J. Membrane Biol. 199, 163–171 (2004)
DOI: 10.1007/s00232-004-0686-7

The Journal of
Membrane
Biology

Catecholamine-induced Regulation in Vitro and ex Vivo of Intralymphocyte Ionized Magnesium

P. Delva, C. Pastori, M. Degan, G. Montesi, A. Lechi
Department of Biomedical and Surgical Sciences, Section of Medicina Interna C, University of Verona, Verona, Italy

Received: 1 October 2003/Revised: 3 May 2004

Abstract. Despite the importance of the adrenergic activity and of the metabolism of magnesium in some important cardiovascular pathologies, very little is known about how intracellular ionized magnesium ($Mg^{2+}{}_i$) is regulated by catecholamines. We made an in-vitro study of the variations in the concentration of ionized magnesium in human lymphocytes using the fluorescent probe furaptra in response to different catecholamines. We also made an ex-vivo study of the changes in intracellular ionized magnesium in lymphocytes in 20 subjects with essential arterial hypertension, 10 treated with 120 mg/d of propranolol and 10 with placebo. Norepinephrine and isoproterenol significantly decrease $Mg^{2+}{}_i$ and this effect is blocked by β-blockers but not by α-blockers. The EC_{50} of the effect of norepinephrine is within the range of concentrations physiologically present in plasma. The substitution of extracellular sodium with choline blocks the decrease in intracellular ionized magnesium induced by norepinephrine, which leads us to suppose that the magnesium-reducing effect of catecholamines is a result of the activation of a Na^+-Mg^{2+}exchanger. We were not able to demonstrate any change in intracellular ionized magnesium after 1 and 17 days of active treatment in essential hypertensives. The impossibility of demonstrating ex vivo the mechanism of catecholamine-mediated regulation that is evident in vitro is perhaps due to our experimental conditions or to substances which in vivo inhibit the action of the catecholamines on magnesium, such as insulin and/or glucose.

Key words: Magnesium — Intracellular magnesium — β-Blockers — Catecholamines — Lymphocyte — Hypertension

Correspondence to: P. Delva; email: pietro.delva@univr.it

Introduction

The contribution of a disregulation in the cellular metabolism of magnesium to some cardiovascular pathologies and, in particular, to ischemic heart disease, cardiac insufficiency and arterial hypertension is still under debate (Gottlieb 1990; Delva et al., 1996, 2000; Barbagallo et al., 1997; Ford, 1999). Epidemiological studies suggest that populations with a low intake of magnesium in their diet develop ischemic heart disease more frequently than others (Liao, Folsom & Brancati, 1998). The relationship between epidemiological and clinical data is, however, not easily understood and, after some initial optimism (Woods et al., 1992) the positive effects of the use of infusions of magnesium in patients with acute myocardial infarct were not confirmed (Seelig & Elin, 1995; The MAGIC Trial, 2002).

There is a large quantity of data in the literature showing that an excess of catecholamines in circulation has a fundamental role in the progression of cardiac insufficiency (Yoshikawa et al., 1996), and in determining the sudden death of patients with coronary heart disease (Eisenberg et al., 1992) and essential arterial hypertension (Folkow, 1982). The protective effect of β-blockers in all of these pathologies is also well known. We also know that the cellular manifestation of a lack of magnesium is similar to the cellular damage induced by catecholamines (Rona, 1985; Seeling 1989).

Some researchers have described a cellular mechanism that could explain the relationship between catecholamines and magnesium. Vormann and Gunther (1987) and Romani and Scarpa (1990) and Romani, Masfella and Scarpa (1993) have described a mechanism of cellular extrusion of magnesium in rat myocytes and hepatocytes, which is stimulated by catecholamines and inhibited selectively by β-blockers. However, this mechanism does not appear to

bring about a significant decrease in the ionized intracellular concentration of magnesium, probably because of the homeostatic action of the intracellular deposits of magnesium (Fatholahi et al., 2000). Scarpa and coworkers have recently shown how insulin antagonizes the catecholamine-mediated effect on the levels of intracellular magnesium (Romani, Mathews & Scarpa, 2000). Despite the interest the definition of this mechanism has aroused, the lack of significant variation in the intracellular concentration of ionized magnesium, that is, the biologically active magnesium, sheds doubt on its physiological role.

This study aims to try to extend what has previously been described in experimental animal models to human cells and also to measure the intracellular concentration of ionized magnesium ($Mg^{2+}{}_i$). We have also tried to evaluate the cellular mechanisms that may be involved. The second part of the study aims to verify ex vivo the catecholaminergic effects shown in vitro on the cellular metabolism of magnesium by subjecting a group of hypertensive subjects to therapy with β-blockers or placebo and measuring the lymphocyte ionized magnesium in response to this treatment.

Both for the in-vitro and the ex-vivo study we used the human lymphocyte as cell model. Because of the difficulties in measuring the adrenoceptor surface density in human cardiovascular tissues as well as because of the close correlation observed between adrenoceptors in the circulating cells and in the solid tissues (Fraser et al., 1981), the lymphocyte was studied in depth in patients with essential arterial hypertension and in subjects with congestive heart failure.

Materials and Methods

Measurement of Free Intralymphocyte Magnesium

Peripheral blood lymphocytes were isolated from healthy blood donors and consent was obtained from the subjects after the nature of the procedure had been explained. We used the method previously described (Delva et al., 1996) based on the fluorescent probe furaptra. The K_D of furaptra for magnesium was calculated as follows. To ensure that the furaptra inside the cell, which was formed by the hydrolysis of acetoxymethyl ester during our loading and de-esterification procedure, had the same K_D as the free acid, we performed in vivo calibration as follows: the extracellular Mg^{2+} was set with Mg-ethylenediaminetetraacetic acid (EDTA) buffers as described by Tsien (1980). Magfura was released in the cytosol with Triton-X 0.1% and excitation spectra with free Mg^{2+} ranging from 0 to 1000 μmol/l were performed with emissions collected at 510 nm. K_D values for binding of Mg^{2+} to furaptra were calculated by non-linear regression analysis of the 335/370 ratio and of free Mg^{2+}. K_D values obtained after permeabilization with Triton-X are given in Table 1 and were compared with those of the free acid form obtained with a procedure similar to that previously described. These data show that the intracellularly generated furaptra has the same K_D as the chemically made furaptra and the result is consistent with the conclusion that the acetoxymethyl ester has been fully hydrolized in the cells loaded in our experimental conditions.

Table 1. Apparent K_D values of furaptra and fura-2 for Mg^{2+} and Ca^{2+}

Furaptra K_D for Mg^{2+} (mM)		Fura-2 K_D for Ca^{2+} (μM)	
Chemically made	intracellularly-generated	chemically made	intracellularly-generated
2.0	2.10	149	175

Values represent the means of three independent experiments

Measurement of Free Intralymphocyte Calcium ($Ca^{2+}{}_i$)

The intracellular Ca^{2+} ($Ca^{2+}{}_i$) of lymphocytes was measured using Fura-2 (Grynkiewicz, Poenie & Tsien, 1985) as was previously described (Delva et al., 1996). The K_D value for Fura-2 was obtained as follows: the extracellular Ca^{2+} was set with Ca^{2+}-ethylene-glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) buffers. Fura-2 was released in the cytosol with Triton-X 0.1% and excitation spectra with free Ca^{2+} ranging from 0 to 39.8 μM were performed with emissions collected at 510 nm. K_D values for the binding of Ca^{2+} to Fura-2 were calculated by a non-linear-regression analysis of the 340/380 ratio and free Ca^{2+}. K_D values obtained after permeabilization with Triton-X are given in Table 1 and were compared with those of the free acid form. These data show that intracellularly generated Fura-2 has the same K_D as chemically made Fura-2.

The intra-assay and inter-assay variability (coefficient of variation) determinations were 5.8% and 3.9% for $Mg^{2+}{}_i$ and 4.6% and 12.7% for $Ca^{2+}{}_i$.

Measurement of Intralymphocyte Adenosine 5′-Triphosphate (ATP_i)

ATP_i in human lymphocytes was measured using the procedure described by Nieminen et al. (1990).

Ex-Vivo Study

We enrolled 20 subjects with light or moderate arterial hypertension (12 men and 8 women). Secondary causes of arterial hypertension were excluded using clinical, biohumoral, radiological and hormonal criteria. The patients had never taken any anti-hypertensive drugs before the study and were not being treated with any other drugs before or during the study. The study was approved by the local Ethics Committee and all the subjects gave their informed consent. The hypertensive subjects were divided randomly in a single-blind into two groups: on propranolol (120 mg/d; 40 mg three times a day) or placebo. The group of patients under active treatment was made up of 10 subjects, (6 men and 4 women, age mean ± SD: 45 ± 14; Body Mass Index (BMI): 26 ± 1). The group on placebo was made up of 10 subjects (6 men and 4 women, age mean ± SD: 48 ± 13; BMI: 25 ± 3). The first day (T0: immediately before taking the drug for the first time), 24 hours after taking the drug (T1) and after 17 days of treatment (T17), the patients' blood pressure and heart rate were measured and blood was sampled in order to measure intracellular ionized magnesium. The subjects, at T0, T1 and T17, were placed in a supine position, a cannula was inserted into an anterocubital vein 0.5 h before the blood sampling. Blood pressure was determined in supine position

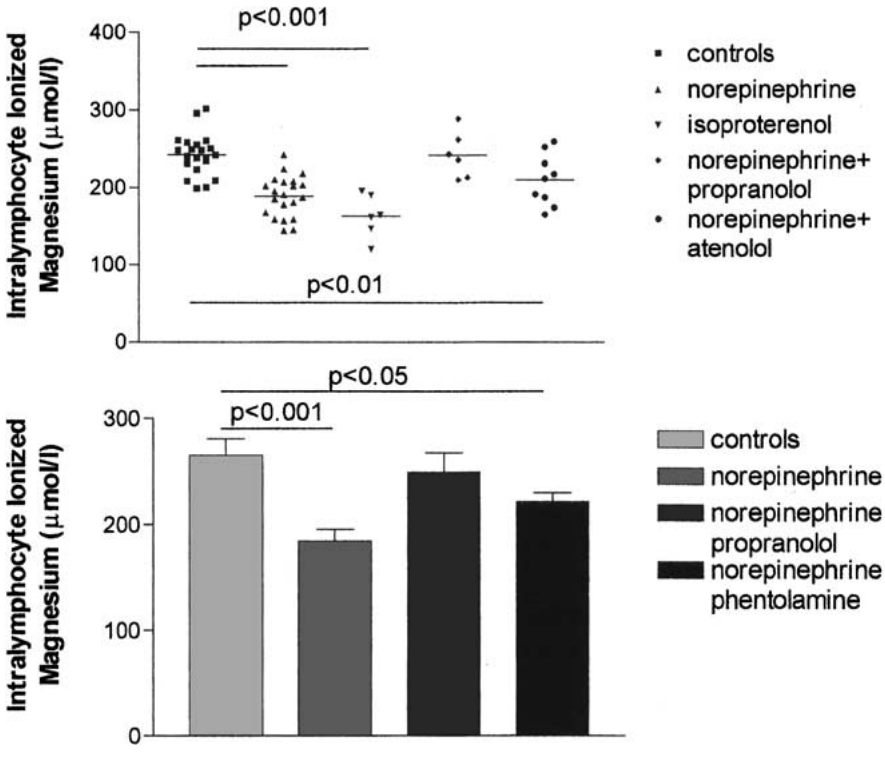

Fig. 1. *Upper panel.* Scatter diagram showing the in vitro effects of isoproterenol (10 μM) and norepinephrine (10 μM) on intralymphocyte ionized magnesium ($Mg^{2+}{}_i$) and the different effects of a non-selective β-blocker (propranolol; 2 μM) and a selective β1-blocker (atenolol; 15 μm) on the norepinephrine-induced $Mg^{2+}{}_i$ decrease. *Lower panel.* Bar graph showing the effect of a β-blocker (propranolol) and an α-blocker (phentolamine; 20 μM) on the norepinephrine-induced $Mg^{2+}{}_i$ decrease. Values are mean ± SE.

by taking the average of three measurements with a mercury sphygmomanometer.

Statistical Analysis

Results are expressed as means ± SD if no other method is specified. For multiple comparison a one-way ANOVA with Bonferroni's correction for multiple comparisons was used and then a *t*-test was performed.

Results

The in Vitro Effect of Catecholamines on Lymphocyte $Mg^{2+}{}_i$

In Vitro Effect of Norepinephrine and Isoproterenol on Intralymphocyte $Mg^{2+}{}_i$

The steady-state $Mg^{2+}{}_i$ in human lymphocytes was (n = 21) 242 ± 27 μM. The incubation of lymphocytes for 2.5 h with 10 μM norepinephrine or 10 μM isoproterenol substantially decreased this value (ANOVA F = 16.04): norepinephrine-treated cells, n = 21, 189 ± 26 μM, P = 0.001, 95% confidence interval: 36–70 μM; isoproterenol-treated cells, n = 6, 163 ± 28 μM, P = 0.001, 95% confidence interval: 53–105 μM), as shown in Fig. 1.

In Vitro Effect of Norepinephrine on Intralymphocyte $Ca^{2+}{}_i$

The steady-state $Ca^{2+}{}_i$ in human lymphocytes was (n = 12) 38 ± 12 nM. The incubation of lymphocytes for 2.5 h with 10 μM norepinephrine substantially increased this value (n = 12) 71 ± 13 nM, P = 0.0001, 95% confidence interval: 36–70 nM.

Effect of β- and α-Blockers on Catecholamine-induced Lymphocyte $Mg^{2+}{}_i$ Decrease

The effect of two β-blockers, one β1−selective, atenolol (15 μM) and one non-selective, propranolol (2 μM), on the norepinephrine-induced $Mg^{2+}{}_i$ decrease was evaluated. As shown in Fig. 1, only the non-selective β-blocker, propranolol, inhibits the norepinephrine-induced $Mg^{2+}{}_i$ decrease, while atenolol does not show any effect (control cells, n = 21, 242 ± 27 μM, norepinephrine-treated cells, n = 21, 189 ± 26 μM, P = 0.001 *vs.* control cells; norepinephrine-treated cells plus atenolol, n = 9, 209 ± 33 μM, P = 0.01 *vs.* control cells, 95% confidence interval: 10–55 μM; norepinephrine-treated cells plus propranolol, n = 6, 242 ± 30 μM, n.s. *vs.* control cells).

Figure 1 shows the different effects of a β-blocker, propranolol, and an α-blocker, phentolamine (20 μM), on the catecholamine-induced lymphocyte $Mg^{2+}{}_i$ decrease. Only propranolol completely inhibits the norepinephrine-induced $Mg^{2+}{}_i$ decrease, while phentolamine has a limited inhibitory effect on the decrease, which does not reach statistical significance (ANOVA, F = 6.92; control cells, n = 6, 265 ± 39 μM; norepinephrine-treated cells, n = 6, 185 ± 27 μM, P = 0.001 *vs.* control cells, % confidence interval: 41–120 μM; norepinephrine plus propranolol, n = 5, 250 ± 40 μM; norepinephrine plus

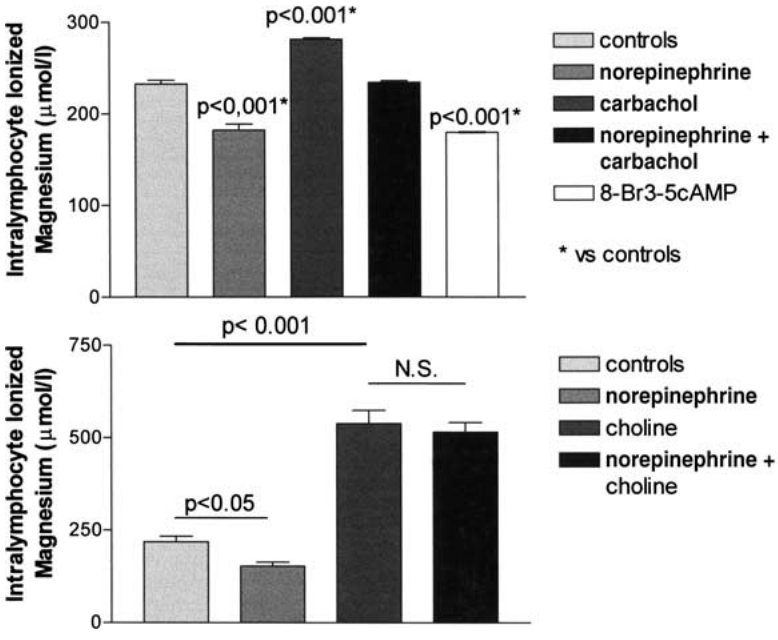

Fig. 2. *Upper panel.* Bar graph showing the effect of carbachol (100 μM) on the norepinephrine-induced $Mg^{2+}{}_i$ decrease and of 8-Br-cAMP on intralymphocyte $Mg^{2+}{}_i$. *Lower panel.* Bar graph showing the effect of extracellular Na^+ substitution (choline) on the norepinephrine-induced $Mg^{2+}{}_i$ decrease. Values are mean ± SE.

phentolamine, $n = 6$, 221 ± 21 μM, $P = 0.05$ *vs.* control cells, 95% confidence interval: 4–83 μM).

Effect of Muscarinic Receptor Stimulation on Lymphocyte $Mg^{2+}{}_i$

Figure 2 shows the effect of a muscarinic receptor agonist, carbachol, (100 μM), on lymphocyte $Mg^{2+}{}_i$. Carbachol significantly increases lymphocyte $Mg^{2+}{}_i$ compared to untreated lymphocytes. In the same figure we can see that the addition to the medium of both norepinephrine and carbachol has no effect on $Mg^{2+}{}_i$.

Characterization of the Catecholamine-induced Lymphocyte $Mg^{2+}{}_i$ Decrease

The dose-effect curve of the effect of norepinephrine on lymphocyte $Mg^{2+}{}_i$ is shown in Fig. 3. The EC_{50} is approximately 0.1 μM of norepinephrine.

As far as the time course of the effect of norepinephrine on lymphocyte $Mg^{2+}{}_i$ is concerned, no effect is evident until the 40th minute is reached and its maximal effect occurs after approximately 60 minutes, as shown in Fig. 3.

We investigated the possibility that a Na^+-Mg^{2+} exchange is involved in the norepinephrine-induced $Mg^{2+}{}_i$ decrease by substituting extracellular Na^+ with choline. In this situation the Na^+-Mg^{2+} exchanger cannot operate, thus allowing us to evaluate the role of this transport system. In the same set of experiments the effect of norepinephrine in the presence or absence of choline in the extracellular medium was also evaluated. As shown in Fig. 2, norepinephrine is capable of decreasing $Mg^{2+}{}_i$ only if sodium is present in the extracellular milieu. In cells exposed to choline, in substitution for sodium, $Mg^{2+}{}_i$ is hugely increased compared to lymphocytes incubated in medium with sodium, and no norepinephrine effect is then detectable on $Mg^{2+}{}_i$ (ANOVA $F = 78.5$; cells in sodium medium, $n = 9$, 218 ± 46 μM; cells in sodium medium plus norepinephrine, $n = 9$, 152 ± 32 μM, $P < 0.05$ *vs.* cells in sodium medium, 95% confidence limits 4–126 μM; cells incubated in choline medium, $n = 9$, 538 ± 89 μM, cells in choline medium plus norepinephrine, $n = 9$, 515 ± 80 μM, n.s. *vs.* cell in choline medium, $P < 0.001$ *vs.* cells in sodium medium with or without norepinephrine, 95% confidence limits 252–588 μM).

The Effect of Norepinephrine on Intralymphocyte ATP

In our experimental conditions, the incubation of lymphocytes for 2.5 h with 10 μM norepinephrine did not produce any statistically significant modifications in ATP_i (ATP_i control cells: $n = 6$: 4.31 ± 0.73 mM; norepinephrine-treated cells, $n = 6$; 4.78 ± 0.85 mM, n.s.).

The Effect of Propranolol on Lymphocyte $Mg^{2+}{}_i$ in Hypertensive Patients

Clinical Data

Table 2 summarizes the main clinical characteristics of the two groups of essential hypertensive patients studied.

In hypertensive patients, treatment with propranolol (both at time T1 and time T17) was characterized by a statistically significant decrease of both blood pressure (systolic and diastolic) and heart rate

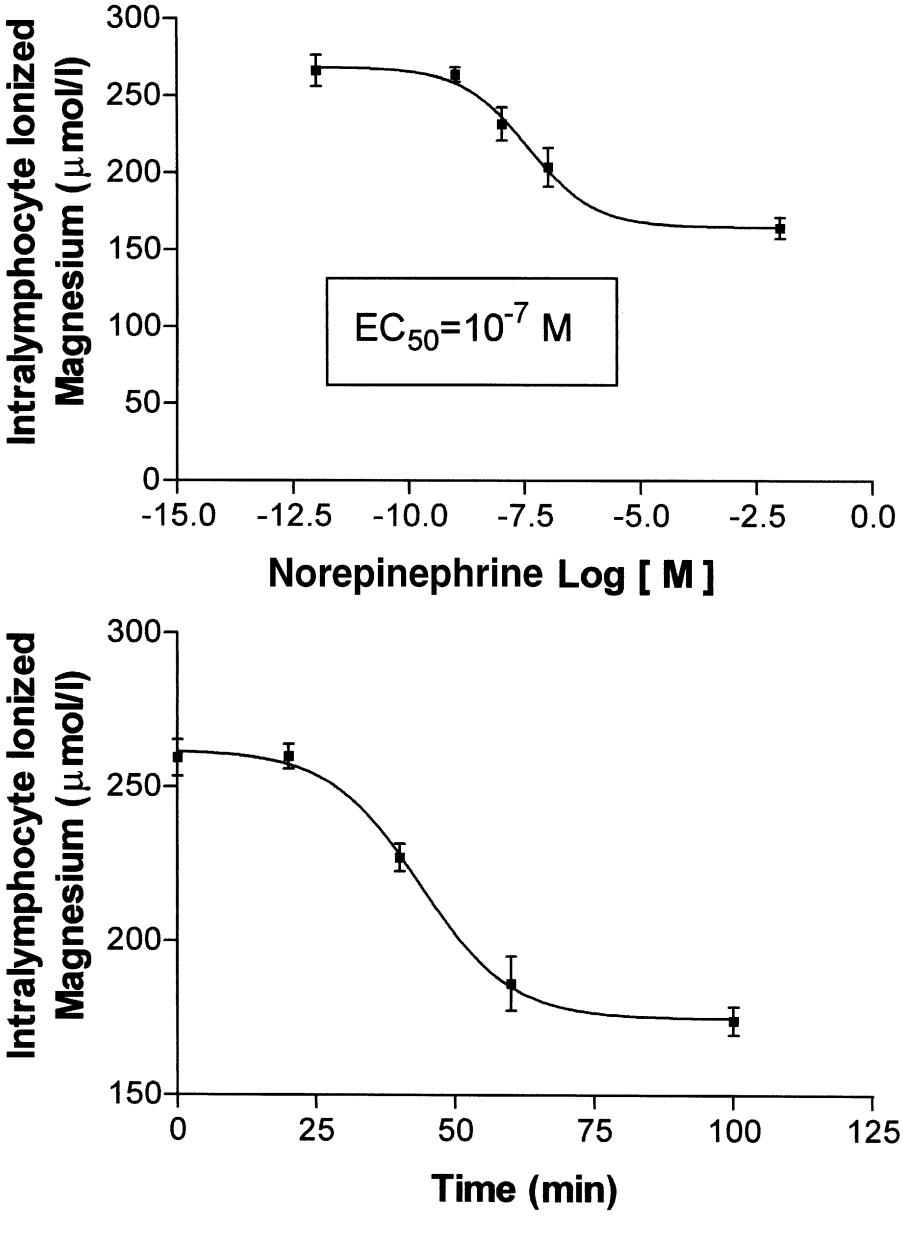

Fig. 3. *Upper panel.* Dose-response curve of the effect of increasing extracellular norepinephrine concentration on lymphocyte $Mg^{2+}{}_i$. *Lower panel.* Time course of the effect of norepinephrine (10 μM) on $Mg^{2+}{}_i$. Values are mean ± SE

compared to pre-treatment values. These statistical differences were not evident in the group of hypertensive patients treated with placebo.

Lymphocyte $Mg^{2+}{}_i$, $Ca^{2+}{}_i$, in Essential Hypertensive Patients

As shown in Fig. 4 (upper curves), neither active treatment with propranolol nor placebo induces any statistically significant variation in lymphocyte $Mg^{2+}{}_i$ after 24 h (T1) or after 17 d (T17) as compared to pre-treatment values (T0) in hypertensive patients (propranolol, T0: 241 ± 87 μmol/l, T1: 224 ± 84 μmol/l, T17: 212 ± 50 μmol/l, ANOVA test $F = 0.42$, n.s.; placebo, T0: 215 ± 71 μmol/l, T1: 176 ± 34 μmol/l, T17: 211 ± 73 μmol/l, ANOVA test $F = 0.52$, n.s.).

The same lack of statistical differences was found for intralymphocyte $Ca^{2+}{}_i$ (propranolol, T0: 46 ± 25 nmol/l, T1: 39 ± 10 nmol/l, T17: 38 ± 10 nmol/l, ANOVA test $F = 0.3$, n.s.; placebo, T0: 39 ± 8 nmol/l, T1: 38 ± 5 nmol/l, T17: 42 ± 11 nmol/l, ANOVA test $F = 0.2$, n.s.; *see* lower curve in Fig. 4).

Discussion

In the first part of this study, in which we considered the effect of catecholamines studied in vitro on human lymphocytes, we saw how two sympathomimetic

Table 2. Main clinical data of the two groups of patients studied

	Propranolol Treatment			Placebo Treatment		
	T0	T1	T17	T0	T1	T17
Systolic Blood Pressure (mm Hg)	150 ± 8	136 ± 6*	133 ± 7*	149 ± 7	151 ± 5	146 ± 6
Distolic Blood Pressure (mm Hg)	93 ± 7	86 ± 5*	85 ± 5*	94 ± 8	96 ± 6	92 ± 6
Heart rate (bpm)	72 ± 5	58 ± 11*	53 ± 4**	74 ± 8	76 ± 8	70 ± 9

T0 = pre-treatment, T1 = after 1 day treatment, T17 = after 17 day treatment
*$p < 0.01$ vs T0; **$p < 0.001$ vs T0;

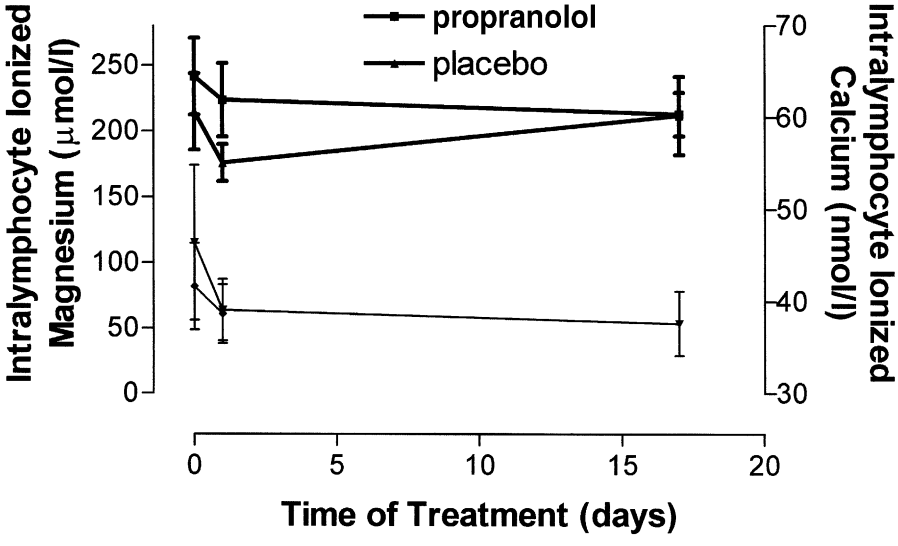

Fig. 4. Variations of intralymphocyte $Mg^{2+}{}_i$ (*thick lines*) and $Ca^{2+}{}_i$ (*thin lines*) during active (propranolol) treatment and placebo at T0 (pre-treatment), T1 (after 1 day treatment) and T17 (after 17 days treatment). Values are mean ± SE.

substances, isoproterenol and norepinephrine, significantly reduce the concentration of intracellular ionized magnesium.

The decrease may seem modest in absolute terms, in that it amounts to about 60–80 μmole of magnesium, yet it is important as a percentage in that it represents about 25–30% of the intracellular ionized magnesium concentration at steady state. We consider this to be interesting because it shows for the first time the effect of catecholamines on $Mg^{2+}{}_i$ in human-derived cells. The effect we observed confirms what Romani and Scarpa (1990, 1993) have described in perfused rat liver and heart, and in rat hepatocytes and myocytes. However, these authors suggest that the catecholamines mainly affect the whole magnesium content and, to a lesser degree, the ionized quota, with a variation of about 100 μmole of magnesium, which makes up about 25% of the ionized cell magnesium in the myocyte, a percentage which was, however, not considered quantitatively sufficient to affect cell metabolism (Fatholahi et al., 2000). We do not consider that the literature provides unequivocal information on the role of small variations in the concentration of intracytoplasmatic ionized magnesium on cell metabolism, and the data gathered so far, which suggest that magnesium is not a rate-limiting step in glycolysis and in oxydative phosphorylation, have been inferred from animal cells (Laughlin & Thompson, 1966; Rodrìguez-Zavala & Moreno-Sànchet, 1998).

In order to rule out the possibility that the variation in $Mg^{2+}{}_i$ induced by the norepinephrine was in part induced by changes in the ionized cell calcium, we measured the concentration of this ion in the presence of norepinephrine and found a variation in the calcium that went in the opposite direction to that of the magnesium. We can therefore rule out the possibility that the variations in $Mg^{2+}{}_i$ are due to the effect of cell calcium.

The effect of norepinephrine on lymphocyte $Mg^{2+}{}_i$ is totally blocked when the cells are treated with the non-selective β-blocker propranolol. The lymphocytes are characterized by a β_2-type receptor population (Brodde et al., 1981) and therefore the β_1-selective β-blocker atenolol did not prevent the decrease in $Mg^{2+}{}_i$ induced by norepinephrine. Although our experimental model did not allow us to distinguish between the β_1- or β_2-catecholaminergic effect on $Mg^{2+}{}_i$, we would like to point out that the effect of the infusion of catecholamines in rats produced an increase in plasmatic magnesium through a β_2-selective mechanism (Keenan, Romani & Scarpa, 1995).

It seems that the α-adrenergic antagonist phentolamine is not able to antagonize the effect of nor-

epinephrine on $Mg^{2+}{}_i$. We can therefore infer that the effect of norepinephrine on lymphocyte $Mg^{2+}{}_i$ is a specific cell event that occurs after binding with type-β adrenergic receptors. cAMP plays an important role in the intracellular signaling pathway, which starts by binding norepinephrine to the membrane-receptor. Indeed, conditions that mimic the intracellular increase in cAMP are characterized by an increase in the efflux of Mg^{2+} from the cell (Huang, Smith & Zahler, 1982). We therefore tested the lymphocytes in vitro with the permeable equivalent of cAMP, 8-Br-cAMP, which, on entering the cell, increases the concentration of cAMP, and with carbachol, a muscarinic agonist, which reduces the cell concentration of cAMP. The results show that the decrease in cAMP with 100 μM carbachol significantly increases $Mg^{2+}{}_i$ and that its increase with 8-Br-cAMP significantly reduces $Mg^{2+}{}_i$ (*see* Fig. 2, *upper panel*). Furthermore, when we compare the effects of norepinephrine and of 8-Br-cAMP in the same set of experiments, the decrease in $Mg^{2+}{}_i$ produced by 8-Br-cAMP also appears to be quantitatively similar to that produced by norepinephrine (*see* Fig. 2, *upper panel*). It is therefore likely that the second messenger involved in the chain of events that links the β-adrenergic receptor to the variation in $Mg^{2+}{}_i$ is cAMP.

As for the mechanism by which norepinephrine decreases $Mg^{2+}{}_i$, this could be a result of an extrusion of $Mg^{2+}{}_i$ via the Na^+-Mg^{2+} exchanger of the plasmatic membrane, which is still considered to be the most important membrane transport system of magnesium. This transport mechanism exchanges extracellular sodium for intracellular magnesium and is therefore inhibited by the absence of sodium on the outside of the membrane. We treated the lymphocytes with norepinephrine in the presence and absence of extracellular sodium and under the latter conditions, the effect of the norepinephrine on $Mg^{2+}{}_i$ was no longer measurable. Moreover, this absence of extracellular sodium was distinguished by $Mg^{2+}{}_i$ levels that were more than twice the steady-state concentration, thus indirectly confirming the important role of the Na^+-Mg^{2+} exchanger in determining the concentration of $Mg^{2+}{}_i$. This represents indirect evidence that the activity of the Na^+-Mg^{2+} exchanger may have profound effects on intracellular Mg^{2+} concentration; furthermore, this transport system is operating at a basal rate in the presence of physiological extracellular sodium concentration and adrenergic stimulation increases its rate to such an extent as to decrease $Mg^{2+}{}_i$ levels. Taking into account the opposite effects of norepinephrine and carbachol on $Mg^{2+}{}_i$, we may hypothesize a homeostatic mechanism for intracellular ionized magnesium in which the main regulators are agonists of the sympathetic and parasympathetic nervous system whose action on $Mg^{2+}{}_i$ is mediated by a regulation of the rate of the membrane Na^+-Mg^{2+} exchanger.

The role of intracellular ATP, the principal magnesium buffer, does not seem to be decisive in the regulation of catecholamine-mediated decrease of $Mg^{2+}{}_i$, in that the concentration of cellular ATP measured during our experiments was not changed by the action of norepinephrine, which confirms the previous results on cardiac myocytes (Silverman et al., 1994).

To summarize then, norepinephrine appears to influence the content of $Mg^{2+}{}_i$ in the human lymphocyte through specific binding to type-β adrenergic membrane receptors and the Na^+-Mg^{2+} membrane exchanger is activated probably through an increase in cAMP, which, by increasing the efflux of Mg^{2+}, reduces its intracellular concentration.

The other question we have tried to answer is that of the physiological role of the mechanism of catecholamine-mediated regulation of $Mg^{2+}{}_i$. We may make a first assessment on the basis of the dose-response curve of noradrenaline on $Mg^{2+}{}_i$ and of the time course of the effect. The EC_{50} of the dose-response curve is 10^{-7} M, which is a value within the physiological range of concentration of norepinephrine in vivo in healthy subjects. From the time course we can see that the effect of norepinephrine does not begin immediately but takes place after about 30–40 minutes. These in vitro data are, however difficult to demonstrate in vivo, as shown in the second part of the study, which focuses on the effect of the administration of propranolol on $Mg^{2+}{}_i$ in a group of hypertensive subjects. In the literature there are, as far as we know, no previous findings about the effect of β-blockers on ionized cellular magnesium. On the basis of our findings in vitro, we expected that in patients in treatment with propranolol the blocking of the cell-depleting effect of catecholamines on this ion would produce an increase in $Mg^{2+}{}_i$ compared to placebo. However, we did not find this increase either the first day or after 17 days of administration of propranolol.

Thus, in vivo, a catecholamine-mediated regulation of $Mg^{2+}{}_i$ does not seem to be evident, at least in the experimental conditions considered in the present study. Furthermore, the present data does not give us a reply to the question of the cause/s of the discrepancy between the results obtained in vitro and ex vivo.

The literature agrees about the up-regulation of β-adrenergic lymphocyte receptors under treatment with non-selective beta-blockers such as propranolol (Aarons et al., 1980). Since during treatment with propranolol, the number of β-adrenergic receptors increases, the plasmatic concentration of propranolol obtained in our study may be insufficient to inhibit the Na^+-Mg^{2+} membrane exchanger. Moreover, we may speculate that the presence in vivo of other known and unknown $Mg^{2+}{}_i$ regulators may mask the action of the catecholamines. Among the known

regulating factors, we refer in particular to the action of insulin, which has been described as capable of interfering with the catecholamine-mediated regulation of $Mg^{2+}{}_i$ (Romani et al., 2000) and to that of glucose, which may modify the concentration of $Mg^{2+}{}_i$ (Delva, Degan & Lechi, 2001). Furthermore, it is conceivable that acute and chronic effects of catecholamines are different as far as effects on $Mg^{2+}{}_i$ are concerned.

In conclusion, we have shown for the first time in cells of human derivation the ability of sympathomimetic substances to modify in vitro the concentration of $Mg^{2+}{}_i$ through a mechanism of specific receptor binding and, probably, the activation of a sodium-mediated Mg^{2+} efflux, possibly through the mediation of a cAMP increase. This mechanism, which seems to be identical to that described by Romani et al. (2000) in rats, if present in vivo may constitute an important regulator of intracellular magnesium concentration. Unfortunately, in our experimental conditions in vivo we were not able to evidence it. It is nonetheless clear that the question should be looked into further, particularly in relation to the importance of magnesium in mechanisms underlying many cardiovascular pathologies.

The present study was supported by Fondi di Ateneo per la Ricerca Scientifica, University of Verona (Italy).

References

Aarons, R.D., Nies, A.S., Gal, J., Hegstrand, L.R., Molinoff, P.B. 1980. Elevation of β-adrenergic receptor density in human lymphocytes after propranolol treatment. *J. Clin. Invest*. **65:** 949–957

Barbagallo, M., Resnick, L.M., Dominguez, L.J., Licata, G. 1997. Diabetes mellitus, hypertension and ageing: the ionic hypothesis of ageing and cardiovascular-metabolic diseases. *Diabetes Metab*. **23:**281–294

Brodde, O.E., Engel, G., Hoyer, D., Bock, K.D., Weber, F. 1981. The β-adrenergic receptor in human lymphocytes: subclassification by the use of a new radio-ligand, ± ^{125}iodocyanopindolol. *Life Sci*. **29:**2189–2198

Colucci, W.S., Alexander, R.W., Williams, G.H., Rude, R.E., Holman, B.L., Konstam, M.A., et al 1981. Decreased lymphocyte beta-adrenergic-receptor density in patients with heart failure and tolerance to the beta-adrenergic agonist pirbuterol. *N. Engl. J. Med*. **305:**185–190

Delva, P., Pastori, C., Degan, M., Montesi, G., Lechi, A. 1996. Intralymphocyte free magnesium in a group of patients with essential hypertension. *Hypertension* **28:**433–439

Delva, P., Pastori, C., Degan, M., Montesi, G., Brazzarola, P., Lechi, A. 2000. Intralymphocyte free magnesium in patients with primary aldosteronism: aldosterone and lymphocyte magnesium homeostasis. *Hypertension* **35:**113–117

Delva, P., Degan, M., Lechi., A. 2001. Preliminary communication on the effect of extracellular glucose on intracellular ionized magnesium in human lymphocytes. *In:* Rayssiguier, Y, Mazur, A, Durlach, J., (Editors) Advances in Magnesium Research: Nutrition and Health. pp 59–62, John Libbey & Co Ltd, London

Eisenberg, M.J. 1992. Magnesium deficiency and sudden death. *Am Heart J*. **124:**544–549

Fatholahi, M., LaNoue, K., Romani, A., Scarpa., A. 2000. Relationship between total and free cellular Mg^{2+} during metabolic stimulation of rat cardiac myocytes and perfused hearts. *Archiv. Biochem. Biophys*. **374:**395–401

Folkow, B. 1982. Physiological aspects of primary hypertension. *Physiol. Rev*. **62:**347–504

Ford, E.S. 1999. Serum magnesium and ischaemic heart disease: findings from a national sample of US adults. *Int. J. Epidemiol*. **28:**645–651

Fraser, J., Nadeau, J., Robertson, D., Wood, A.J. 1981. Regulation of human leukocyte beta receptors by endogenous catecholamines: relationship of leukocyte beta receptor density to the cardiac sensitivity to isoproterenol. *J. Clin. Invest*. **67:**1777–1784

Gottlieb, S.S., Baruch, L., Kuki, M.L., Bernstein, J.L., Fischer, M.L., Packer, M. 1990. Prognostic importance of the serum magnesium concentrations in patients with congestive hearth failure. *J. Am. Coll. Cardiol*. **16:**827–831

Grynkiewicz, G., Poenie, M., Tsien, R.Y. 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem*. **260:**3440–3450

Huang, R.D., Smith, M.F., Zahler, W.L. 1982. Inhibition of forskolin-activated adenylate cyclase by ethanol and other solvents. *J. Cyclic Nucleot. Res*. **8:**395–394

Keenan, D., Romani, A., Scarpa, A. 1995. Differential regulation of circulating Mg^{2+} in the rat by β_1 and β_2-adrenergic receptor stimulation. *Circ. Res*. **77:**973–983

Laughlin, M.R., Thompson, D. 1966. The regulatory role for magnesium in glycolytic flux of the human erythrocyte. *J. Biol. Chem*. **271:**28977–28983

Liao, F., Folsom, A.R., Brancati, F.I. 1998. Is low magnesium concentration a risk factor for coronary heart disease? The Atherosclerosis Risk in Communities (ARIC) Study. *Am. Heart J*. **136:**480–490

Nieminen, A.L., Gores, G.J., Dawson, T.L., Herman, B., Lemasters, J.J. 1990. Toxic injury from mercuric chloride in rat hepatocytes. *J. Biol. Chem*. **265:**2399–2408

Rodrìguez-Zavala, J.S., Moreno-Sànchez, R. 1998. Modulation of oxidative phosphorylation by Mg^{2+} in rat heart mitochondria. *J. Biol. Chem*. **273:**7850–7855

Romani, A., Scarpa, A. 1990. Norepinephrine evokes a marked Mg^{2+} efflux from liver cells. *FEBS Lett*. **269:**37–40

Romani, A., Marfella, C., Scarpa, A. 1993. Regulation of magnesium uptake and realease in the heart and in isolated ventricular myocytes. *Circ. Res*. **72:**1139–1148

Romani, A., Matthews, V., Scarpa, A. 2000. Parallel stimulation of glucose and Mg^{2+} accumulation by insulin in rat hearts and cardiac ventricular myocites. *Circ. Res*. **86:**326–333

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

Seelig, M.S., Elin, R.J. 1995. Editorial: Reexamination of Magnesium Infusions in Myocardial Infarction. *Am. J. Cardiol*. **76:**172–173

Seeling, M. 1989. Cardiovascular consequences of magnesium deficiency and loss: pathogenesis, prevalence and manifestations-magnesium and chloride loss in refractory potassium repletion. *Am. J. Cardiol*. **63:**4G–21G

Silverman, H.S., Di Lisa, F., Hui, R.C., Miyata, H., Sollott, S.J., Hanford, R.G., Lakatta, E.G., Stern, M.D. 1994. Regulation of intracellular free Mg^{2+} and contraction in single adult mammalian cardiac myocytes. *Am. J. Physiol*. **266:**C222–C233

The Magnesium in Coronaries (MAGIC) Trial Investigators. 2002. Early administration of intravenous magnesium to high-risk patients with acute myocardial infarction in the Magnesium in

Coronaries (MAGIC) Trial: a randomised controlled trial. *Lancet* **360:**1189–1196

Tsien, R.Y. 1980. New calcium indicators and buffers with high selectivity against magnesium and protons: design, synthesis, and properties of prototype structures. *Biochemistry* **19:**2396–2404

Vormann, J., Gunther, T. 1987. Amiloride-sensitive net Mg^{2+} efflux from isolated perfused rat hearts. *Magnesium* **6:**220–224

Woods, K.L., Fletcher, S., Roffe, C., Haider, Y. 1992. Intravenous magnesium sulphate in suspected acute myocardial infarction: results of the second Leicester Intravenous Magnesium Intervention Trial (LIMIT-2). *Lancet* **339:**1553–1558

Yoshikawa, T., Handa, S., Anzai, T., Nishimura, H., Baba, A., Akaishi, M., et al. 1996. Early reduction of neurohormonal factors plays a key role in mediating the efficacy of β-blocker therapy for congestive heart failure. *Am. Heart. J.* **131:**329–336