_i$ in the latter half of isoprenaline application, whereas it seems likely that during short-term applications of isoprenaline, $[Mg^{2+}]_i$ would only increase due to the 'release' of Mg^{2+} from ATP. Ouabain, which is also known to have positive inotropic action, increased $[Mg^{2+}]_i$ in 25 min, accompanied by reduction of [ATP]. In contrast to isoprenaline, during the subsequent 75 min application, the increase in $[Mg^{2+}]_i$ was either sustained or increased gradually (unpublished observation, Nishimura & Nakayama). These results are consistent with the interpretation that the late decrease in $[Mg^{2+}]_i$ induced by isoprenaline is specific to β-adrenoceptor stimulation.

Intracellular Mg^{2+} buffers are also likely to have a binding affinity to Ca^{2+} . Therefore an increase in $[Ca^{2+}]_i$ may increase $[Mg^{2+}]_i$ due to competition. This effect has been investigated by use of n.m.r. techniques (Rotevatn *et al.*, 1989) and fluorescent dyes (Murphy *et al.*, 1989). In heart cells, it has been reported that $[Mg^{2+}]_i$ was increased by the removal of extracellular Na^+ in the presence of Ca^{2+} , whether extracellular Mg^{2+} is present or not, suggesting that the increase in $[Mg^{2+}]_i$ was induced through an increase in $[Ca^{2+}]_i$ via Na^+-Ca^{2+} exchange. In the present study, isoprenaline increased cardiac contractility throughout application. One would suspect that an increase in $[Ca^{2+}]_i$ by isoprenaline may modulate $[Mg^{2+}]_i$. The increase in $[Mg^{2+}]_i$ observed in the first 25 min after application of isoprenaline may in part be due to an increase in $[Ca^{2+}]_i$. However, in the latter half of prolonged application of isoprenaline, decreases in $[Mg^{2+}]_i$ were observed. Thus, modulation of $[Mg^{2+}]_i$ elicited by changes in $[Ca^{2+}]_i$ appears not to be significant when the physiological Na^+ -gradient is maintained. When isoprenaline was applied together with propranolol, a small transient increase in $[Mg^{2+}]_i$ (Figure 4a, statistically not significant) was observed during the initial 25 min, accompanied by little change in [ATP]. This may be explained by intracellular competition between Mg^{2+} and Ca^{2+} .

There are many papers concerning the effects of extracellular Mg^{2+} on cardiac function (Shine, 1979). In contrast,

little is known about changes in cardiac function upon increases or decreases in $[Mg^{2+}]_i$. However, according to the results obtained using material isolated from heart tissue, we can postulate the effects of $[Mg^{2+}]_i$ on cardiac function. In skinned muscle experiments, increases in $[Mg^{2+}]_i$ shift the $[Ca^{2+}]_i$ -tension relation towards higher $[Ca^{2+}]_i$ (Godt, 1974; Fabiato & Fabiato, 1975; Best *et al.*, 1977). Furthermore, patch clamp techniques have recently revealed that increases in $[Mg^{2+}]_i$ inhibit L-type Ca^{2+} channel currents, whilst decreases in $[Mg^{2+}]_i$ facilitate such currents, especially when stimulated by adenosine 3':5'-cyclic monophosphate (cyclic AMP) (White & Hartzell, 1988). These results suggest that the decreases in $[Mg^{2+}]_i$ observed during prolonged applications of isoprenaline contribute to the inotropic effect of β-adrenoceptor stimulation. On the other hand, in S49 murine lymphoma cells, it has been shown that the guanine nucleotide-binding regulatory component of adenylyl cyclase is activated only by high concentrations of Mg^{2+} (Cech & Maguire, 1982), but that hormonal stimulation reduces the Mg^{2+} requirement below the normal concentrations of $[Mg^{2+}]_i$ (Iyengar & Birnbaumer, 1982). If cardiac cells possess similar mechanisms for the regulation of adenylyl cyclase, a fall of $[Mg^{2+}]_i$ would reduce cyclic AMP production and subsequently may diminish the effects of β-adrenoceptor stimulation. In this case, our observation of a fall in $[Mg^{2+}]_i$ during the prolonged application, may be interpreted as a feedback mechanism of desensitization, and would correlate with a reduction in ventricular pressure and heart rate (Table 2). At present, it is difficult to address the role of $[Mg^{2+}]_i$ in such mechanisms. Thus, the physiological implications of changes in $[Mg^{2+}]_i$ on contractility as well as regulatory mechanism(s) of $[Mg^{2+}]_i$ during applications of isoprenaline merit further investigation.

In the present study, a transient increase and subsequent decrease in $[Mg^{2+}]_i$ were observed during prolonged applications of isoprenaline. These changes can be explained by apparently conflicting evidence which has been reported in short-term stimulation of β-adrenoceptors: $[Mg^{2+}]_i$ is transiently increased by Mg^{2+} 'release' via reduction of MgATP through a positive inotropic effect (Headrick & Willis, 1989); a large efflux of Mg^{2+} may be due to an $[Mg^{2+}]_i$ regulatory mechanism activated by cyclic AMP (Romani & Scarpa, 1990).

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