Magnesium homeostasis and its role in the exocrine pancreas

Homeostasis de magnesio y su papel en el páncreas exocrino

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ABSTRACT

Magnesium is an abundant divalent cation that plays an important physiological and biochemical role at different levels. It is involved in several physiological processes during cellular regulation, so it is important to understand the homeostasis of this cation in the pancreas. This study shows the role of magnesium in the pancreas acting on the regulation of insulin secretion, altering pancreatic and blood histamine levels, and on the ability of acinar cells to synthesize new proteins. It is also reported the effect of a perturbation of extracellular magnesium on the pancreatic enzyme, protein, and juice secretion in response to acetylcholine, cholecystokinin-octapeptide and activation of intrinsic secretomotor nerves.

We have shown the distribution of magnesium within pancreatic acinar cells, focusing on the transport mechanisms for this cation in the exocrine pancreas, which we have examined by means of several techniques.

Finally, our results have demonstrated that magnesium may play an important regulatory role in the mobilization of calcium during the exocytotic process in the exocrine pancreas.

KEY WORDS: Magnesium, Pancreas, homeostasis, pancreatic juice, exocrine pancreas, transport, calcium.

RESUMEN

El magnesio es un abundante cation divalente que juega un importante papel fisiológico y bioquímico a diferentes niveles. Está implicado en varios procesos fisiológicos durante la regulación celular, por lo que es importante entender la homeostasis de este catión en el páncreas. Este estudio muestra el papel del magnesio en el páncreas actuando sobre la regulación de la secreción de insulina, alterando los niveles de histamina en páncreas y sangre, y la habilidad de las células acinares para sintetizar nuevas proteínas. También mostramos el efecto de una alteración del magnesio extracelular sobre enzimas y proteínas pancreáticas, y secreción de jugo pancreático como respuesta a la acetilcolina, colecistoquinina-octapéptido y a la activación de nervios secretomotores intrínsecos.

Hemos mostrado la distribución del magnesio dentro de la célula acinar pancreatica, centrándonos en los mecanismos de transporte de este catión en el páncreas exocrino utilizando diversas técnicas.

Nuestros resultados han demostrado que el magnesio puede jugar un importante papel regulados en la movilización del calcio durante el proceso de exocitosis en el páncreas exocrino.

PALABRAS CLAVE: Magnesio, páncreas, homeostasis, jugo pancreático, páncreas exocrino, transporte, calcio.

BIOLOGICAL ROLES OF MG^{2+}

The role of the abundant divalent cation magnesium (Mg^{2+}) in the exocytotic process has long been overshadowed by the advancements in current knowledge of the second messenger role of Ca^{2+} (1). However, Mg^{2+} is involved in several physiological and

biochemical processes. It is an important co-factor for over 300 enzymes (2). Mg^{2+} is involved in the synthesis and replication of RNA and DNA (3) and with muscle contraction (4), and the secretion of enzymes (1, 5, 6) and hormones (7-9). Mg^{2+} also plays an important physiological role in transmembrane movements of ions (eg. Na^+: K^+-ATPase, Ca^{2+}-ATPase, K^+H^+-ATPase, K^+:Na^+:Cl^- and Na^+:Cl^- transporter and Ca^{2+}:Na^+ and HCO_3:Cl^- exchanges) and regulation of ion channel activities eg. Na^+, K^+ and Ca^{2+} (10-13). Furthermore, Mg^{2+} is also involved with metabolic pathways, protein synthesis, bioenergetic and structural properties of cells, stabilization of membrane and electrical potentials across cells (see 14 for reviews).

MG^{2+} AND THE PANCREAS

Since Mg^{2+} is involved in several physiological processes during cellular regulation, then it is of some importance to understand the homeostasis of this abundant divalent cation in the pancreas. In 1966, Grodsky and Bennett (9) first demonstrated that the release of insulin by the perfused pancreas following glucose infusion was dependent upon both extracellular Mg^{2+} and Ca^{2+}. Elevated extracellular Mg^{2+} had an inhibitory effect on secretory responses compared to Ca^{2+} alone. Similar results were also obtained by Curry et al, (8) in the perfused rat pancreas. These actions suggested that Mg^{2+} may play an important physiological role in the regulation of insulin secretion by altering the sensitivity of the beta cells of the islet of Langerhans to glucose.

DIETARY MG^{2+} AND PANCREATIC FUNCTION

Sarnar and Snodgrass (15) first demonstrated that exocrine pancreatic function was preserved in young and adult rats fed on chronic dietary deficiency of Mg^{2+} (10 mg Kg^{-1} diet) for 90-120 days. Mg^{2+} level in the pancreas of young rats was much higher compared to rats fed on normal diet (1800 mg Kg^{-1} diet) although plasma Mg^{2+} was severely reduced. In 1974, Greger and Schwartz (16) repeated these experiments and showed similar results regarding pancreatic secretion. However, they also demonstrated that pancreatic and blood histamine levels were markedly elevated in hypomagnesemic animals compared to rats fed on normal Mg^{2+} diet. Furthermore, they also showed that the ability of acinar cells to synthesize newly synthesized proteins were severely reduced in hypomagnesemic rats.

More recently, we have also obtained similar results employing weanling rats which were fed on a low (20 and 39 mg Mg^{2+} Kg^{-1} diet) compared to rats fed on normal diet (516 mg Mg^{2+} Kg^{-1} diet). Although plasma Mg^{2+} decreased by over 90% pancreatic Mg^{2+} level was increased by 162 " 12% (n=10) compared to the pancreas of rats fed on normal Mg^{2+} diet. These results indicate that the pancreas can conserve Mg^{2+} during low Mg^{2+} condition and thus protects itself from severe hypomagnesemic conditions at least during dietary experimental hypomagnesemia. However, in rats which are fed on low Mg^{2+} and low protein (25%) diet, light and electron microscopic examinations have revealed that acinar cells of the pancreas were packed with zymogen granules, suggesting disturbances in the discharge (rather than in production) of pancreatic enzymes. (17). The mitochondria and the lumina of the rough endoplasmic reticular were swollen. The nuclei had an irregular outline, the chromatin was aggregated into irregular granules and the nucleolemma of the nucleolus was fibrillar. The et al (17) indicated that the disturbance in the release of enzymes from the pancreas in response to a low Mg^{2+} and protein diet may be due to a maldigestion of the low dietary proteins and the disturbance of exocytosis in the pancreas of Mg^{2+} deficient rats may result in preparental use of all the available Mg^{2+} for enzyme synthesis so that no Mg^{2+} is available to regulate the energy-dependent discharge of zymogen granules.

MG^{2+} AND PANCREATIC JUICE SECRETION

Mg^{2+} is released with enzymes in both saliva (5) and pancreatic juice (18). Mg^{2+} is believed to be distributed around rough microsomes in acinar cells (19) and also stored in zymogen granules. The divalent cation is released with the digestive enzymes and pancreatic juice during basal condition and upon stimulation with secretagogues (5, 20). Moreover, the pancreatic zymogen membrane seems to contain a strong Mg^{2+} requiring (phosphate) activity towards the hydrolysis of all nucleosides and di and tri-phosphates. The protein appears to be intrinsic with its active site localized on the internal face of the zymogen granular membrane (21). A perturbation of extracellular Mg^{2+}, $[Mg^{2+}]_o$, has been shown to have little or no effect on basal secretion but it has profound effect on pancreatic enzyme, protein and juice secretion in response to acetylcholine (ACh) (1, 22), cholecystokinin-octapeptide (CCK-8) (6) and activation of intrinsic secretomotor nerves (23, 24). Elevated $[Mg^{2+}]_o$ inhibits secretagogue-evoked secretory responses whereas low $[Mg^{2+}]_o$ has the opposite effect. Perturbation of $[Mg^{2+}]_o$, results in changes in intracellular free Mg^{2+} concentration $[Mg^{2+}]_i$ which seems important to the stimulus secretion-coupling process. In elevated $[Mg^{2+}]_o$, $[Mg^{2+}]_i$ rises whereas in low $[Mg^{2+}]_o$, $[Mg^{2+}]_i$ decreases only slightly (6, 25, 26).

RELATIONSHIP BETWEEN MG^{2+} AND CA^{2+} SIGNALLING IN ACINI

Mg^{2+} is now believed to be linked closely with the mobilization of Ca^{2+} both from cellular stores and from extracellular medium (6, 27). For example, the metabolism of inositol trisphosphate (IP_3) which stimulates the release of Ca^{2+} from rough endoplasmic reticulum (RER) depends upon the Mg^{2+} dependent enzyme, 5 phosphatase (28, 29). In conditions where little Mg^{2+} was present, this enzyme would be inhibited in its hydrolysis of IP_3. As a consequence increasing level of IP3 would stimulate mobilization of Ca^{2+}. This would promote secretion and provide increased $[Ca^{2+}]_i$ for extrusion. In high Mg^{2+} conditions reduced IP_3 and therefore $[Ca^{2+}]_i$ would be expected. The major Ca^{2+} release in pancreatic acinar cells is now believed to occur through the caffine-sensitive Ca^{2+} channel (30). In sarcoplasmic reticulum (SR) of skeletal muscle this channel is highly sensitive to $[Mg^{2+}]_i$ (31). Recent work on the pancreas also suggests that the channel is Mg^{2+}-dependent and is inhibited by Mg^{2+} in millimolar range. Conversely, in low Mg^{2+} conditions the open state of this channel is promoted (32). A perturbation of $[Mg^{2+}]_o$ has marked effect on secretagogue-evoked Ca^{2+} influx, changes in $[Ca^{2+}]_i$ and Ca^{2+} oscillations. Elevated $[Mg^{2+}]_o$ inhibits $^{45}Ca^{2+}$ uptake, changes in $[Ca^{2+}]_i$ and the amplitude and frequency of Ca^{2+} oscillations whereas low Mg^{2+} facilitates $^{45}Ca^{2+}$ uptake, increases in $[Ca^{2+}]_i$ and Ca^{2+} oscillations (1, 6, 26, 27, 33). More specifically, Mg^{2+} has been shown to be the co-factor for a membrane-bound Ca^{2+}-ATPase directly acting to bring intracellular Ca^{2+} levels to a point of stasis following stimulation by such secretagogues as ACh and CCK-8 (2, 34-38). Taken together these observations clearly indicate that Mg^{2+} may play an important regulatory role in the mobilization of Ca^{2+} during the exocytotic process in the exocrine pancreas.

MG^{2+} TRANSPORT IN THE EXOCRINE PANCREAS

Mg^{2+} (ions) are present in abundant quantities in the cytoplasm of living cells including pancreatic acinar and ductal cells existing in either the free $[Mg^{2+}]_i$ or the bound form $[Mg_b^{2+}]$ to biological ligands such as proteins, RNA, ATP and Mg^{2+} activated enzymes (39). Measurements of total Mg^{2+} as well as free intracellular Mg^{2+} can be obtained by atomic absorbance spectrophotometry, nuclear magnetic resonance (NMR), spectrofluorimetry using acetoxymethyl ester (AM) and tetrapotassium salts, null point for plasma membrane permeabilization, magnesium sensitive electrodes, metallochromic dyes eg. Arsenazo III and Antipyrylazo III, radiotracer methods (eg. using $^{28}Mg^{2+}$) and enzyme dependance (eg. citrate/isocitrate and glucolysis) (25, 27, 40-45). Using

the method of atomic absorbance spectroscopic method, we have shown that such secretagogues as ACh and CCK-8 as well as electrical field stimulation of intrinsic secretometor nerves can result in significant increases in Mg^{2+} efflux from superfused pancreatic segments compared to basal release (25, 27, 33, 46). On the other hand, stimulation of pancreatic segments with secretin resulted in an uptake of Mg^{2+} compared to basal level (46). From these studies it is apparent that pancreatic secretagogues eg. (ACh & CCK-8) which utilize cellular Ca^{2+} as a mediator caused Mg^{2+} efflux whereas secretagogue (eg. secretin) which exerts its secretory effect via cyclic AMP metabolism caused an uptake of Mg^{2+} by pancreatic acinar cells. Spectroscopic method has also been used successfully to characterize Mg^{2+} transport in red blood cells (10, 11, 47-49) as well as the pancreatic acinar cells and segments (6, 25, 27, 50). ACh and CCK-8-evoked Mg^{2+} efflux in superfused pancreatic segments is sensitive to extracellular sodium removal (with choline as substitute) (6, 27, 50) but unaffected by either of the Ca^{2+} channel antagonist, diltiazem, verapamil, gallopamil or lanthanum chloride (27, 51).

Another important technique employed in charaterizing Mg^{2+} transport is to load the cell with Mg^{2+} using A23187 and elevated extracellular Mg^{2+} (12 mM $MgSO_4$) and then measure passive Mg^{2+} efflux from the loaded cells. (47-49). Resting $[Mg^{2+}]_i$ in unloaded cell is 0.74 " 0.02 mM (n=12). When cells were incubated with either 12 mM $MgSO_4$ alone or 12 mM $MgSO_4$ plus 6 mM A23187 for 30 min at 37°C $[Mg^{2+}]_i$ increased to 0.84 " 0.05 mM (n=6) and 1.58 " 0.03 mM (n=5), respectively. These results clearly indicate that treatment of pancreatic acinar cells with A23187 in elevated $[Mg^{2+}]_o$ resulted in a two fold increase in $[Mg^{2+}]_i$. The resting $[Mg^{2+}]_o$ in a nominally free Mg^{2+} and Ca^{2+} buffer in the loaded cells was 160.2 " 12.6 mM (n=6). Treatment of the Mg^{2+} loaded cells with 0.8 mM digitonin to permeabilize the plasma membrane caused $[Mg^{2+}]_o$ to increase from the resting value of 160.2 " 12.6 mM (n=5) to 262.2 " 13.7 mM (n=6). On addition of Triton-X 100 to Mg^{2+} loaded cell suspensions $[Mg^{2+}]_o$ increased further to 272.02 " 15.0 FM (n=6). These data suggest that treatment of acinar cells with A23187 did not seem to cause any redistribution of internal Mg^{2+} from intracellular organelles especially since the level of free Mg^{2+} in the cytoplasm of the loaded cells did not increase significantly following permeabilization of intracellular organelles with Triton-X-100. It is possible that most of the internal Mg^{2+} is located in stores or bound to cellular substrates.

Mg^{2+} loaded pancreatic acinar cells can release Mg^{2+} in a time dependent manner in a Mg^{2+} deficient medium with maximal release occuring within 40 min. Pre-treatment of the Mg^{2+} loaded acini with either bumetanide, SITS or ouabain had no significant effect on Mg^{2+} efflux. In contrast, when acini were pretreated with either 10 mM dinitrophenol, 10-4M amiloride, 1 mM lidocaine or 1 mM quinidine there were significant ($P<0.001$) decreases in Mg^{2+} efflux. Moreover, replacement of extracellular sodium with either N-methyl-D-glucamine (NMDG), TRIS or choline results in significant ($P<0.001$) inhibition of Mg^{2+} efflux. These results clearly demonstrate that passive Mg^{2+} extrusion (efflux) from pancreatic acinar cells may not be associated with either the Na^+:K^+-ATPase pump, the Na^+:K^+:Cl^- co-transporter or the ion exchanger but instead with a Na^+-sensitive Mg^{2+} transport system (6, 27, 50).

In addition to atomic absorbance spectroscopy to measure total Mg^{2+}, we have also successfully employed the fluorescent bioprobes magfura-2 acetomethyl ester (AM) and magfura-2 tetrapotassium salt to measure free intracellular and extracellular Mg^{2+}, respectively in pancreatic acinar cells. Magfura-2 tetrapotassium salt is the cell impermeant form of magfura-2 and it is used to measure Mg^{2+} efflux from acinar cells following stimulation with secretagogues. In order to obtain successful results it is necessary to undertake the measurement in a nominally free extracellular Ca^{2+} physiological salt solution containing 1 mM EGTA. Stimulation of pancreatic acinar cells with either ACh (10^{-5}M) or CCK-8 (10^{-8}M) resulted in a gradual and significant release (efflux) of Mg^{2+} from pancreatic acinar cells reaching a maximum after about 300-350 sec following stimulation in either normal 1.03 " 0.04 mM (n=14) or a nominally Mg^{2+} free 0.05 " 0.02 mM (n=13) physiological salt solution. In contrast, the paracrine hormone histamine (10-4) had no significant effect on Mg^{2+} release (41).

The use of magfura-2 (AM) has been used successfully in a number of studies including pancreatic acinar cells to measure intracellular free Mg^{2+} concentrations (6, 25-27, 33, 41, 44, 52-58). Basal $[Mg^{2+}]_i$ in normal and nominally Mg^{2+} free solution were 0.82 " 0.02 mM (n=65)

and 0.76 " 0.002 mM (n=16), respectively. Stimulation of either pancreatic acinar cell suspensions or single pancreatic acinar cells at 37°C with either ACh or CCK-8 resulted in an initial transient rise (release 0.98 " 0.03 mM (n=30)) within the first 30 sec followed by a continuous decline (efflux) below resting value until a new steady-state (0.53 " 0.03 mM (n=30) is reached. In the case of perfused single acinar cell on removal of the secretagogue, $[Mg^{2+}]^i$ returned to pre-stimulated value (uptake) (6, 27, 33, 41, 54, 56). The initial transient rise in $[Mg^{2+}]_i$ evoked by secretagogues is still retained in the absence of $[Mg^{2+}]_o$ indicating that $[Mg^{2+}]_o$ is not associated with this initial response. Furthermore, at ambient temperature the initial rise in $[Mg^{2+}]_i$ following secretagogue stimulation is absent (25, 26) indicating that the initial release in $[Mg^{2+}]_i$ is temperature-dependent. In an attempt to assess whether secretagogue-evoked initial rise in $[Mg^{2+}]_i$ in magfura loaded pancreatic acinar cells was due to Ca^{2+}, the cells were also loaded with BAPTA (1,2 bis (2-aminophenoxy) ether, N, N, N1, N1 - tetra acetic acid) to chealate $[Ca^{2+}]_i$. On secretagogue stimulation $[Mg^{2+}]_i$ rises to the same magnitude compared to the absence of BAPTA indicating that the initial increase in Mg^{2+} may not be due to changes in cellular Ca^{2+} (54). It is now known that Mg^{2+} is stored in the mitochondria of cells and it is released into the cytoplasm upon stimulation (59). From the cytoplasm the Mg^{2+} is extruded from the cell to the extracellular medium by a sodium-dependent magnesium transporter (10, 11, 48, 49).

In pancreatic acinar cells either ACh, CCK, thapsigargin or ionomycin but not histamine can elicit marked decreases in intracellular free $[Mg^{2+}]_i$. On the other hand either secretin, forskolin or dibutyryl cyclic AMP can evoke an elevation in $[Mg^{2+}]_i$ (6, 25-27, 33, 41, 56). Similarly, pretreatment of magfura-2 loaded acinar cells with elevated Mg^{2+} (10 mM) can also result in a elevation in $[Mg^{2+}]_i$ whereas in low extracellular Mg^{2+} $[Mg^{2+}]_i$ is decreased only slightly (6, 25, 26). Following stimulation of magfura loaded acini with Ca^{2+} mobilizing secretagogue (eg. either ACh or CCK-8) addition of another Ca^{2+} mobilizing secretagogue (eg. either CCK or ACh) had no further effect on $[Mg^{2+}]_i$. This result indicates that the Ca^{2+} mobilizing secretagogues such as ACh, CCK-8, ionomycin and thapsigargin are stimulating Mg^{2+} release from a common intracellular pool (33, 41, 56). The secretagogue-evoked decrease in $[Mg^{2+}]_i$ in magfura-2 loaded acini is unaffected by either of the Ca^{2+} channel blockers verapamil, gallopamil, diltiazem or lanthanum chloride (27), the Na^+:K^+:Cl blocker, bumetanide and the Na^+:K^+ -ATPase inhibitor, ouabain (50) but sensitive to sodium removal (with either TRIS, Choline or NMDG as substitute (6, 27, 50). These results employing fluorimetric method to measure Mg^{2+} transport indicate that Mg^{2+} efflux is not associated with either the Ca^{2+} channel, the Na^+:K^+: Cl^- co-transporter or the Na^+:K^+-ATPase pump but with a sodium-dependent Mg^{2+} transporter. Furthermore, these findings corroborate our previous studies employing spectroscopic techniques to investigate Mg^{2+} mobilization.

DIGITAL IMAGING OF $[MG^{2+}]_I$

Single mouse pancreatic acinar cells are clearly polarized in that the secretory pole of the cells contained large numbers of zymogen granules whereas the basal pole appeared clear with few granules. Image of magfura-2 for spatial and temporal changes in $[Mg^{2+}]_i$ indicates that at rest, the intensity of the fluorescence ratio (330/370) was non-uniform and suggests that Mg^{2+} is non-uniformally distributed within pancreatic acinar cells. In a representative of 4 different acinar cells $[Mg^{2+}]_i$ was more localized towards the secretory pole before stimulation with CCK-8. When these cells were continuously perfused with 10 pM CCK-8, $[Mg^{2+}]_i$ transiently increased throughout the entire cell within the first 30 sec. At this moment the intensity at the secretory pole was higher than at the basal pole. The intensity shortly returned to resting values and was followed by a gradual decline phase in which $[Mg^{2+}]_i$ was significantly reduced around the entire basolateral membrane. These results corroborate our fluorimetric studies of Mg^{2+} homeostasis in pancreatic acinar cells (60).

CONCLUSIONS

The results have demonstrated that Mg^{2+} may play an important regulatory role in the mobilization of Ca^{2+} during the exocytotic process in the exocrine pancreas. Since hypomagnesemia is associated with the pathogenesis of pancreatitis, (61-63) then our understanding of Mg^{2+} transport (its release, its efflux and uptake) and moreover, the ability of elevated Mg^{2+} to reduce secretagogue-evoked pancreatic juice secretion may have some physiological relevance in the treatment of pancreatitis (eg. elevating the symptoms associated with pancreatitis). Studies in progress are now focused on drugs which can either inhibit Mg^{2+} extrusion or facilitate Mg^{2+} uptake.

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