Paradoxical Block of Parathormone Secretion Is Mediated by Increased Activity of Ga Subunits*

Ursula Quitterer‡‡, Michaela Hoffmann‡, Marc Freichel¶, and Martin J. Lohse‡

From the ‡Institut für Pharmakologie und Toxikologie, Universität Würzburg, 97078 Würzburg, Germany and the ¶Institut für Pharmakologie und Toxikologie, Universität des Saarlandes, 66421 Homburg, Germany

The paradox of blunted parathormone (PTH) secretion in patients with severe hypomagnesemia has been known for more than 20 years, but the underlying mechanism is not deciphered. We determined the effect of low magnesium on in vitro PTH release and on the signals triggered by activation of the calcium-sensing receptor (CaSR). Analogous to the in vivo situation, PTH release from dispersed parathyroid cells was suppressed under low magnesium. In parallel, the two major signaling pathways responsible for CaSR-triggered block of PTH secretion, the generation of inositol phosphates, and the inhibition of cAMP were enhanced. Desensitization or pertussis toxin-mediated inhibition of CaSR-stimulated signaling suppressed the effect of low magnesium, further confirming that magnesium acts within the axis CaSR-G-protein. However, the magnesium binding site responsible for inhibition of PTH secretion is not identical with the extracellular ion binding site of the CaSR, because the magnesium deficiency-dependent signal enhancement was not altered on CaSR receptor mutants with increased or decreased affinity for calcium and magnesium. By contrast, when the magnesium affinity of the Ga subunit was decreased, CaSR activation was no longer affected by magnesium. Thus, the paradoxical block of PTH release under magnesium deficiency seems to be mediated through a novel mechanism involving an increase in the activity of Ga subunits of heterotrimeric G-proteins.

Parathormone (PTH) secretion from the parathyroid gland is suppressed by high extracellular calcium and magnesium (1). The calcium-sensing receptor (CaSR) is responsible for the calcium-dependent inhibition of PTH secretion (2). Direct binding of calcium or magnesium activates the CaSR (3). Activation of the CaSR triggers Ga/i/Gq-mediated signaling pathways (4). Several mutations have been identified with increased activation of this receptor (5, 6). CaSR mutants with increased affinity/potency for the agonist calcium and in part enhanced constitutive activity led to permanent inhibition of PTH secretion (7). Therefore, patients with activated CaSR mutants suffer from hypoparathyroidism. A similar phenotype of blunted PTH secretion is seen in patients with severe magnesium deficiency (8–10). This finding is unexpected since the effects of high magnesium on parathyroid hormone secretion are similar to those of calcium, and therefore, low magnesium should be expected to result in increased PTH secretion. And indeed, in contrast to patients, rats respond to severe hypomagnesemia with increased secretion of PTH (11, 12). It is known that hypomagnesemia reflects intracellular magnesium deficiency (9). Thus, the site of magnesium action has been assumed to lie intracellularly (9). However, causality between blunted PTH secretion and magnesium deficiency is not established, although the magnesium paradox has been known for more than 20 years (8). In search for the mechanism we investigated the relationship between magnesium deficiency, PTH secretion, and CaSR-mediated signaling. We present evidence that increased activity of Ga subunits leads to enhanced signaling mediated by constitutive activation of the human CaSR. Enhanced CaSR-mediated signaling may thus constitute the link between severe hypomagnesemia and blunted PTH secretion.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—Human embryonic kidney cells (HEK-293) were cultivated and transfected with plasmids encoding the wild-type and the different CaSR mutants (7, 13–15) under the control of the cytomegalovirus promotor as described (16).

Construction of Ga, and CaSR Mutants—For mutagenesis of Gaα, we used the Escherichia coli expression vector pQE60 containing the Gaα1-cDNA with an internal nucleotide sequence after amino acid 121 encoding a hexahistidine tag. The codon coding for arginine 209 of Gaα1 was exchanged to cysteine by site-directed mutagenesis (16). For mutagenesis of the human CaSR, an additional restriction site for XhoI was introduced (silent mutation of leucine 276). The mutated cDNAs were sequenced entirely to confirm the identity of the mutants.

Expression and Purification of Gaα, and Gaβ,–The Gaα and Gaβ proteins (wild-type and R209C) were expressed in E. coli BL21(DE3) and purified by Ni2+-agarose according to the manufacturer’s protocol (Qiagen). Purified proteins were desalted and concentrated by centrifugation through a Centricon concentrator with an exclusion limit of 30,000 Da (Amicon). After the addition of 20% (v/v) glycerol, the purified proteins were frozen in liquid nitrogen and stored in aliquots at −80 °C at a protein concentration of 2–10 µg/µl.

Purification of Gaα, from Bovine Brain—Gaα from bovine brain was prepared as described (17).

Expression of Gaα in Sf9 Cells—Gaα was expressed in Sf9 cells together with Gβγ, using recombinant baculoviruses. Forty-eight hours after infection, cells were harvested, and membranes of baculovirus-infected cells were prepared as described (16). Membranes were directly assayed for [35S]GTPyS binding in comparison to control membranes expressing Gβγ alone, since Gaα was not stable during further purification.

Preparation of HEK-293 Membranes—Membranes of HEK-293 cells transfected with the cDNA encoding the human CaSR were prepared at 4 °C by sucrose density gradient centrifugation as described (18). The membrane pellet was treated with 5 µL urea, washed, and stored at a protein concentration of 0.1–0.5 µg/µl at −80 °C.

Determination of [35S]GTPyS Binding to Ga Subunits—Basal

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‡ To whom correspondence should be addressed: Institut für Pharmakologie und Toxikologie, Versbacher Strasse 9, 97078 Würzburg, Germany. Tel: 49-(0)931-201-3982; Fax: 49-(0)931-201-3539; E-mail: tophi29@rzbox.uni-wuerzburg.de.

¶ The abbreviations used are: PTH, parathormone; CaSR, calcium-sensing receptor; [Mg2+]i, extracellular free Mg2+ concentration; [Mg2+]ex, extracellular free Mg2+ concentration; GTPγS, guanosine 5′-3′,O′-thiotriphosphate.
Block of PTH Secretion under Magnesium Deficiency

In patients with severe hypomagnesemia, PTH secretion is blocked (8–10). To analyze whether this inhibition of PTH secretion is not seen before the Mg2+ concentration tested. Then 10 mM LiCl was added. After 20 min at 37 °C, cellular inositol phosphates were extracted and determined.

Determination of Cellular Inositol Phosphate Levels—Inositol phosphate levels of dispersed parathyroid or of HEK-293 cells were determined as described (19) with minor modifications. Before the experiment, cells were washed with buffer (138 mM NaCl, 0.5 mM CaCl2, 5 mM KCl, 20 mM Na+-HEPES, pH 7.4) containing the indicated concentration of MgCl2 and stored in the same buffer for 15 min to equilibrate the cells with the Mg2+ concentration tested. Then 10 mM LiCl was added. After 20 min at 37 °C, cellular inositol phosphates were extracted and determined.

FIG. 1. Effect of magnesium on PTH release from dispersed parathyroid cells. A and B, dispersed parathyroid cells (1 × 106) were incubated for 10 min in incubation buffer with the indicated concentration of Mg2+ and Ca2+, and PTH release was determined by radioimmunoassay. Basal PTH release at 0.5 mM Ca2+ and 1 mM Mg2+ was 4.9 ± 1.5 ng/106 cells/10 min. C, inhibition of basal cAMP-levels (100%) in dispersed parathyroid cells incubated for 10 min in buffer with 0.5 mM Ca2+ and the indicated concentration of Mg2+. D, inositol phosphate levels of dispersed parathyroid cells labeled with myo[3H]inositol for 12 h. Cells were incubated in buffer with 0.5 mM Ca2+ and the indicated concentrations of Mg2+ for 20 min at 37 °C.

Maximum stimulation (100%) was determined with 5 mM CaCl2. Results are the means ± S.D. (n = 4).

RESULTS

Effect of Magnesium Deficiency on PTH Release from Human Parathyroid Cells—In patients with severe hypomagnesemia, PTH secretion is blocked (8–10). To analyze whether this in vivo paradox is related to the release of PTH from parathyroid cells, we determined the effect of low magnesium on PTH secretion in vitro on dispersed parathyroid cells. As a control for calcium-dependent stimulus-secretion coupling of the parathyroid tissue from patients with hyperparathyroidism, stimulation of the CaSR by calcium was measured. Calcium as a CaSR agonist blocked the release of PTH with an EC50 of 1.5 ± 0.2 mM (n = 4) when the Mg2+ concentration was 1 mM (Fig. 1A). This EC50 value is in close correlation with the set-point values reported previously for human adenomas or primary hyperplasias (23, 24). Severe magnesium deficiency was simulated by decreasing the extracellular Mg2+ to 0.1 mM. When Mg2+ was decreased, PTH secretion was blocked independently of the extracellular Ca2+ concentration (Fig. 1A). These findings resemble the in vivo situation in patients; magnesium deficiency blocks PTH release, leading to concomitant hypocalcemia (8–10), and calcium replenishment cannot overcome this inhibition of PTH secretion (9). The IC50 value of magnesium for the inhibition of PTH release was 0.25 ± 0.04 mM, and inhibition of PTH release is not seen below the Mg2+ concentration falls below 0.5 mM (Fig. 1B). This finding correlates again with in vivo data demonstrating that the magnesium levels in patients that induce PTH secretion block are generally very low, with concentrations varying between 0.4 and 1.0 mM (8–10).
Generation of cAMP was inhibited to about 80–85% that of the maximum inhibition observed after stimulation with the CaSR agonist calcium (Fig. 1C and not shown), and basal inositol phosphates were increased to 30–35% that of the maximum stimulation by magnesium or calcium (Fig. 1D). The IC₅₀ values of the magnesium deficiency-dependent signal enhancement were similar as for the PTH secretion, i.e. 0.19 ± 0.03 mM for the cAMP pathway and 0.2 ± 0.02 mM for the inositol phosphate production (Fig. 1, C and D). These findings demonstrate that magnesium deficiency affects similar signaling pathways as those activated by the CaSR.

**Desensitization of CaSR-mediated Signaling Affects PTH Release under Magnesium Deficiency**—Is the CaSR necessary for mediating the effect of magnesium deficiency on PTH release? To address this question, we desensitized the CaSR on parathyroid cells by prolonged calcium stimulation. Desensitization of the CaSR was visible by a substantially decreased response to the agonist magnesium (6 mM) and calcium (2 mM), i.e. after desensitization, magnesium and calcium inhibited PTH release by only ~10% (Fig. 2, lower panel) compared with a ~60% inhibition of PTH release in nondesensitized control cells (Fig. 2, upper panel). Interestingly, the effect of magnesium deficiency on PTH release was suppressed similar to the response to the CaSR agonists (Fig. 2, lower panel). This finding strongly suggests that active cell surface-localized CaSRs are a prerequisite for the effect of magnesium deficiency on PTH release.

**Effects of Low Magnesium on Inositol Phosphate Generation Mediated by Basal Activation of Recombinantly Expressing Human CaSR**—Since the previous experiments suggested that the site of magnesium action lies within the axis CaSR-G-protein-effector, we further analyzed the mechanism of the magnesium paradox in a transfected cell system. Direct effects of magnesium on the signaling of the CaSR were analyzed compared with control cells without this receptor. Basal inositol phosphate levels of HEK-293 cells expressing the CaSR increased up to 2.8-fold when the Mg²⁺ concentration in the buffer was decreased from 1 to 0.1 mM, whereas mock-transfected cells without CaSR expression did not show any significant increase in basal inositol phosphate levels under these conditions (Fig. 3A). The IC₅₀ value of magnesium was 0.18 ± 0.02 mM. The CaSR was responsible for the increase in basal inositol phosphate levels under magnesium deficiency, since basal inositol phosphate levels increased with increasing CaSR expression levels (Fig. 3B). Thus, the effect of magnesium deficiency on basal activation of recombinantly expressed human CaSR paralleled the effects of magnesium deficiency on parathyroid cell signaling.

**CaSR-mediated Inhibition of Adenylyl Cyclase**—CaSR-mediated inhibition of adenylyl cyclase activity was determined. In HEK-293 cells, forskolin-stimulated adenylyl cyclase activity was inhibited by the CaSR. Maximal inhibition was obtained with 5 mM CaCl₂ in buffer with 0.5 mM or 0.1 mM MgCl₂ (not shown). Low magnesium alone resulted in partial inhibition (Fig. 3C). The CaSR-mediated inhibition of cAMP levels at 0.1 mM MgCl₂ was 64 ± 8% that of the maximum inhibition by 5 mM CaCl₂ (not shown). The IC₅₀ value of magnesium was 0.25 ± 0.05 mM (Fig. 3C). In contrast, the forskolin-stimulated cAMP levels of mock-transfected control cells were barely affected by a decrease in magnesium (Fig. 3C). Together these findings demonstrate that the Gₐ- and Gₛ-mediated pathways triggered by constitutive activity of the CaSR were affected by magnesium deficiency in HEK-293 cells similarly as in dispersed parathyroid cells (cf. Fig. 1).

**Effect of Magnesium on the Activation of a CaSR Mutant with Altered Ion Binding Properties**—Magnesium is an agonist of the CaSR. Therefore we asked whether the extracellular magnesium binding site(s) of the CaSR is (are) involved in mediating the signal enhancement under magnesium deficiency. Different CaSR receptor mutants were expressed (Fig.
stimulation of the wild-type CaSR by 5 mM CaCl₂. Results are the
and the mutated CaSR F128L (Fig. 4

% of maximum,

the EC₅₀ value for the activation of CaSR F128L by magnesium

B

was 20–25% that of the signal of the wild-type receptor at 0.5 mM and at 0.1 mM Mg²⁺ (Fig. 4B, panel III). By contrast, the IC₅₀ value of the magnesium decrease-dependent signal enhancement was not altered. Thus, a decrease in the basal activity of the CaSR is accompanied by a decrease in the absolute extent of the signal enhancement under magnesium deficiency but no change in the IC₅₀ value of magnesium. Together these findings confirm that magnesium deficiency enhances signaling mediated by the basal activity of the CaSR.

Comparison of the Signals Generated by the Basal Activity of the Rat and the Human CaSR—Is the basal activity of the CaSR related to the magnesium paradox of blunted PTH secretion in vivo? Since the paradox of blunted PTH secretion under severe magnesium deficiency has been observed in patients but not in rats (11, 12), we compared the basal activity of the rat and the human CaSR. With 0.5 mM Mg²⁺, the increase in basal inositol phosphate levels of rat CaSR-expressing cells was only 15–20% that of the increase of cells expressing the human CaSR (Fig. 4B, panels V and VI). Interestingly, a decrease in the extracellular magnesium also led to an enhancement of the signaling mediated by basal rat CaSR activity, with an IC₅₀ value for magnesium similar to that of the human CaSR (Fig. 4B, panel V). However, the absolute extent of the signal enhancement of the rat CaSR at 0.1 mM Mg²⁺ was again only 15–20% that of the human CaSR (Fig. 4B, panel V). Equally effective levels of the human and the rat CaSR were expressed in these experiments, as determined by maximum stimulation with 10 mM Mg²⁺ (Fig. 4B, panel VII) and by similar concentration response relationships with EC₅₀ values of 2.8 ± 0.3 and 5.6 ± 1 mM for calcium (not shown) and magnesium (Fig. 4B), respectively. Together these experiments demonstrate that differences in PTH secretion under magnesium deficiency reported for patients and rats correlate with differences in the basal activity of the respective CaSRS and with differences in the absolute extent of the signal enhancement under magnesium deficiency. The findings that the IC₅₀ values of magnesium for the signal enhancement were similar between the human and the rat CaSR and between different CaSR mutants with different basal activity or with different affinity for extracellular magnesium binding suggest a common magnesium binding site different from the extracellular ion binding site of the CaSR.

CaSR-mediated Activation of G-proteins—Since the extracellular magnesium binding site of the CaSR did not seem to be involved in the magnesium paradox, we asked whether magnesium acted on the intracellular side of the CaSR, at the CaSR-G-protein interface. To address this question, CaSR-mediated activation of G-proteins was determined by the receptor-stimulated enhancement of [³⁵S]GTPγS binding to recombinant Gα₁₆-protein. A decrease of the Mg²⁺ concentration from 1 to 0.1 mM or 0.01 mM increased the rate of CaSR-mediated binding of [³⁵S]GTPγS nearly 2-fold (Table I and Fig. 5A). The IC₅₀ value for the magnesium-dependent inhibition of CaSR-stimulated GTPγS binding was 0.18 ± 0.04 mM (Table I). Thus, magnesium suppresses the CaSR-mediated G-protein activation and the CaSR-stimulated activation of Gα₁₆ and
... CaSR-stimulated \( [35S] \)GTP\(^{-}\)S binding is given, i.e. the difference between the \( [35S] \)GTP\(^{-}\)S bound to CaSR-transfected cell membranes and control membranes. Results are the means \( \pm \) S.E. (n = 6).

**FIG. 5. CaSR-stimulated \([35S]\)GTP\(^{-}\)S binding.** Membranes of HEK-293 cells transfected with the CaSR-cDNA were prepared and reconstituted with wild-type \( G_{\alpha} \) (A) or mutant \( G_{\alpha129NC} \) (B). CaSR-stimulated GTP\(^{-}\)S binding was determined at \( t = 5 \) min in the absence of Ca\(^{2+} \) in buffer containing the indicated concentrations of MgCl\(_2\). The CaSR-stimulated increase in \( [35S] \)GTP\(^{-}\)S binding is given, i.e. the difference between the \( [35S] \)GTP\(^{-}\)S bound to CaSR-transfected cell membranes and control membranes. Results are the means \( \pm \) S.E. (n = 6).

**TABLE I**

| \( G_{\alpha} \)  | \( IC_{50} \) [Mg\(^{2+}\)] (\( mM \)) | \( t_{1/2} \) (1 mM Mg\(^{2+}\)) | \( t_{1/2} \) (0.01 mM Mg\(^{2+}\)) |
|------------------|---------------------------------|---------------------------------|---------------------------------|
| Wild type        | 0.18 \( \pm \) 0.04             | 9.1 \( \pm \) 0.5              | 4.9 \( \pm \) 0.4              |
| R209C            | \( >2 \)                        | 4.8 \( \pm \) 0.3              | 4.5 \( \pm \) 0.4              |

**TABLE II**

| \( G_{\alpha} \) subunit | \( IC_{50} \) (mM) | \( I_{\max } \) (%) |
|---------------------------|-------------------|---------------------|
| Wild type                 | 0.18              | 80                  |
| \( G_{\alpha129NC} \)     | 2.8               | No inhibition       |
| \( G_{\alpha} \) (bovine brain) | 0.13 \( \pm \) 0.04 | 57 \( \pm \) 5      |
| \( G_{\alpha} \) (SF9)    | 0.16 \( \pm \) 0.07 | 68 \( \pm \) 7      |

**Inhibition of \( G_{\alpha} \), and the Release of PTH from Parathyroid Cells**—To further analyze whether \( G_{\alpha} \) proteins are involved in mediating the magnesium paradox of blunted PTH secretion, we determined the effect of \( G_{\alpha129NC} \)-protein inhibition on parathyroid cells (Fig. 7). Pertussis toxin treatment abolished the effect of high magnesium (6 mM) in suppressing PTH release (Fig. 7, B versus A). Pertussis toxin also abolished the effect of low magnesium (0.1 mM) on PTH release (Fig. 7B). These findings demonstrate that \( G_{\alpha129NC} \) proteins are essentially involved in mediating the magnesium paradox of PTH release.

**Pertussis Toxin Treatment Reveals Magnesium Effects of \( G_{\alpha} \)-Mediated Signaling**—The magnesium binding site on the \( G_{\alpha} \) subunit that seems responsible for the enhancement of CaSR-mediated signaling under magnesium deficiency is conserved between various \( G_{\alpha} \) subunits (26). In accordance with this fact, GTP\(^{-}\)S binding to various \( G_{\alpha} \) subunits was inhibited by magnesium with similar concentration response relationships (Table II). However, in parathyroid cells, magnesium deficiency selectively enhanced CaSR-mediated inhibitory signaling, whereas the stimulation of PTH secretion by isoprotrenol was not enhanced under magnesium deficiency (Fig. 7C). Since \( G_{\alpha129NC} \) proteins are the most abundant \( G_{\alpha} \) subunits in the cell, we asked whether inhibition of \( G_{\alpha129NC} \) proteins could reveal effects of low magnesium on \( G_{\alpha} \)-mediated signaling. And indeed, when \( G_{\alpha129NC} \) proteins were blocked by pertussis toxin,
isoproterenol-stimulated PTH release was slightly but significantly enhanced under 0.1 mM Mg$_{2+}$ compared with 1 mM or 6 mM Mg$_{2+}$ (Fig. 7D). Thus, in parathyroid cells the enhancement of G$_{q,i}$-mediated signaling under magnesium deficiency is overcome by the increased activity of G$_{a_i}$ proteins.

**Effect of Magnesium Deficiency on Signals Generated by Activation of Different G$_{q,i}$-coupled Receptors**—If the magnesium binding site responsible for the enhancement of CaSR-mediated signaling under magnesium deficiency is localized on the Ga subunit, signals mediated by the basal activity of receptors other than the CaSR should be similarly enhanced under magnesium deficiency. To analyze whether magnesium deficiency enhances the basal activity of G$_{q,i}$-coupled receptors in intact cells, we determined the effect of magnesium suppression on G$_{q,i}$ subunits in intact cells.

**Effect of Magnesium Deficiency on Signals Generated by Activation of Different G$_{q,i}$-coupled Receptors:** The basal or agonist-stimulated increase in inositol phosphate levels of HEK-293 cells expressing the angiotensin II AT$_1$ or the bradykinin B$_2$ receptor was determined in buffer supplemented with 0.5 mM CaCl$_2$ and 1.0 mM Mg$_{2+}$ (A) or 0.1 mM Mg$_{2+}$ (B and C). In panel C, the cells received 1.0 mM Mg$_{2+}$ at the time point indicated by an arrow.

**TABLE III**

| Receptor | Constitutive activity (1 mM Mg$_{2+}$) | Constitutive activity (0.1 mM Mg$_{2+}$) | EC$_{50}$ (1 mM Mg$_{2+}$) | EC$_{50}$ (0.1 mM Mg$_{2+}$) |
|----------|----------------------------------|----------------------------------|----------------|----------------|
| AT$_1$   | 4 ± 0.5                          | 11 ± 1                           | 5.1 ± 0.3      | 1.7 ± 0.2      |
| B$_2$    | 3 ± 0.1                          | 9 ± 0.5                          | 1.9 ± 0.2      | 0.9 ± 0.3      |

**DISCUSSION**

Cloning of a CaSR-cDNA (3) and the identification of several CaSR mutants from patients with hypo- and hyperparathyroidism (1, 5–7) clearly established the mechanistic relationship between serum calcium and magnesium and PTH secretion. Despite this progress, the paradox of blunted PTH secretion under magnesium deficiency is still an open question. Previous work suggested that the target of magnesium action was intracellular (9). Since PTH synthesis was not affected by magnesium deficiency (32) and PTH levels rose within minutes after parenteral magnesium replacement (33), the defect was pinpointed to the level of PTH secretion.

And indeed, we found that in vitro PTH secretion from parathyroid cells was blocked under low magnesium similarly to that reported in patients. Since the effect of low magnesium was dependent on the axis CaSR-G-protein, i.e. desensitization of the CaSR or inhibition of G$_{a_i}$-proteins by pertussis toxin also suppressed the effect of magnesium deficiency on PTH secretion, we reconstructed this system of CaSR-G-protein in vitro in a cell line other than the parathyroid cell. The characteristics of the magnesium deficiency-mediated signal enhancement of basal CaSR activity were similar in parathyroid and in CaSR-expressing HEK-293 cells. The magnesium binding site was localized on the Ga subunit; the presence of a G$_{a_i}$ mutant with decreased affinity for magnesium abolished the effect of magnesium on CaSR-mediated G-protein activation, whereas on CaSR mutants with increased or decreased affinity for magnesium, the IC$_{50}$ value of the magnesium deficiency-mediated signal enhancement did not change. Together these findings let us conclude that the enhancement of G-protein activation under magnesium deficiency enhances signals mediated by constitutive CaSR activity. The absolute extent of this enhancement is greater for the human CaSR than for the rat CaSR because the human receptor has a higher constitutive activity. This difference between the rat and the human CaSR leads to blunted PTH secretion under severe hypomagnesemia in patients but not in rats. Although our data suggest that the magnesium binding site responsible for this effect is located on the Ga, we cannot rule out the possibility that the human CaSR differs from the rat CaSR by an additional yet unknown magnesium binding site acting in concert with the magnesium binding site on the Ga subunit.

Magnesium-dependent inhibition of guanine nucleotide exchange by stabilizing guanine nucleotide binding is a common feature of small GTP-binding proteins (29). Our finding that magnesium suppresses the release of GDP on Ga subunits parallels the role of magnesium on c-Ras mechanistically (34).
In contrast to GDP-bound Ras, which has a more than 10-fold higher affinity for magnesium than Ga (34), the affinity of magnesium for GDP-bound Ga subunits is in the submillimolar range. Therefore pathophysiological alterations in the magnesium homeostasis will affect selectively the activity of heterotrimeric G-proteins. Guanine nucleotide exchange of Ga, Go, Ga, and Ga is similarly affected by magnesium. Therefore the detected mechanism of magnesium stabilizing the GDP-bound Ga may define a novel function of magnesium on heterotrimeric G-proteins.

Why does magnesium deficiency in vivo selectively enhance signaling mediated by the CaSR although low magnesium enhances guanine nucleotide exchange of many different G-proteins. Therefore the enhancement enhances guanine nucleotide exchange of many different G-proteins, which is true for parathyroid cells, where magnesium effects on Ga-mediated signaling has also been found for Ga-dependent secretion processes other than PTH (35). Concerning Ga-mediated signaling, minor or absent effects of magnesium deficiency on Ga-mediated signaling is also seen for GDP-bound Ras, which has a more than 10-fold range. Therefore pathophysiological alterations in the magnesium range. Therefore pathophysiological alterations in the magnesium

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Ursula Quitterer, Michaela Hoffmann, Marc Freichel and Martin J. Lohse

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