Korean J Physiol Pharmacol
Vol 2: 49—54, February, 1998

Effects of Histamine H_2-Receptor Stimulation on Mg^{2+} Efflux in Perfused Guinea Pig Heart

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Mg^{2+} is an important regulator of many cardiac functions. However, regulation of intracellular Mg^{2+} activity in the heart is not well characterized. To assess the effect of histamine H_2-receptor stimulation on intracellular Mg^{2+} regulation, changes in extracellular Mg^{2+} concentration were examined under a variety of conditions in perfused guinea pig hearts. Mg^{2+} in the cardiac perfusate was measured by atomic absorbance spectrophotometry. The histamine (10^{-6} M) inuced a marked Mg^{2+} efflux from the heart. The H_2-receptor antagonists, cimetidine (10^{-5} M), ranitidined (10^{-5} M), but not a H_1-receptor antagonist, diphenhydramine (3×10^{-6} M), completely blocked the histamine-induced Mg^{2+} efflux. The Mg^{2+} efflux could also be induced by forskolin (3×10^{-6} M), 8-Cl-cAMP (2×10^{-4} M), permeable cAMP analogue, or dimaprit, (10^{-5} M). However, the carbachol (10^{-5} M) considerably decreased the efflux of Mg^{2+}. In the presence of papaverine (10^{-5} M), a phosphodiesterase inhibitor, dimaprit-induced Mg^{2+} efflux was potentiated. These results suggest that a significant Mg^{2+} efflux from perfused guinea pig heart by histamine can be induced by the histamine H_2-receptor stimulation and it is suggested that cytosolic cAMP may be linked.

Key Words: Histamine, Magnesium, Dimaprit, cAMP, Heart, Guinea pig

INTRODUCTION

Magnesium (Mg^{2+}) is the second or third most abundant intracelluar cation. While it is well known about the roles of intracelluar Mg^{2+} in cell functions (Murphy, 1991; White & Hartzell, 1989), the mechanism of Mg^{2+} regulation is poorly understood. In erythrocytes, the existence of Na^+-Mg^{2+} exchanger for Mg^{2+} regulation has been identified by Gunther (1984, 1985); this antiporter could account for Mg^{2+} extrusion in cardiac and liver cells (Vormann & Gunther, 1987; Gunther et al, 1991; Murphy, 1991). In eukaryocytes, major cellular Mg^{2+} redistribution under hormonal stimulation in liver cells, muscle cells, and cardiac cells (Bond et al, 1987; Jakob et al, 1989; Somlyo et al, 1985; and Romani et al, 1990a; 1991; 1992), respectively and norepinephrine-induced intracellular cAMP produce a large Mg^{2+} efflux from perfused hearts, livers, isolated myocytes or hepatocytes (Romani et al, 1990a; 1990b). A major mobilization of Mg^{2+} could be demonstrated upon the direct addition of cAMP to hepatocytes or rat liver mitochondria (Jakob et al, 1989; Romani and Scarpa, 1990b; Romani et al, 1991).

Histamine is an ubiquitous biogenic amine that affects a diverse array of physiological and behavioral responses in animals and men. Histamine is a potent stimulant of adenylate cyclase activity in cardiac tissues (Johnson, 1982), and there is strong evidence that the cardiac effects of H_2-receptor stimulation are, like β_1-adrenoceptor responses, mediated via cAMP by activated adenylate cyclase (Cabanie & Godfraind, 1988). Its role in cardiac Mg^{2+} regulation has not been elucidated. This study examines the cardiac effects of histamine receptor stimulation on Mg^{2+} efflux via cAMP.

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METHODS

Perfused hearts

Male guinea pigs (350~400 g) were used. The animal is then rapidly decapitated and bled after intraperitoneal injection of phentobarbital sodium (25 mg/kg) treatment, which provides a clearer field for dissection and reduces blood clots (De Young et al, 1989). Hearts (1.1~1.2 g) were removed and immersed in room-temperature buffer containing (mM); NaCl 120, KCl 3, $CaCl_2$ 1.25, $MgCl_2$ 1.2, KH_2PO_4 1.2, glucose 10, $NaHCO_3$ 12, and HEPES 10 (pH 7.3 with O_2 : CO_2 97 : 3). After immersing, hearts were suspended to the cannula of Langendorff apparatus (open system) for perfusion. Perfusion rate was 7 ml/min through a cannula inserted in the aorta. After 20 minutes of perfusion, the buffer was replaced with a same buffer containing 0 mM Mg^{2+} (low Mg^{2+}, contaminant Mg^{2+}, or Mg^{2+}-free). The use of low Mg^{2+} in the buffer was necessary to obtain adequate sensitivity for measuring changes in Mg^{2+} content of the perfusate by atomic absorbance spectrophotometry (Romani et al, 1993). After 10 minutes perfusion with a Mg^{2+}-free buffer, the effluent perfusate was continuously collected, pooled test tubes each containing 60 seconds of perfusate, and the Mg^{2+} content was measured by atomic absorbance spectrophotometry (AA, Sunil Lab., Korea).

Chemicals

All drugs were prepared as concentrated stock solution and diluted to their final concentration with the perfusate. Histamine and carbachol were purchased from Sigma Chemical Co. Dimaprit, cimetidine, diphenhydramine, ranitidine, papaverine, 8-(4-chlorophenylthio)-cAMP and forskolin were purchased by RBI.

RESULTS

Effects of histamine on Mg^{2+} efflux in perfused guinea pig hearts

Fig. 1 shows Mg^{2+} efflux in the absence (Fig. 1A) and presence (Fig. 1B) of 10^{-6} M histamine. In order to measure the possible efflux of Mg^{2+} from heart, infusion of Mg^{2+} was stopped after 20 min of preperfusion. The collected perfusate was assayed as described in "Materials and Methods". In control hearts (Fig. 1A), the Mg^{2+} efflux measured as Mg^{2+} content of perfusate was gradually diminished in the time dependent manners, and reached steady state about 10 ± 2 μM. By contrast, in stimulated hearts (Fig. 1B) the addition of 10^{-6} M histamine evoked Mg^{2+} efflux. Under the same experimental conditions, a series of controls (not shown) determined that Mg^{2+} efflux was specific to histaminergic stimulation and was not secondary to an increase in heart rate and/or positive inotropic effects.

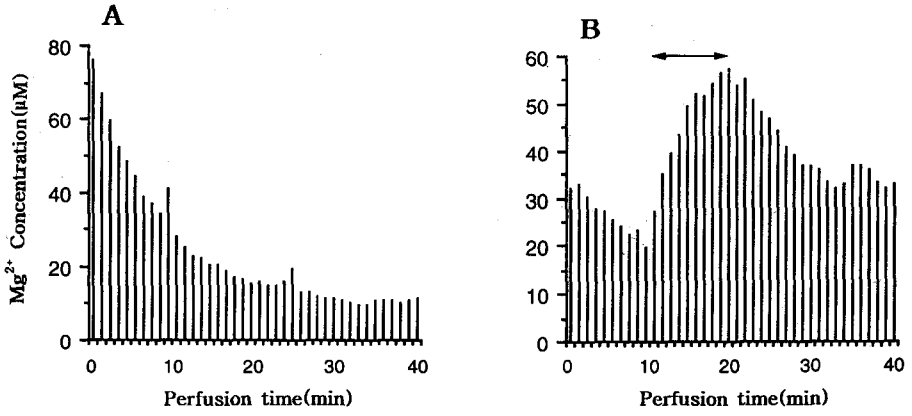

Fig. 1. Mg^{2+} efflux in control perfused guinea pig hearts (A) and after addition of 10^{-6} M histamine (B). The arrows indicate the period that the hearts were perfused with the drugs. One experiment typical of five, both for control and histamine stimulated hearts is shown.

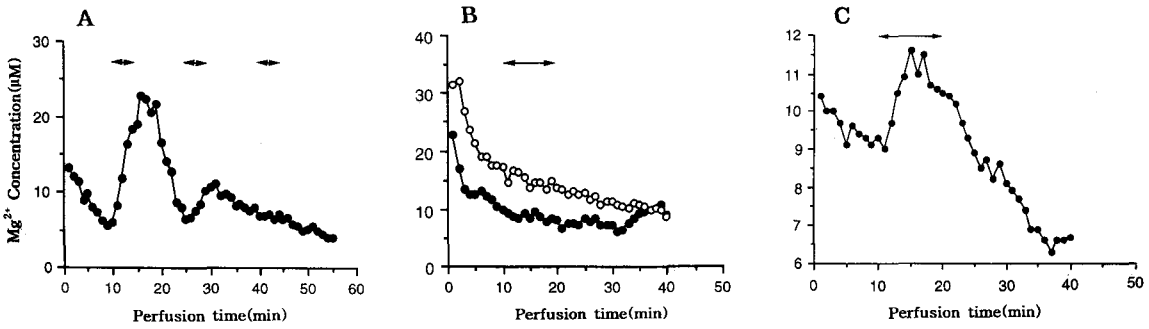

Fig. 2. Mg^{2+} efflux induced by successive 10^{-6} M histamine additions in perfused guinea pig hearts (A). One experiment typical of three is shown. Inhibitory effects of H_2-blocking agents on the Mg^{2+} efflux in guinea pig hearts (B). This figure represents a typical experiment out of four each for the histamine stimulation in the presence of H_2-antagonist, 10^{-5} M cimetidine (○) or 10^{-5} M ranitidine (●), and H_1-antagonist, 3×10^{-6} M diphenhydramine (C).

Effects of histaminergic antagonists on histamine-induced Mg^{2+} efflux

Fig. 2A shows Mg^{2+} efflux after repetitive short stimulation with 10^{-6} M histamine. Where indicated arrow bars that histamine was added in the perfusing buffer. Each stimulation evoked a progressively smaller Mg^{2+} efflux. This results could be accounted for by either a down-regulation of histaminergic receptors or a depletion of magnesium from intracellular pool(s).

As cardiac cell membrane contains H_1, H_2 and H_3-receptors, the Mg^{2+} efflux effects in heart may be mediated by different receptors. In guinea pig heart, H_2-receptors mediate a positive chronotropic effect in atria (Levi et al, 1982) and a positive inotropic effect in ventricular muscles (Hatori & Kanno, 1985). So we used specific histaminergic antagonists to clarify type(s) of receptors involved in the stimulation of Mg^{2+} efflux by histamine. Specific histaminergic H_1 antagonist such as 3×10^{-6} M diphenhydramine had little effect in preventing Mg^{2+} efflux by histamine (Fig. 2C). By contrast, 10^{-5} M cimetidine and 10^{-5} M ranitidine(specific histaminergic H_2 antagonists) completely blocked the Mg^{2+} efflux induced by histamine in perfused guinea pig hearts (Fig. 2B).

Effects of H_2-histaminergic stimulation on Mg^{2+} efflux

As shown in Fig. 2B, pretreatment with H_2-antagonists blocked the efflux of Mg^{2+} by histamine, indicating that histamine had an effect of Mg^{2+} release via activation of H_2-receptors. Because the targets of histaminergic stimulation are the H_1 and H_2- receptors located in the cell membrane, we used a specific histaminergic agonist to identify the type of receptor involved in the Mg^{2+} efflux after histaminergic stimuli. Fig. 3 shows that 10^{-5} M dimaprit, a selective H_2-agonist, produced a large efflux of Mg^{2+}. The addition of 10^{-5} M ranitidine blocked the Mg^{2+} efflux induced by dimaprit in another perfused hearts.

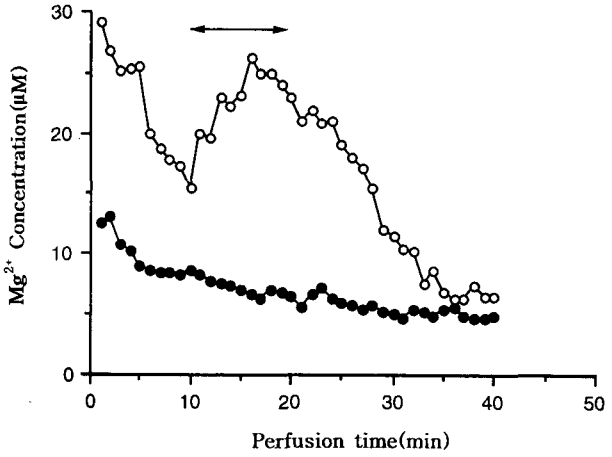

Fig. 3. Inhibition by H_2-histaminergic receptor blocking agents on the Mg^{2+} efflux induced by 10^{-6} M dimaprit in perfused guinea pig hearts. Where indicated, dimaprit was added in the absence (○) or presence (●) of ranitidine. One experment typical of four is shown.

Effects of cyclic AMP on Mg^{2+} mobilization

As shown in Fig. 4, the Mg^{2+} efflux increased with 3×10^{-6} M forskolin (adenylate cyclase activator), which directly increases an intracellular cAMP levels in hearts. However, the addition of 10^{-5} M carbachol (muscarinic agonist), which significantly decreases cAMP levels, results in a decrease of efflux

of Mg^{2+} in perfused hearts. These results suggest that the effect of Mg^{2+} mobilization depends on the intracellular cAMP levels. In order to clear the relationship between cAMP level and Mg^{2+} efflux. We used 2×10^{-4} M 8-Cl-cAMP, permeable cAMP analogues, increased Mg^{2+} efflux in perfusate (Fig. 5A). The inhibition of phosphodiesterase may also have an increase in intracellular cAMP levels because its inhibition by papaverine can inhibit the conversion of cAMP to AMP in cytosol. we observed the effect of 10^{-5} M dimaprit in the presence of 10^{-5} M papaverine (Fig. 5B). In this conditions, 10^{-5} M dimaprit-induced Mg^{2+} efflux was potentiated (Fig. 5B). This result suggest that the Mg^{2+} efflux by histamine H_2-receptors stimulation was due to the increase in cytosolic cAMP levels in hearts.

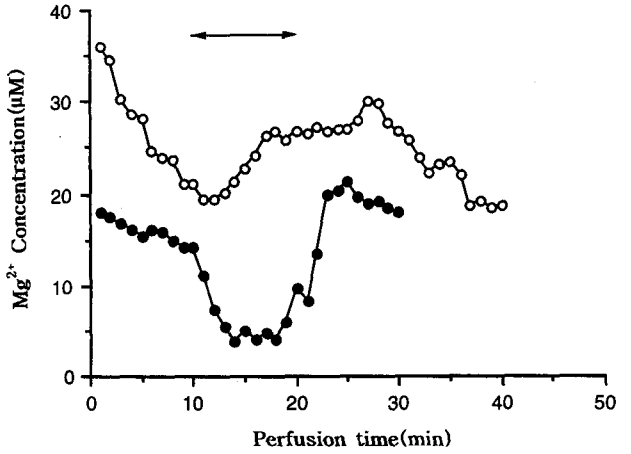

Fig. 4. Effects of 10^{-5} M carbachol (●) and 3×10^{-6} M forskolin (○) on Mg^{2+} efflux in perfused guinea pig hearts. This figure represents a typical experiment out of four each for the carbachol and the forskolin stimulation.

DISCUSSION

Histamine produces cardiac actions by interacting directly with specific receptors in various animal species both in *vivo* and in *vitro* (See Levi et al, 1982). However species and regional differences exist with respect to the distribution of H_1-and H_2-receptors mediating the cardiac responses to histamine. In the guinea pig heart, H_2-receptors are responsible for the positive chronotropic and inotropic effects of histamine in right atria and ventricles (Hattori & Kanno, 1985; Hatori et al, 1990).

Histamine has been shown to increase cAMP production via the stimulation of H_2-receptors (Johnson, 1982; Macall & Lui, 1986) but to activate inositol phospholipid hydrolysis via the stimulation of H_1-receptor in heart (Sakuma et al, 1988). Furthermore, it has been reported that hormone could change a cellular Mg^{2+} directly by affecting the transport of Mg^{2+} (Erdos & Maguire, 1987; Romani and Scarpa, 1990a, 1990b). Alternatively, hormons could directly affect cellular Mg^{2+} through alterations of H^+, Ca^{2+} and Na^+, which could compete with Mg^{2+} for intracelluar binding sites. In S49 lymphoma cell, Grubbs and Maguire (1983) showed that adrenergic β -agonists stimulated ^{28}Mg uptake, recently Romani et al, (1992) showed a similar response to PKC acti-

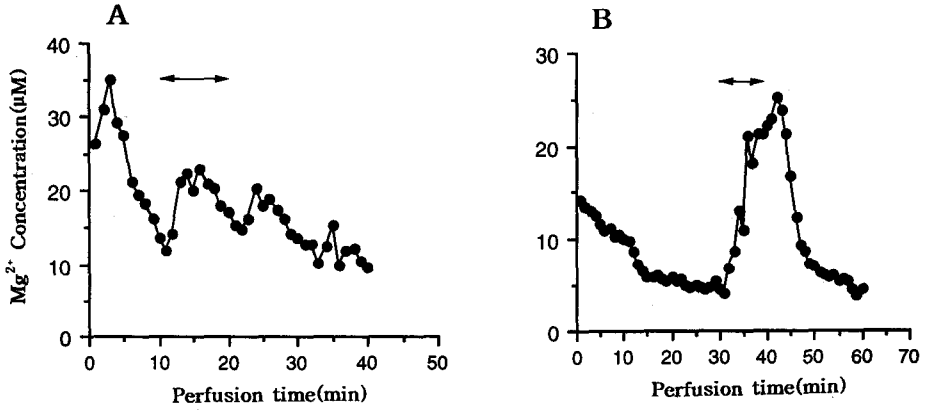

Fig. 5. Stimulatory effect of cAMP analog, 2×10^{-4} M 8-(4-chlorophenylthio)-cAMP sodium (A) or potentiation of 10^{-5} M papaverine, phosphodiesterase inhibitor (B) on the dimaprit-induced Mg^{2+} efflux in perfused guinea pig hearts. The arrows indicate the period that the hearts were perfused with the drugs.

vators in myocytes and hepatocytes. Romani and Scarpa (1990a) reported that noradrenaline causes Mg^{2+} efflux from perfused rat heart and myocytes, a finding supported by the recent observations of Vormann and Gunther (1992), which show that the addition of dibutyryl cAMP in thymocytes causes an increase in Mg^{2+} efflux through the activation of Na^+ -Mg^{2+} antiport by cAMP. However, no studies examining the H_2-histaminergic regulation of Mg^{2+} efflux have been published.

The present data, obtained from perfused guinea pig hearts, indicate the presence of a mechanism of hormonal regulation involved in Mg^{2+} transport across the cell membrane. We have recently shown that a Mg^{2+} release is induced by phenylephrine and that the PKC activator produced a Mg^{2+} uptake in perfused guinea pig hearts (Kang et al, 1995). Our data show that histamine increased markedly Mg^{2+} efflux by the H_2-receptor stimulation and that the Mg^{2+} efflux is clearly related to the cAMP levels of the hearts (Fig. 1B and 5). Additionally, the histamine-induced Mg^{2+} mobilization may be followed by an increase in heart rate and/or inotropic effects. In rat hearts, Romani and Scarpa (1990a) reported that Mg^{2+} efflux was due to specific adrenergic stimulation and was not secondary to an increase in heart rate and/or inotropic effects. The increase of heart rate did not increase Mg^{2+} efflux and the increase of inotropic effect also result in no detectable Mg^{2+} efflux above base line. In this study, also the increase in frequency by electrical stimulation was not enhanced the Mg^{2+} efflux (not shown).

To identify which types of receptor were involved in the stimulation of Mg^{2+} efflux by histamine, we used specific histamine receptor agonist and antagonists. In presence of diphenhydramine, histamine evoked Mg^{2+} efflux same as only histamine addition. By contrast, H_2-antagonists (cimetidine or ranitidine) completely blocked the Mg^{2+} efflux by histamine (Fig. 1, 2). In support of the involvement of H_2-receptor in Mg^{2+} efflux by histaminergic stimulation, We found that dimaprit (specific H_2-agonist) increased Mg^{2+} efflux. When compared with the Mg^{2+} efflux by dimaprit, the dimaprit-induced Mg^{2+} efflux in the presence of ranitidine was abolished by blockade of H_2-receptor, which indicates that the Mg^{2+} efflux is mediated by H_2-receptor (Fig. 3). If effects of histamine on Mg^{2+} efflux are related to activation of H_1-receptor it is probably resultant to uptake rather than an efflux of Mg^{2+}, since H_1-receptor activation by histamine mediated phospholipase C in plasma membrane, produced IP_3 and diacylglycerol, diacylglycerol activated protein kinase C. Stimulation of Mg^{2+} uptake is observable in the presence of phorbol ester (PDBU, PMA), a specific activator of protein kinase C (Romani et al, 1992; Kang et al, 1995), and we also observed that Mg^{2+} efflux by histamine have little effect in the presence of specific H_1-antagonist (Fig. 2C).

The mechanism of Mg^{2+} mobilization under hormonal stimulation has also been investigated by Scarpa group (Romani & Scarpa, 1990a, 1990b; Romani et al, 1991, 1992, 1993, 1995). They have recently reported that β-adrenergic stimulation of cardiac cells induces a major extrusion of Mg^{2+} from the cells. This efflux can be mimicked by stimulating isolated cardiac ventricular myocytes with different permeable cAMP analogues or with forskolin, which directly stimulates adenylate cyclase activity. Histamine also produced a large increase in cAMP content through stimulation of H_2-receptors (Johnson, 1982). Thus the increase in intracellular cAMP content via histamine H_2-receptors stimulation as well as the direct additions of permeable cAMP analogues or forskolin induce a Mg^{2+} efflux. Since our experiments were carried out at very low concentrations of external Mg^{2+}, the presence of a concentration gradient between the inside and the outside of the cell membrane may have resulted in an amplification of Mg^{2+} efflux. It is noteworthy that under those conditions, carbachol was able to stimulate Mg^{2+} uptake against a concentration gradient (Fig. 4). Carbachol, a muscarinic agonist, inhibits cAMP formation by activating G_i proteins. Thus, we have hypothesized that Mg^{2+} efflux produced by histaminergic stimulation increases H_2-receptor-stimulated Mg^{2+} release dependently of the cAMP levels. Another possible explanation for the cAMP-induced Mg^{2+} release may be the enhancement of Mg^{2+} efflux caused by dimaprit (Fig. 5B). Intracellular cAMP levels is regulated by many ligands (like as β-agonist, H_2-agonist, muscarinic agonist *etc.*). Specially phosphodiesterase acts on the conversion of cAMP to AMP in cytosol. When compared with the Mg^{2+} efflux by dimaprit, the degree of the Mg^{2+} efflux by dimaprit was large in presence of papaverine, a phsphodiesterase inhibitor. This result suggest that the Mg^{2+} efflux by H_2-histaminergic stimulation was due to the increase in cAMP levels in perfused hearts.

In conclusion, our data show that there is an appar-

ent correlation between intracellular cAMP levels and Mg^{2+} efflux in hearts. The observed Mg^{2+} efflux could be the result of regulation by cAMP of one or several Mg^{2+} transport pathways in hearts. In considering the recent studies and our data on Mg^{2+} regulation, many other experiments need to be carried out to define in more detail the mechanism involved in the nature of Mg^{2+} transport and to examine a regulation of intracellular Mg^{2+} by drugs or hormones.

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