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Extracellular Mg^{2+} Inhibits both Histamine-Stimulated Ca^{2+}-Signaling and Exocytosis in Human Tracheal Secretory Gland Cells

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The effects of extracellular Mg^{2+} concentration have been investigated on the histamine-stimulated exocytotic process of human tracheal secretory gland (HTG) cells. The exocytosis of secretory granules (SG) was observed concomitantly with dynamic changes of intracellular Ca^{2+} ($[Ca^{2+}]_i$) and Mg^{2+} concentrations ($[Mg^{2+}]_i$). The rate of SG exocytosis was appraised by the decrease of quinacrine fluorescence emission. Dynamic changes of $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$ in HTG cells were determined by the combined use of UV-microspectrofluorometry with Mag-Indo-1 and Indo-1 probes, respectively. High Mg^{2+} medium significantly inhibited the histamine-stimulated secretion. The influence of the extracellular and intracellular Mg^{2+} concentrations on $[Ca^{2+}]_i$ was analyzed. Basal $[Mg^{2+}]_i$ increased from 0.8 mM in a Mg^{2+}-free medium to 1.7 mM in 10 mM Mg^{2+} medium. Histamine induced a $[Mg^{2+}]_i$ increase which is dependent on extracellular Mg^{2+} concentration. The histamine stimulated $[Ca^{2+}]_i$ rise was reduced in the presence of elevated Mg^{2+} extracellular medium and inhibitory effects of extracellular Mg^{2+} were concomitant with changes in $[Mg^{2+}]_i$. Our data suggest that the inhibition by extracellular Mg^{2+} of stimulated exocytosis is dependent on both the increase of $[Mg^{2+}]_i$ and the inhibition of cytosolic Ca^{2+} influx. © 1998 Academic Press

Key Words: **secretion; Ca^{2+}; Mg^{2+}; histamine; tracheal gland cells; microspectrofluorometry.**

The involvement of inflammatory mediators in human airway diseases displaying excessive airway mucus secretion such as asthma, chronic bronchitis or cystic fibrosis is now well established (1). In cellular mechanisms of stimulus-secretion coupling, intracellular Ca^{2+} has been designated to play a central role in the exocytosis of secretory granules (SG) in different secretory cell types (2,3). In human tracheal gland secretory cells (HTG), we have recently displayed evidence that dynamic changes in intracellular Ca^{2+} ($[Ca^{2+}]_i$) are one major step in the cellular events from the secretagogue-receptor interaction to the mucus exocytosis (4-6). In airway inflammation, histamine is a secretagogue known to exert its effect directly by Ca^{2+}-related mechanisms (7). The induced $[Ca^{2+}]_i$ rise takes the form of a Ca^{2+} wave, followed by a smooth decay back to baseline. In most instances, the regulation of exocytosis would be dependent on the influx of extracellular Ca^{2+}, Ca^{2+} binding proteins and protein phosphorylation (8,9).

In parallel to the Ca^{2+}-dependent signaling pathway linked to the exocytotic process, it has been recently proposed that intracellular free Mg^{2+} acts as a second messenger to mediate physiological responses (10,11). In contrast to the highly regulated Ca^{2+}, little is known about the intracellular homeostasis of Mg^{2+} that is present in the cell at much higher concentrations than that of Ca^{2+} (10^{-3} M versus 10^{-7} M). Recently, the combined use of Mag-Indo-1 and laser confocal UV-microspectrofluorometry allowed us to monitor spatial and temporal dynamics changes of $[Mg^{2+}]_i$ in single human tracheal gland cells (12). This previous study demonstrated that the level of $[Mg^{2+}]_i$ was dependent both on the extracellular Mg^{2+} concentration and was modulated by hormones and transmitters. High Mg^{2+} extracellular solutions have been described to inhibit both Ca^{2+} signals and cellular functions in, rat pancreatic acinar cells (13), rat pituitary lactotropes (14) and rat parietal cells (15). It appeared of some interest to i) analyze the influence of Mg^{2+} levels on the Ca^{2+}-dependent exocytosis in HTG cells and, ii) determine the role of Mg^{2+} in the regulation of the mobilization of Ca^{2+} during the stimulus-secretion coupling process in these secretory cells.

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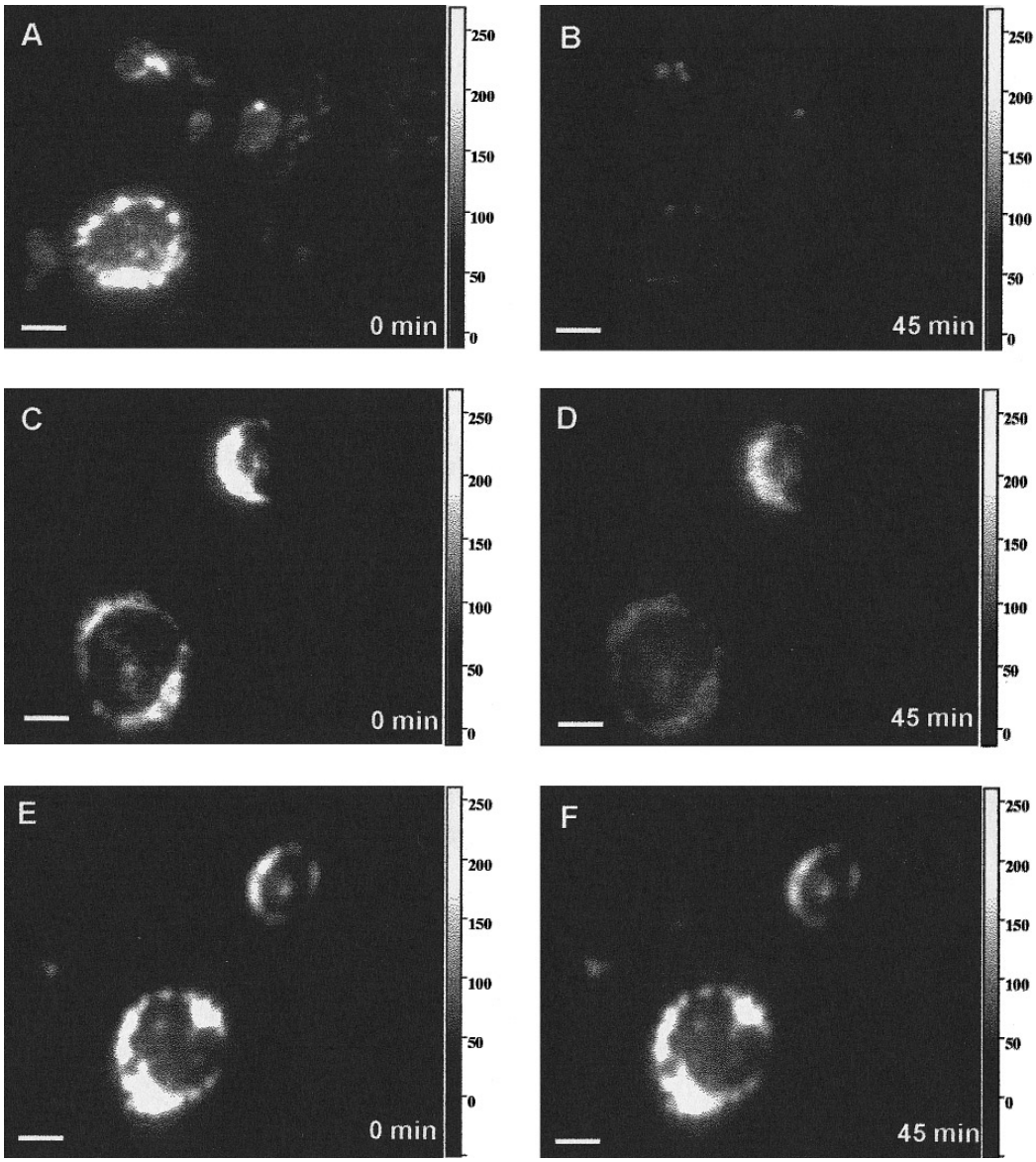

FIG. 1. Histamine-induced SG movements and exocytosis. Videomicroscopic series of fluorescence changes in HTG cells preloaded with quinacrine. A and B: 100 μM histamine was added at time 0 in 1.4 mM extracellular Mg^{2+}. C and D: 100 μM histamine was added at time 0 in 10 mM extracellular Mg^{2+}. E and F: control kinetics in 1.4 mM extracellular Mg^{2+} without histamine addition. The grey scale levels correspond to fluorescence emission in arbitrary units. Bar = 10 μm.

We report here the effect of elevated extracellular Mg^{2+} on the degranulation rate induced by histamine, in HTG cells. The role of extracellular Mg^{2+} on both $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ histamine-stimulated dynamics changes was analyzed in relationship with the degranulation rate observed in HTG cells.

MATERIALS AND METHODS

Chemicals. Mag-indo-1/AM, Indo-1/AM and quinacrine were purchased from Sigma Chemical Co. (St. Louis, MO).

Cell culture. Primary cultures of human tracheal secretory gland (HTG) cells were performed as previously described (16). Cells were grown on 20 x 20 mm type 1 collagen coated glass coverslips in RPMI 1640 medium supplemented with 2% Ultroser G (Biosepra, Villeneuve la Garenne, France) in the presence of 2 mM glutamine and antibiotics (penicillin 100U/ml, streptomycin 100 μg/ml) under a humidified atmosphere of 5% CO_2 -95% air at 37°C. The medium was changed after 2 days and the cells were incubated until they became confluent and quiescent (6-7 days). In these culture conditions, cells exhibit characteristics of epithelial and serous secretory cell type (4,16). Two days before measurements, a defined culture medium (a RPMI 1640 medium supplemented with insulin: 1 $\mu g.\mu l^{-1}$, Epithelial Growth Factor: 10 $ng.\mu l^{-1}$, retinol: 10 $ng.\mu l^{-1}$, hydrocortisone: 0.5 $\mu g.\mu l^{-1}$ and transferrine: 1 $\mu g.\mu l^{-1}$) was used.

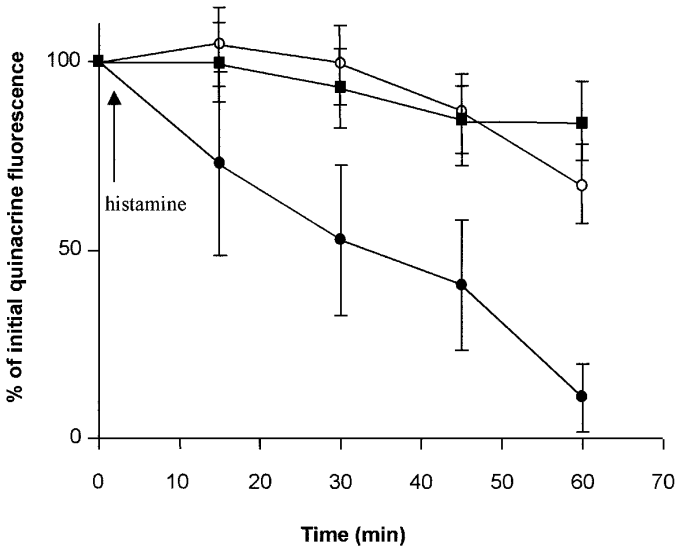

FIG. 2. Time related changes of SG exocytosis in HTG cells induced by histamine (100 μM) in 1.4 mM extracellular Mg^{2+} (●), without histamine in 1.4 mM extracellular Mg^{2+} (O), with histamine in 10 mM extracellular Mg^{2+} (■). The decrease of fluorescence intensity due to secretagogue addition is expressed as % of initial quinacrine fluorescence (time zero). Each point represents the mean ± S.D. of quinacrine fluorescence intensity from 60 cells.

Indo-1 and Mag-indo-1 loading. Quiescent cells were washed twice with Physiological Salt Solution (PSS) (NaCl: 140 mM, KCl: 5.9 mM, NaH_2PO_4: 1.2 mM, $NaHCO_3$: 4.17 mM, Hepes: 10 mM, Glucose: 11 mM and $MgCl_2$: 0, 0.5, 1.4, 5 or 10 mM, the pH was adjusted at 7.4 with NaOH) and incubated in PSS medium containing 5μM Indo-1/AM for 30 min. or Mag-indo-1/AM for 45 min. at 37°C (12).

Microspectrofluorometry. Fluorescent emission spectra within an isolated living cell were recorded using a UV confocal microspectrofluorometer (Dilor, Lille, France). An optical microscope (Olympus BH2) equipped with a water immersion objective lens (100X. N.A. 0.95, State Optical institute of St-Petersburg, Russia) was used for this purpose. This objective lens was specially developed for the total transmission of UV radiation down to 300 nm. The axial chromatic aberration was corrected, so that both excitation (351 nm) and emission (400-500 nm) voxels were superposed. The thickness of the optical section was controlled by varying of the opening of the square pinhole from 50 to 1000 μm. The 351 nm laser line (Ar^+, 2065A model, spectra Physics) was focused with a measured power of 0.5μW at the sample. Fluorescence emission in the 360-560 nm range was spectrally dispersed by a diffraction grating, and was detected with an optical multichannel analyzer consisted of an air-cooled CCD detector.

Dynamic changes of $[Ca^{2+}]_i$ or $[Mg^{2+}]_i$ were monitored as follows: measurements were taken in different locations from one cell (or different cells) and after the final measurement, the process was restarted from the initial point. To do so, an (X, Y) motorized stage (Märzhäuser, model MCL-2 with increments of 0.1 μm), coupled to a computer was utilized. This allowed to store the (X, Y) positions of several (up to 10) chosen points. During this time course, each point was in turn positioned for laser irradiation and for measurement of the emission spectrum. The acquisition of one spectrum and the corresponding stage motion can be performed in a time scale of

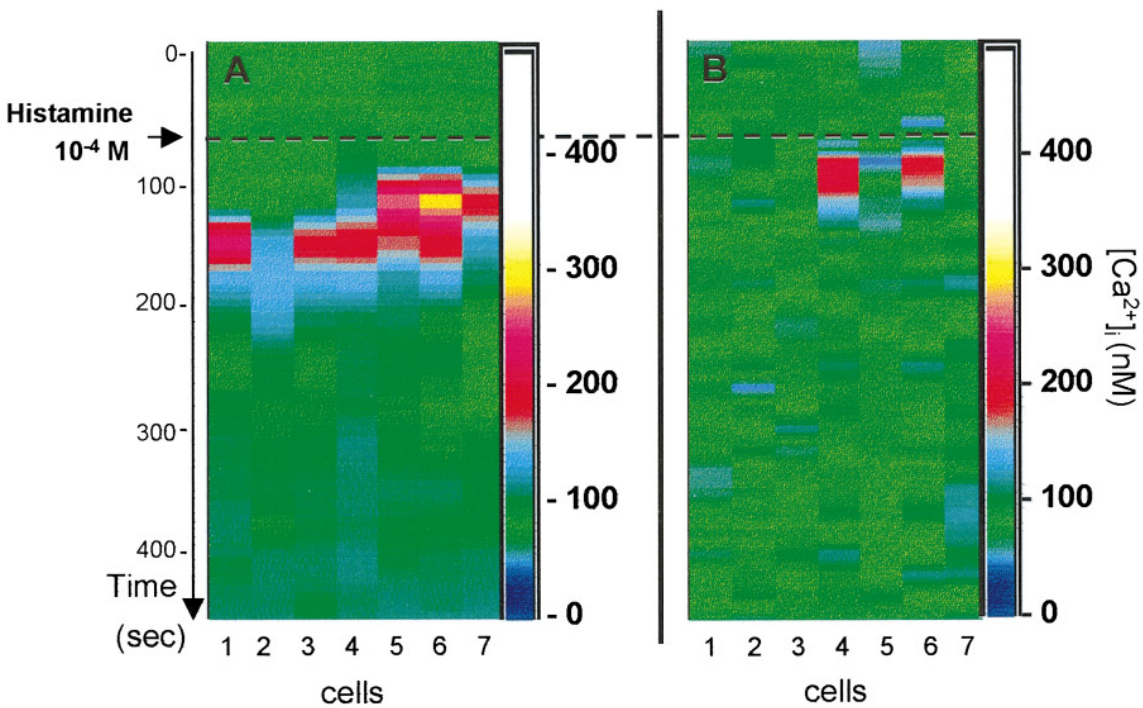

FIG. 3. Histamine-induced $[Ca^{2+}]_i$ increases in HTG cells. Time courses of $[Ca^{2+}]_i$ transients were recorded from 7 adjacent HTG cells (column 1-7) before and after addition of 100 μM histamine in 0 mM extracellular Mg^{2+} (A) and in 10 mM extracellular Mg^{2+} (B). In each column, $[Ca^{2+}]_i$ is measured during 1 second, every 7 seconds. Arrow indicates the histamine addition. The color scale represents $[Ca^{2+}]_i$.

100 ms to a few seconds, depending on the desired temporal resolution (5,6).

Determination of $[Mg^{2+}]_i$ *and* $[Ca^{2+}]_i$. Intracellular free ions (Ca^{2+} or Mg^{2+}) concentrations were calculated according to the following equation (17):

$$[\text{Free ion}]_i = \beta.K_d.(R_{min}-R)/(R-R_{max}),$$

where R is the ratio of fluorescence intensity of the sample at 410 and 500 nm; R_{max} and R_{min} represent the R ratios for the dye emissions when bound or free of ions respectively; β is the emission intensity ratio of the dye at 500 nm in free and bound forms; and K_d is the apparent dissociation constant.

Measurements of secretory granule exocytosis. Measurements of SG exocytosis with quinacrine in HTG cell types has been previously described (6). Cultured HTG cells on glass coverslips were loaded overnight with quinacrine by adding 0.1 μM of the dye to the culture medium. To detect changes in quinacrine fluorescence emission, the measurements were made using a microfluorometer equipped with a Xenon lamp. Excitation was realized at 360-400 nm and emitted light was collected above 450 nm. Control parameters were set up in order to obtain a clear image of the cell on the monitor and a fluorescence intensity of 250 (arbitrary units) in a secretory granule region of the cell. Time-lapse sequences were recorded at scanning rates of 2 sec per image every 5-min for a 40 min-period on videotape. Partial and total release of each fluorescent secretory granule was defined as % of initial fluorescence emission intensity.

Control fluorescence emission measurements from cells bathed in secretagogue-free red-phenol RPMI 1640 medium were carried out before each series of experiments. To be certain that the rate of fluorescent SG release monitored by fluorescence videomicroscopy was associated with SG degranulation, exocytosis of antileukoprotease (ALP), a serous gland cell secretory protein was previously verified by in situ immunostaining and ELISA methods (6).

RESULTS

Influence of extracellular magnesium on secretion. The fluorescent base quinacrine has been previously used to monitor exocytosis in secretory cells (18). This dye specifically accumulates in acidic vesicles, thus enables to monitor simultaneously motions and exocytosis of secretory granules (SG) by fluorescence videomicroscopy (Fig. 1). The addition of 100 μM histamine resulted in an marked decrease in quinacrine emission after a 45 minute period (Figs. 1A and 1B) as compared to control cells (Figs. 1E and 1F). A weak decrease of quinacrine emission intensity was observed in controls, thus showing a weak photobleaching of the dye. As compared with 1.4 mM extracellular Mg^{2+} ($[Mg^{2+}]_e$) physiological conditions (Figs. 1A and 1B), high $[Mg^{2+}]_e$ (10 mM) markedly prevented the fluorescence diminution of quinacrine during the exposure of HTG cells to histamine (Figs. 1C and 1D).

The decrease in quinacrine fluorescence was then plotted as a function of time after exposure of HTG cells with different extracellular Mg^{2+} concentrations. The stimulation with histamine (100 μM) was shown to induce a time-dependent release of SG. In 1.4 mM $[Mg^{2+}]_e$ condition, we observed a large decrease in quinacrine fluorescence intensity down to almost complete extinction after a 60 min stimulation period (Fig. 2). In high $[Mg^{2+}]_e$ condition, the rate of decrease of fluorescence intensity was close to the one observed from control experiment (without addition of histamine).

Influence of extracellular magnesium on the dynamic changes of intracellular calcium $[Ca^{2+}]_i$. As histamine induces both the SG exocytosis and a transient $[Ca^{2+}]_i$ increase in HTG-cells (4), a possible interaction between intracellular Mg^{2+} and Ca^{2+} has been studied. The histamine-induced Ca^{2+} rise was observed for 0 and 10 mM $[Mg^{2+}]_e$. Figure 3 shows the profiles of dynamic changes in $[Ca^{2+}]_i$ observed by quantitative UV laser microspectrofluorometry from 7 adjacent HTG cells. Without Mg^{2+} in extracellular medium, a fast increase of $[Ca^{2+}]_i$ was observed in 6 of the 7 analyzed cells after histamine addition. This was followed by a decrease back to the basal levels (Fig. 3A). Besides, with 10 mM $[Mg^{2+}]_e$, only 2 of the 7 adjacent HTG cells evoked a high $[Ca^{2+}]_i$ rise (Fig. 3B). Percentages of the HTG cells responding to histamine addition showed a significant $[Ca^{2+}]_i$ increased peak (>25 nM) are reported in Figure 4. 95% of cells evoked a transient increase of $[Ca^{2+}]_i$ in 0 mM $[Mg^{2+}]_e$ condition. This percentage of responding cells decreased for higher $[Mg^{2+}]_e$, down to 60% for 10 mM $[Mg^{2+}]_e$.

Influence of $[Mg^{2+}]_e$ *on* $[Mg^{2+}]_i$. Changes in $[Mg^{2+}]_e$ from 1.4 mM (physiological conditions) to 10 mM caused an increase in basal $[Mg^{2+}]_i$ from 1.3 $\pm$ 0.2 mM to 1.8 $\pm$ 0.3 mM (inset of Fig. 5). These variations in $[Mg^{2+}]_i$ were less important in comparison with those of $[Mg^{2+}]_e$ and are in agreement with data from our previous report (12).

Since a clear effect of $[Mg^{2+}]_e$ on both basal $[Mg^{2+}]_i$ and on histamine-stimulated $[Ca^{2+}]_i$ was demonstrated, we next investigated the dependence of $[Mg^{2+}]_i$ as a function of $[Mg^{2+}]_e$ after exposure of HTG cells to histamine (Fig. 5). Dynamic changes in $[Mg^{2+}]_i$ were reproducible for each of the $[Mg^{2+}]_e$ assessed. After a 100 second time period of exposure to histamine, a significant increase in $[Mg^{2+}]_i$ was observed in 1.4, 5 and 10 mM $[Mg^{2+}]_e$ conditions. The variations of $[Mg^{2+}]_i$ were more important with high extracellular magnesium concentrations; at 1.4 mM $[Mg^{2+}]_e$, the $[Mg^{2+}]_i$ variations were low (Δ= 0.3 mM), whereas Δ= 0.6 mM was noted for 10 mM $[Mg^{2+}]_e$.

DISCUSSION

During the last years, Mg^{2+} was designated to play a more important role than co-substrate with nucleic acids or enzyme cofactor (19). Several proteins such as ionic channels (20), ATPases (21) and cell cycle proteins (22) are indeed either activated or inhibited by changes in free Mg^{2+} concentrations, displaying the importance of fluctuations in cytosolic free Mg^{2+} as a consequence

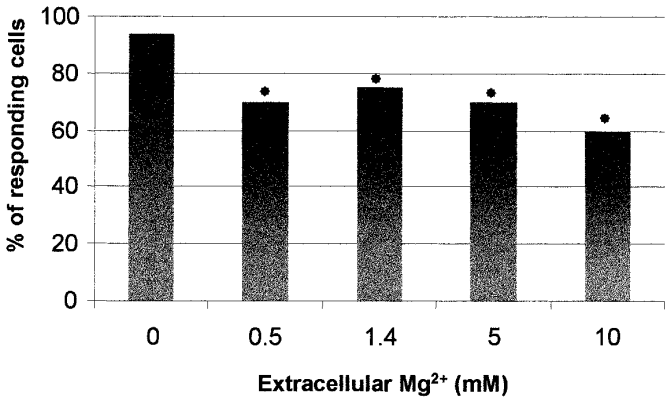

FIG. 4. Response of $[Ca^{2+}]_i$ changes in HTG cells to 100 μM histamine in various extracellular Mg^{2+} concentrations. The percentage of cells showing transient $[Ca^{2+}]_i$ increase higher than 25 nM was determined in 0, 0.5, 1.4, 5 and 10 mM extracellular Mg^{2+}. Each percent has been determined from 30 analyzed cells. (*) Significant difference ($p < 0.05$) as compared with 0 mM Mg^{2+}.

of hormonal stimulation. This modulator role of Mg^{2+} in the cell is supported by i) the existence of regulation mechanisms of intracellular Mg^{2+} (Mg^{2+} transporters, sequestering compartments, transient changes of Mg^{2+}) (20), and ii) the modulation of enzyme activity or ionic channels by changes of Mg^{2+} (23).

In the present study, the addition of 10 mM Mg^{2+} to the medium allowed the HTG cell to fill their Mg^{2+} stores from 0.7 to 1.4 mM. We have previously shown the time-resolved changes in Mg^{2+} by addition of high Mg^{2+} in the medium (12). Indeed, a 6 min time period was required for the HTG cell to present a stable $[Mg^{2+}]_i$ level. However, intracellular Mg^{2+} concentration does not reach the external Mg^{2+} level (fig. 5), thus resulting in a Mg^{2+} gradient across the plasma membrane. This study confirms that HTG cells possess regulation mechanisms to maintain $[Mg^{2+}]_i$ in a physiological range against high $[Mg^{2+}]_e$ medium.

Several activities of enzymes, channels or cellular constituents have been described to be modulated by changes of $[Mg^{2+}]$ (21) and a recent report described that extracellular Mg^{2+} affects stimulus-secretion coupling processes in various cell types (24). In HTG cells, we clearly show that Mg^{2+} inhibits both the histamine-stimulated Ca^{2+} rise and the SG exocytosis rate. A similar phenomenon has been observed in carbachol-stimulated rat parietal cells (25), underlining that this inhibitory effect on SG degranulation would be dependent on the impaired Ca^{2+} influx. Recently, we have reported that the SG exocytosis of HTG cells evoked by a histamine treatment and other secretagogues was clearly dependent on an influx from extracellular Ca^{2+} and not of discharge of internal Ca^{2+} stores (6). Several alternatives could be suggested to account for the modulation role of Mg^{2+} to balance the movements of Ca^{2+} across the plasma membrane under hormonal stimulation. First, Mg^{2+} seems to affect the Ca^{2+}-regulated pathways and the direct inhibitory activity of Mg^{2+} on Ca^{2+} channels could occur. An increase in $[Mg^{2+}]_i$ has been reported to inhibit both L-type Ca^{2+} channels in cardiac myocytes (26) and Ca^{2+} channel current in pregnant rat myometrical cells (27). Second, a Mg^{2+} countertransport has been proposed to modulate the active Ca^{2+} uptake in the intracellular Ca^{2+} stores and the Ca^{2+} release (28). Third, the direct effect of Mg^{2+} on adenylate cyclase activity could be also mentioned, due to the importance of this enzyme in cell signal transduction. In fact, it has been recently demonstrated that elevated Mg^{2+} inhibits the late phase of cAMP formation (28). One site of the Mg^{2+} dependent activation is located at the level of the G-protein. An increase of Mg^{2+} level activates the G-proteins, which exerts a regulatory effect on $[Ca^{2+}]_i$ and is able to block the influx of extracellular Ca^{2+} as demonstrated in cardiac cells (29). Finally, Mg^{2+} could also play an important role in the activation of the Mg^{2+}-dependent calcium pump located in the membranes of endoplasmic reticulum, to interfere with ATPase reactions such as phosphorylation or dephosphorylation of the enzyme (20).

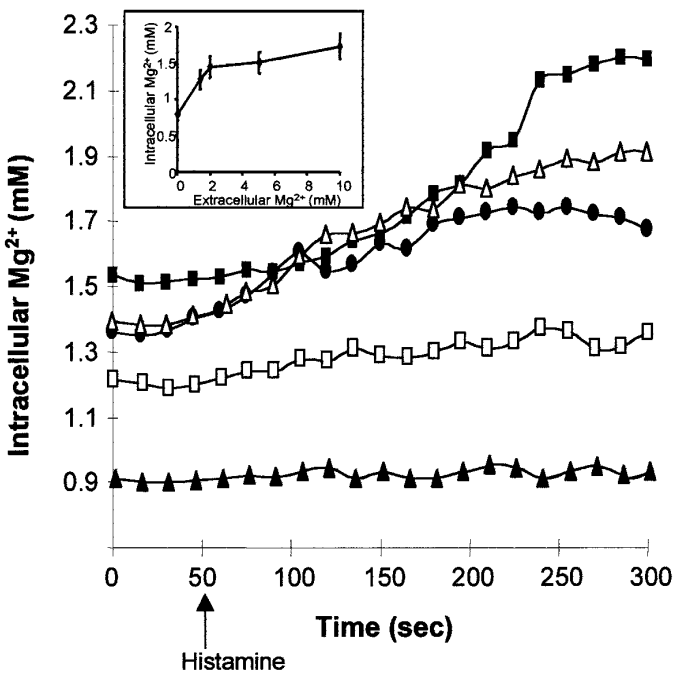

FIG. 5. Histamine-induced $[Mg^{2+}]_i$ dynamics in HTG cells. Time courses of $[Mg^{2+}]_i$ variations were recorded before and after addition of 100 μM histamine in 0 (△), 0.5 (□), 1.4 (●), 5 (△) and 10 mM (■) extracellular Mg^{2+}. Each point is a mean obtained from 20 cells. Inset: Intracellular Mg^{2+} as a function of extracellular Mg^{2+}. $[Mg^{2+}]_i$ were determined in 0, 1.4, 2, 5 and 10 mM Mg^{2+} extracellular medium. Each point represents mean of intracellular Mg^{2+} concentrations measured from 60 cells.

Furthermore, recent studies suggested that the effects of elevated Mg^{2+} levels are not restricted to signaling processes but can have more profound influence on secretion (23). In a previous study, any interference of $[Ca^{2+}]_i$ in the determination of $[Mg^{2+}]_i$ dynamic changes were detected in HTG cells (12). Here, we observed that histamine may induce Mg^{2+} dynamic changes dependent of extracellular Mg^{2+} conditions, but similar to histamine stimulated response (Fig. 5). Thus, these new data enhance the idea of the major modulator role of magnesium ion in HTG cells. Because the Mg^{2+} increase correspond to a response just after the addition of histamine, the inhibition of degranulation processes of SG may be the result of an active Mg^{2+} controlled inhibitory action.

To summarize, this study shows the potent effect of elevated $[Mg^{2+}]_e$ via elevated $[Mg^{2+}]_i$ both on histamine stimulated exocytosis and on Ca^{2+} influx. The candidate protein complexes associated with dynamic changes in $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ involved in the regulation of the secretion in HTG cells remain to be defined.

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