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Ga^{3+} INHIBITS PARATHYROID HORMONE RELEASE WITHOUT INTERACTING WITH THE Ca^{2+} RECEPTOR OF THE PARATHYROID CELL

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Gallium nitrate is an antihypercalcemic agent with established actions on bone. The effects of $Ga(NO_3)_3$ on parathyroid hormone (PTH) release, cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) and cAMP production of enzymatically dispersed parathyroid cells from bovine as well as normal and pathological human parathyroid glands have now been studied. Ga^{3+} at 200 μM inhibited PTH release whereas 600 μM NO_3^- had no effect. The inhibition was additive to that obtained by elevating extracellular Ca^{2+}. Unlike Ca^{2+}, Ga^{3+} failed to increase $[Ca^{2+}]_i$ or reduce cAMP formation. The results indicate that Ga^{3+} inhibits PTH release by a mechanism other than activation of the cation receptor of the parathyroid cells. This mechanism may contribute also to inhibition by other cations.

Ca^{2+} is the primary physiological regulator of parathyroid hormone (PTH) release. Elevation of extracellular Ca^{2+} results in biphasic increase of the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$; 1-3), with causal relationship to inhibition of PTH release (4). A decrease of the cAMP content of the parathyroid cells may contribute to the Ca^{2+} inhibition of secretion (5). The changes of $[Ca^{2+}]_i$ (3,6) and probably also cAMP (7) are elicited by Ca^{2+} binding to an external cation receptor. Besides Ca^{2+} a number of di- (3,7,8), tri- (6,7,9) and polyvalent (10,11) cations induce the characteristic changes of $[Ca^{2+}]_i$, cAMP and PTH release.

Gallium nitrate has been utilized for the treatment of hypercalcemia due to malignant tumors (12,13) including parathyroid carcinoma (14) and Paget's disease of the bone

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(15,16). The hypocalcemic effect has been attributed to inhibited resorption and enhanced formation of bone (12,17,18). Although Ga^{3+} is a trivalent cation, no influences have previously been observed on the *in vitro* release of PTH (19).

The present study indicates that Ga^{3+} inhibits PTH release by a mechanism dissociated from rise of $[Ca^{2+}]_i$ and decrease of cAMP. It is proposed that trivalent cations may inhibit secretion also by binding to other sites in the plasma membrane than the Ca^{2+} receptor.

MATERIALS AND METHODS

Bovine parathyroid glands were collected from adult cattle 15-20 minutes after slaughter. Pathological human tissue was obtained from adenomatous and hyperplastic glands during surgery of hypercalcemic patients with adenomatous and uremic HPT, respectively. Biopsies of normal human glands were acquired from euparathyroid subjects during surgery for atoxic goiter. Cell suspensions were prepared as previously described (20).

The cells were loaded for 30 min at 37° C with 0.5-1.0 μM of acetoxymethyl ester of the Ca^{2+} indicator fura-2 (Calbiochem, La Jolla, CA), rinsed and incubated further for 10-20 min in absence of indicator. These incubations and the $[Ca^{2+}]_i$ measurements were performed in a medium containing 25 mM Hepes (pH 7.4), 3 mM glucose, 0.1 % bovine serum albumin, 125 mM Na^+, 5.9 mM K^+, 0.5 mM Mg^{2+} and 0.5 mM Ca^{2+} with Cl^- as the sole anion. $[Ca^{2+}]_i$ of individual cells was analyzed in a microfluorometric system with excitation at 340 and 380 nm as reported elsewhere (20). The 340/380 nm fluorescence excitation ratio was used to calculate $[Ca^{2+}]_i$ as previously described (21) using a K_d of 231 nM (20).

PTH release was determined by duplicate incubations for 30 min at 37° C of 0.5-1.0 x 10^6 cells in 500 μl of the buffer used for $[Ca^{2+}]_i$ measurements, but supplemented with 0.1 % human serum albumin. After centrifugation at 5000 x g for 1 min, PTH in the supernatant was assayed radioimmunologically using a sheep antiserum (Giselle) raised against human PTH, ^{125}I-labelled 44-68(Tyr) human PTH as tracer and human PTH (1-84) as standard. The assay mainly detects the mid-C region of human and bovine PTH (22).

Efflux of cAMP was determined after duplicate incubations of 1.0 x 10^6 cells for 90 minutes at 37° C in the buffer used for PTH determinations but containing 0.5 mM of the phosphodiesterase inhibitor IBMX (Sigma St. Louis, MO). After centrifugation at 5000 x g for 1 minute, the supernatant was analyzed with RIANEN cAMP [^{125}I]RIA kit (Du Pont, Billerica, MA). The cAMP release of 10^6 control cells averaged 21 ± 5.5 pmol/ml after incubation in 0.5 mM Ca^{2+} during 90 min (n=7), and the release of cAMP has been shown to reflect the cAMP content of parathyroid cells (5).

Separate control experiments ascertained that gallium nitrate per se did not interfere with the cAMP or PTH RIAs. Analyses of cells from different patients or batches of bovine glands were considered as independent experiments. Statistical significances were calculated using Student's paired t-test. Results are presented as mean values ± SEM.

RESULTS

The effects of 2, 20 and 200 μM $Ga(NO_3)_3$ on PTH release from pathological human parathyroid cells were tested in the presence of 0.5 mM Ca^{2+}. Secretion was significantly inhibited only at the highest concentration and this action apparently depended on Ga^{3+} since 600 μM $NaNO_3$ had no effect (not shown). Fig. 1 shows that Ga^{3+}-induced reduction

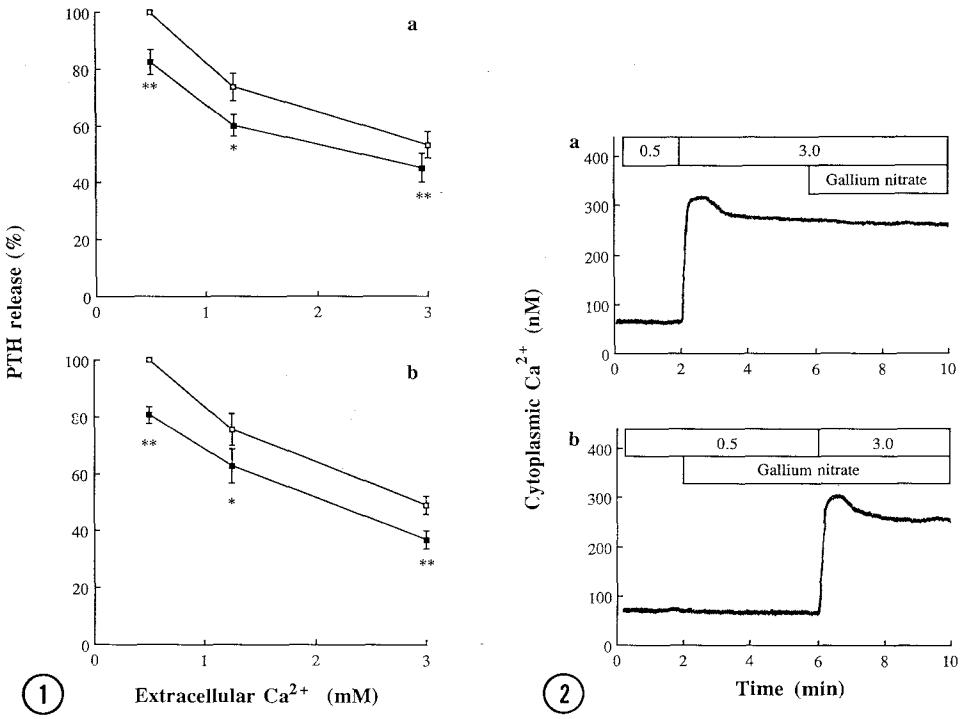

Fig. 1. Effect of gallium on Ca^{2+} inhibition of PTH release from dispersed parathyroid cells of normal bovine glands (a) and of adenomatous and hyperplastic human glands (b). Incubations in the absence (open symbols) and presence (filled symbols) of 200 μM $Ga(NO_3)_3$ are shown. The secretion in medium containing 0.5 mM Ca^{2+} but lacking gallium was set to 100 %. Results are presented as mean values ± SEM for 7-10 (bovine) and 4 (human) experiments. *p<0.05, **p<0.01

Fig. 2. Effects of increasing Ca^{2+} from 0.5 to 3.0 mM and adding 200 μM $Ga(NO_3)_3$ on $[Ca^{2+}]_i$ of normal human parathyroid cells. $Ga(NO_3)_3$ was either added after (a) or before (b) increase of the Ca^{2+} concentration. Representative experiment of three in each case.

of PTH release from bovine and pathological human parathyroid cells is additive to the inhibition obtained when raising Ca^{2+} in the 0.5-3.0 mM range.

In normal human parathyroid cells elevation of Ca^{2+} from 0.5 to 3.0 mM elicited the characteristic biphasic rise of $[Ca^{2+}]_i$ with an initial peak followed by sustained increase (Fig. 2). Analyses of parathyroid cells from 3 normal subjects (Fig. 2) and 7 cases with hyperparathyroidism (not shown) indicated that 200 μM $Ga(NO_3)_3$ had no effect on $[Ca^{2+}]_i$ either in the presence of 0.5 or 3.0 mM Ca^{2+}.

Elevation of Ca^{2+} in the 0.5 to 3.0 mM range induced a pronounced inhibition of cAMP release from pathological human parathyroid cells (Fig. 3). This action was unaffected in the presence of 200 μM $Ga(NO_3)_3$.

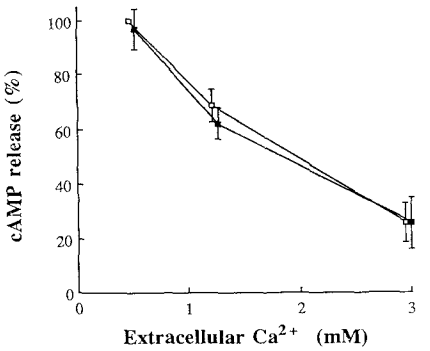

Fig. 3. Release of cAMP from pathological human parathyroid cells in absence (open symbols) and presence (filled symbols) of 200 μM $Ga(NO_3)_3$. The cAMP release in medium containing 0.5 mM Ca^{2+} but lacking gallium was set to 100 %. Results are presented as mean values ± SEM for 7 experiments.

DISCUSSION

In patients with malignancy-associated hypercalcemia unrelated to HPT, treatment with $Ga(NO_3)_3$ seems to increase PTH release as judged from the elevated serum levels (19,23). However, this stimulation may be attributable to the lowering of serum Ca^{2+} obtained with the inhibition of bone resorption. Indeed, gallium administration results in parallel decreases of serum PTH and Ca^{2+} in patients with parathyroid carcinoma (14). In contrast to a previous study analyzing the effect of 100 μM $Ga(NO_3)_3$ on bovine parathyroid cells (19), 200 μM was now found to inhibit PTH release from both bovine and pathological human cells. Unlike the effect of Ca^{2+} and a number of other di- as well as tri- and polyvalent cations, this inhibition was apparently mediated by a mechanism different from activation of the parathyroid cation receptor. Secretion already reduced by rise of Ca^{2+} was consequently further inhibited by Ga^{3+}, which also failed to induce the characteristic elevation of $[Ca^{2+}]_i$ (1-3,10,11) and lowering of cAMP (5,7,10) triggered by the other cationic inhibitors of PTH release.

The inhibitory effect of trivalent lanthanides on secretion is not restricted to the parathyroid cells. In other secretory cells the corresponding action has been attributed to suppression of Ca^{2+} fluxes across the plasma membrane. However, when examining the mode of action of lanthanides, it was discovered that they were equally effective in inhibiting insulin release although the ability to block Ca^{2+} fluxes increased with ionic radius (24). Tm^{3+}, which is among the lanthanides with smallest radius (25), even inhibited secretion without affecting Ca^{2+} uptake. Since Ca^{2+} stabilizes biological membranes in general (26,27), it was proposed that lanthanide binding could induce perturbations of the plasma membrane, which directly inhibit exocytosis. Ga^{3+} has an even smaller ionic radius (0.62 Å; 25) than Tm^{3+} (0.87

Å). Nevertheless it was suggested that the antitumor actions of both Ga^{3+} and La^{3+} (1.15 Å) are mediated by similar structural changes of the plasma membrane (28).

If Ga^{3+} inhibits PTH release by stabilizing the plasma membrane also other trivalent cations should be expected to do so. Since Ga^{3+} is chemically related, absorbed and distributed in a manner similar to Al^{3+} (29), it is pertinent to note that Al^{3+} inhibits PTH release of bovine and porcine parathyroid cells by an unknown mechanism (30,31). Although the more Ca^{2+}-like lanthanides apparently trigger the physiological route for inhibition of PTH secretion, it should not be excluded that a Ga^{3+}-like mechanism is also operating. The involvement of dual independent mechanisms may well explain why the lanthanides inhibit PTH release more profoundly than Ca^{2+} and other divalent cations (7).

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