

# Is there a Role of Intracellular Magnesium in Prevention of Heart Failure?

Panayotis Fantidis\*, Eladio Sanchez, Ijaz Khan, Ibrahim Tarhini, Tomás Pineda, José Antonio Corrales and José Ramón Gonzalez

Servicio de Cirugía Cardiovascular, Hospital Universitario, Infanta Cristina, Elvas s/n, 06080 Badajoz, Spain

## Abstract

Heart failure, characterized by the reduction in left and/or right ventricular function, is the most common cause of mortality and morbidity in almost around the world. This syndrome is induced by  $\beta_1$ -adrenergic receptor ( $\beta_1$ -AR) desensitization, which in turn is caused by the reduction in cellular cAMP concentration. Cellular cAMP concentration is modulated by adenylyl cyclases (ACs), a family of enzymes that are strongly activated by intracellular magnesium and circulating levels of the stress-related hormones.

Short-term activation of  $\beta_1$ -AR signalling pathway by increased levels of these hormones induces an increase in intracellular  $Mg^{2+}$  and cAMP concentrations, which improves cardiac function. Nevertheless, long-term activation of  $\beta_1$ -AR signalling pathway by elevated levels of these hormones reduces intracellular  $Mg^{2+}$  and cAMP levels, which deteriorates cardiac function. Intracellular cAMP signalling has a circadian rhythm (CR) in mammals, and CR may modulate diverse phenomena. The loss of CR may per se be a cause of severe diseases. Magnesium can regulate intracellular cAMP production and through this may modulate the CR. Here we propose that long-term activation of  $\beta_1$ -AR signalling pathway, and/or the use of diuretics, can induce a progressive reduction of intracellular of magnesium and a subsequent gradual reduction of intracellular cAMP levels. This in turn can cause  $\beta_1$ -AR desensitization, the loss of CR of the hormones related with HF, and, finally, HF development. We suggest the therapeutic implications of this mechanism in the prevention of HF.

**Keywords:** Catecholamine; Cortisol;  $\beta_1$ -AR signalling pathway; Intracellular magnesium; Heart failure prevention

## Introduction

Heart failure (HF) is among the most common causes of morbidity and mortality in the so-called Western world [1,2]. It is widely recognised that this illness is an important public health problem that affects millions of people worldwide, and that is the most frequent cause of hospitalization for patients of 65 years and over [1,2].

Acute short-term activation of beta-1 adrenergic receptor ( $\beta_1$ -AR) signaling pathway by elevated circulating catecholamine levels activates the membrane-bound isoforms of ACs enzymes with the consequent increase in intracellular cAMP. In addition all isoforms of ACs are tightly activated by intracellular magnesium. Acute increases of cAMP, by its chronotropic, inotropic, and lusitropic effects, significantly modulate cardiac function [3]. However, long-term activation of  $\beta_1$ -AR signalling pathway by elevated levels of catecholamine account for reduced intracellular cAMP generation and subsequent  $\beta_1$ -AR desensitization and HF development [4-6]. In chronic HF, not only the expression and membrane localization of the  $\beta_1$ -AR is diminished (so called  $\beta_1$ -AR desensitization), but also the balance of receptor coupling to inhibitory versus stimulatory G-proteins shifts, and the activation of ACs required to transduce receptor agonist to cAMP generation, are blunted [4-6].

As we comment below, circadian rhythm (CR), the principal circadian pacemaker in mammals, may modulate diverse phenomena including innate immunity and cell division, and is the endogenous timekeeper that interacts with numerous biological systems. The loss of CR may per se be a cause of severe diseases, and the rhythmicity of suprachiasmatic nucleus, depends on the levels of intracellular calcium and cAMP. Given that mammalian ACs, and subsequent cellular cAMP production, is strongly activated by  $Mg^{2+}$ , and that chronic activation of circulating catecholamine can induce a significant reduction in intracellular magnesium concentration, it might be suggested that magnesium could have an important role in maintaining of CR of the hormones involved in HF.

We propose the mechanism by which magnesium could be involved in the prevention of genesis and of exacerbations of HF; Continually high circulating levels of the stress related hormones, a peculiar feature of HF, can induce decreases in intracellular  $Mg^{2+}$ , which in turn can cause a persistent reduction of cellular cAMP, the loss of CR of the hormones involved in HF,  $\beta_1$ -AR desensitization and HF development. The use of diuretics for the treatment of patients with HF, by increasing the loss magnesium, could participate in the exacerbation of chronic HF. We suggest the therapeutic implications of this mechanism in the prevention of HF.

## Cyclic AMP production

In mammals, intracellular concentration of cAMP, a second messenger that regulates many important cellular functions, is predominantly modulated by the ACs that increase cAMP production, and phosphodiesterases that induce its degradation.

Mammalian ACs play a key role in the cellular response to extracellular signals [6-10]. All the membrane-bound isoforms of ACs enzymes exhibit a basal activity which is enhanced upon binding of the stimulatory G protein (Gs) and reduced upon binding of the inhibitory G protein (Gi) [3]. The mammalian AC gene family contains at least 10 members with several AC isoforms, but only the isoforms AC5 (detrimental effects), and AC6 (beneficial effects), are found in abundance in the heart [7, 8].

**\*Corresponding author:** Panayotis Fantidis, C/ Sierra Nevada 126. 28729 Madrid, Spain, Tel: +34 659007066; E-mail: [pfantidis@yahoo.es](mailto:pfantidis@yahoo.es)

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Beta1-ARs and  $\beta_2$  receptors ( $\beta_2$ -AR) co-exist in the human heart and they are the most powerful physiologic mechanism to increase cardiac performance;  $\beta_1$ -ARs activate  $G_s$  proteins whereas  $\beta_2$ -ARs use both  $G_i$  and  $G_s$  proteins.  $G_s$  signalling stimulate adenylate cyclase, resulting in dissociation of adenosine triphosphate into the second messenger cAMP [7,8].

A second independent source of cAMP in mammalian cells is soluble AC, which is insensitive to heterotrimeric G protein regulation, but is widely modulated by bicarbonate and calcium, and can be found throughout the cytoplasm, in mitochondria, and in the nucleus [7-10]. Cyclic AMP and phosphokinase activities are differentially stimulated in 'membrane' and 'cytosolic' rabbit cardiomyocytes fractions by b-adrenergic and prostaglandin receptor agonists. The  $\beta$ -adrenergic agonist isoproterenol has been shown to stimulate cAMP in both fractions, while prostaglandin increased cAMP exclusively in the cytosolic fraction which is not coupled to positive inotropic response [7-10].

### The role of intracellular $Mg^{2+}$ in the activation of mammalian ACs

Intracellular magnesium, the second most abundant intracellular cation in the body is vital for life, but the mechanisms regulating the transport of this ion into and out of cells remain little known. The thermo toga maritima CorA -Mrs2-Alr1 (CorA-Mrs2-Alr1) family of magnesium channels represents the most prevalent group of proteins enabling magnesium ions to cross membranes [10].

All mammalian ACs are strongly activated by  $Mg^{2+}$ , and inhibited by low concentrations of intracellular free  $Ca^{2+}$  [10,11]. Despite a considerable number of experimental, epidemiological and clinical studies suggesting a role of  $Mg^{2+}$  in the etiology of HF, and although it is widely known that intracellular magnesium depletion may coexist with normomagnesemia [12,13], measurements of intracellular  $Mg^{2+}$  concentration in patients with HF are scarce. A previous study showed that intracellular concentration of magnesium and cAMP in children with HF was significantly lower versus the control group [14]. More recently it has been reported that during the median 14.7 year follow-up, magnesium intake was inversely associated with mortality from ischemic strokes, coronary heart disease, and HF in women [15]. In chronic HF, increased plasma levels of pro-inflammatory cytokines are correlated with both the severity of HF symptoms and clinical outcome, and that also the end-stage failing human myocardium is a source of pro-inflammatory cytokines [16]. Further, previous results showed that intracellular free magnesium is significantly reduced in experimental HF, that magnesium strongly activates ACs, that is the natural antagonist of calcium is involved in many important enzymatic processes, electrolyte balance, skeletal metabolism, arrhythmia, inflammation, and has an important role in the regulation of cation channels in cardiac and smooth muscle cells [17].

Even more importantly, magnesium intracellular concentrations may change following stimulation of  $\beta$ -AR and insulin receptors or during pathophysiological conditions such as ischemia, HF and hypertension [17]. Extrusion of cellular  $Mg^{2+}$  via adrenergic stimulation is the most investigated process, and it has been observed also in cardiomyocytes; cellular magnesium has per se an important influence on myocardial contractility and its mobilization in the heart was associated with  $\beta$ -adrenergic stimulation by adrenaline [18].

Previous report showed that  $\beta_1$ -ARs stimulation slowly decrease the intracellular magnesium concentration, whereas it increases serum magnesium level, and that insulin antagonizes the reduction

of intracellular magnesium induced by  $\beta$ -AR stimulation [19]. Finally, it has been published and discussed in depth previously that intracellular total and free magnesium are compartmentalized between cell organelles and within the cytosol, and also, the percentage of magnesium localized in nuclei, mitochondria, ribosomes and cytosol has been reported [20]. Future studies will determine which of these fractions of intracellular magnesium plays a crucial role in the desensitization of  $\beta_1$ -ARs and HF development.

### Effects of hypercalcemia in the presence of low intracellular magnesium

It is not the purpose of this manuscript to gloss the fundamental role of intracellular stores of  $Ca^{2+}$  in mammalian cell's physiology, and its critical role in physiological and pathological functioning of the heart, which has been discussed in other publications [21,22].

Briefly we wish to highlight that cellular free  $Ca^{2+}$  is a critical second messenger in G-protein-coupled pathways that induce gene transcription, and modulate cardiac cell function.

Beta-adrenergic stimulation results in increased  $Ca^{2+}$  entry into the cell, which leads to increased rates of myofilament contraction and relaxation [21,22]. Alterations in  $Ca^{2+}$  handling by the cardiac myocyte are likely to contribute to the decreased contractility and negative force-frequency relationship seen in HF [21,22]. In addition, calcium might also induce cardiac cell hypertrophy, necrosis, or apoptosis, and can regulate electrical signals that determine the cardiac rhythm via ion currents and exchangers [15,22]. Furthermore, it is known that an increase in intracellular calcium is associated with the transition from reversible to irreversible cell injury [23]. Also, changes in  $Ca^{2+}$  handling often precede the depression of myocardial function [21-23]. In addition, alterations in  $Ca^{2+}$  handling proteins and intracellular kinase have been shown to be involved in the pathogenesis of HF and to promote arrhythmias [23]. Finally, it has been reported that HF is accompanied by a 40% prevalence of sudden cardiac death due to malignant ventricular arrhythmias [21,22].

Magnesium is the natural antagonist of calcium [17], and this ion has a powerful anti-calcifying effect; magnesium transport through the cell membrane inhibited vascular smooth cell calcification [24].

From the above, it might be suggested that a potential reduction of intracellular magnesium levels may potentiate the adverse effects of calcium in cardiomyocytes of patients with HF, in which calcium levels might be already increased by the effect of catecholamine.

### Circadian rhythm (CR) and magnesium

Circulating levels of endogenous glucocorticoids and catecholamine, hormones closely related to HF, are under the influence of the suprachiasmatic nucleus (SCN) of the hypothalamus, the principal circadian pacemaker in mammals and coordinating clocks in other tissues. The circulating levels of these hormones have a diurnal rhythm curve; the zenith of their intracellular concentration is reached in the early morning and the nadir at midnight [25,26].

In addition, CR modulates the circulating levels of many other hormones and diverse phenomena as sleep/wake cycles, glucose homeostasis, innate immunity and cell division etc. [25,26].

Additionally, it has been reported that disruption of CR may have significant effects on human health; e.g. long-term shift workers exhibit increased susceptibility to type 2 diabetes and various cancers [25,26].

Further, CR is a property inherent to mammalian cells that persist

throughout the body in vivo and in isolated tissues/cells for many days in vitro under the influence of SCN, whose rhythmicity depends mainly on levels of intracellular calcium and cAMP [25,26].

The best known CR is that related to the cortisol secretion, the end-hormone of the hypothalamic-pituitary –adrenal (HPA) axis [27]. When the inflammatory stimulus persists and the inflammation has not been controlled by cortisol, then circulating levels of cortisol may remain inappropriately normal [27]. When a molecule whose production follows a CR is continuously stimulated, then its production can lose its cyclic production and, as a consequence, its concentration remains inappropriately normal and this molecule loses its ability to exercise the function it normally carries out [27].

Patients with HF are characterized by increased circulating catecholamine levels, a peculiar feature of HF [4,6]. Given the role of catecholamine in reducing intracellular magnesium concentration [18], the role of magnesium in cAMP production [10,11] and the role of cAMP in the principal circadian pacemaker function in mammals [25,26], it could be suggest that magnesium is essential in normal functioning of CR of the hormones involved in HF, and that its intracellular reduction, through its role in the disruption of CR and  $\beta$ 1-AR desensitization, might have an important role in HF development (Figure 1).

### HPA-axis and heart failure

Various stressful stimuli, intrinsic or extrinsic, can activate the HPA axis and simultaneously the sympathetic nervous system (SNS) inducing increases in circulating levels of catecholamine and the HPA-axis related hormones, ACTH and cortisol.

Recently it has been reported that magnesium deficiency induces HPA-axis deregulation; magnesium deficiency caused an increase in ACTH plasma levels [28].

In patients with HF, besides the increase of catechol amines, circulating levels of cortisol were increased; higher serum levels of both cortisol and aldosterone were independent predictors of increased mortality risk in these patients [29]. Clinical trials have shown that  $\beta$ 1-AR blockers reduce mortality in HF [30-32], and that cortisol can be displaced acutely from the myocardium by mineralocorticoid receptor (MR) antagonists, which may contribute to adverse MR activation in human heart [29]. Cortisol displacements from the heart by mineralocorticoid receptor antagonists have beneficial effects in patients with HF [29] (Figure 2).

The deleterious effects of cortisol on HF could be attributed to the fact that cortisol and aldosterone can reduce intracellular magnesium and membrane adenylyl cyclase activity, and subsequently can induce reduction of intracellular cAMP [29]. In addition, glucocorticoids may increase the concentration of calcium in myocytes [33].

### Final considerations/Therapeutic implications

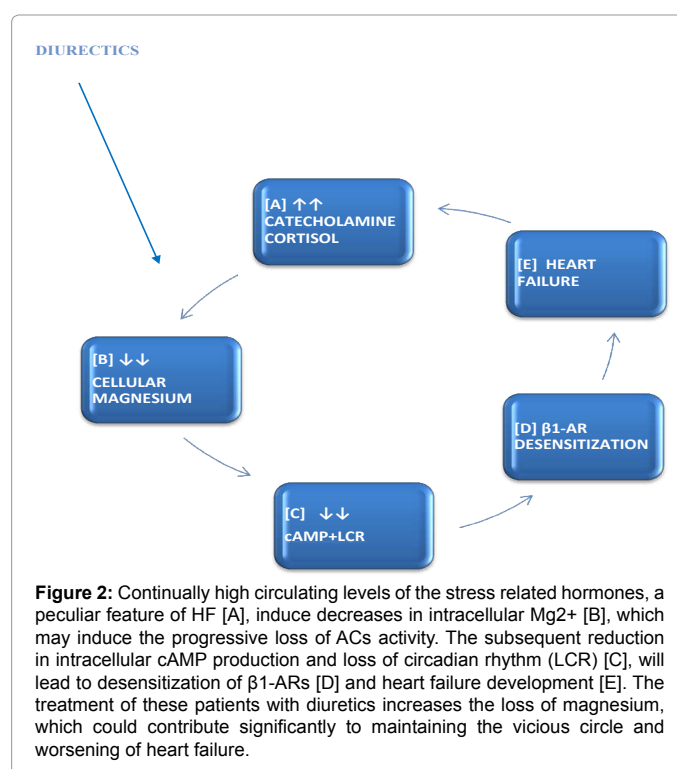
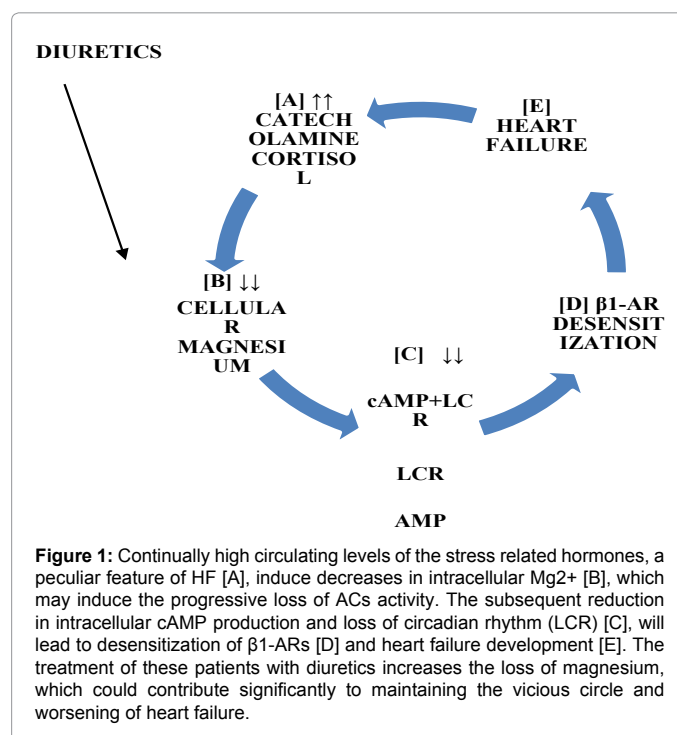
It is widely known that diuretics are used often and at high doses for the treatment of HF. Diuretics can induce, among others, elimination of magnesium [34], which might cause depletion of intracellular magnesium. In the vast majority of patients with heart failure magnesium losses are not restored.

The biggest challenge of this manuscript is to encourage clinicians to measure the intracellular levels of magnesium and cAMP in patients with HF and to compare the clinical evolution of the group of patients in which the loss of magnesium was treated and fully restored, versus

the untreated group with this ion. This mainly will enable us to assess the effect of magnesium on the prevention of HF exacerbations.

Our second goal is to animate clinicians to measure intracellular magnesium and cAMP concentration in patients with acute HF, chronic HF and healthy volunteers.

Statistically significant difference in intracellular concentrations of



these two parameters between patients with acute versus chronic HF, and/or between patients with HF versus healthy subjects, could provide valuable information on the role of magnesium in HF. Additionally, it could be of great interest to know whether there are statistically significant variations in intracellular concentrations of magnesium and cAMP in patients with chronic HF before and after HF exacerbation.

Our third aim; it is assumed that the loss of the CR does not always lead to the development of HF, being necessary to evaluate in which case the loss of the CR release of the hormones involved with HF can lead to the HF development.

Finally, in addition to catecholamine, it has been shown that cortisol and aldosterone reduce intracellular magnesium and membrane ACs activity, and that cortisol and aldosterone have a role in heart failure. A particular area of research could be the use of beta blockers and mineralocorticoids antagonists with or without employing magnesium. This should provide insight into the implications of magnesium as an adjuvant in the treatment of HF, and on the short-term and long-term evolution of this illness.

To reach these goals it is necessary to measure the intracellular levels of magnesium, and cAMP, and requires evaluating the dose of magnesium that must be used in each case to normalize their intracellular levels.

Given the obvious difficulty of using cardiomyocytes to measure intracellular cAMP and magnesium content, we would evaluate whether peripheral mononuclears or polymorphonuclears or other types of cells may reflect more accurately the intracellular magnesium and cAMP levels in cardiomyocytes. It is not the objective of our manuscript to comment about on how to measure intracellular cAMP and magnesium. In our previous works, we describe the procedures used by us to measure intracellular magnesium and cAMP levels in mononuclears and polymorphonuclears [14,38].

At present, magnesium is not an issue of high interest on HF; many clinical trials of positive inotropes have failed in the treatment of HF, and now is an "axiom" that agents that increase cAMP are deleterious to the failing heart [35]. Several questions and comments arise of this negative predisposition; i) Is it necessary to administer inotropes in all patients with HF? If not, in what cases inotropes should be administered and why? ii) Could it be more appropriate that before administering exogenous inotropes, measure the levels of endogenous circulating catecholamine? iii) We believe it is a requirement to restore and/or block the biochemical parameters that are diminished or are in excess in each patient with HF, iv) if any patient it was necessary to use inotropes, then should we not maintain the circadian cycle in their administration?, v) It can be assumed that it is necessary to avoid continuous stimulation of the  $\beta_1$ -AR, because the continuous stimulation of these receptors leads to their desensitization, and induces loss of intracellular magnesium and the lack of response of ACs, vi) What are the arguments of those who believe that we should not maintain the CR in the administration of inotropes? v) We think that in the coming years, since the cloning of ACs isoforms, investigators will try to find reagents that regulate the activity of this enzyme directly in an isoform-dependent manner. The ultimate goal of developing such reagents would be to regulate cAMP signal in an organ-dependent manner. It seems that AC5 especially and AC6 isoforms are the major isoforms in adult cardiomyocytes, and that they can play an important role in acute and chronic HF [35]. vi) Finally, although currently new inotropic agents which are expected to improve clinical outcomes

are under evaluation [36], other avenues of research should also be explored the management of HF.

Many years ago we demonstrated polymorphonuclears magnesium deficiency in patients with bronchial asthma between attacks [37]. On the basis of our results, we proposed the use of magnesium for the treatment of bronchial asthma [37]. At that time the interest for magnesium in bronchial asthma, both in the pathogenesis of the illness and treatment of the crisis of bronchial asthma, was null. But, after publication of our work numerous studies were published demonstrating the effectiveness of the use of magnesium, especially in adults and children who fail to respond to initial bronchodilator therapy or where there is life threatening or near fatal asthma [38-40].

If subsequent clinical and experimental researches show that magnesium plays an important role in both the genesis of HF as in the treatment and prevention of exacerbations of HF, this would stimulate the development of devices that will be rechargeable and could be placed subcutaneously. This device could, via a sensor and a microcomputer, determine the intracellular magnesium levels and if it is necessary, would inject by micro needles the amount of magnesium that at all times the patient needs.

## Conclusion

On the basis of the existing literature, we propose that the constant increase of catecholamine and cortisol induces a continuous decrease of intracellular magnesium. This causes a progressive decrease in intracellular cAMP production, which in turn desensitizes  $\beta_1$ -AR and induce the loss of CR of the hormones related with HF, with the subsequent development of HF. The inevitable use of diuretics further increases the loss of magnesium, which could have a role in the exacerbations of chronic HF. This hypothesis opens new ways to study the factors that influence the progression of HF and to evaluate new ways for the prevention and treatment of this illness.

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