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A PARATHYROID-RELATED PEPTIDE INDUCES TRANSCALTACHIA (THE RAPID, HORMONAL STIMULATION OF INTESTINAL Ca^{2+} TRANSPORT)

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PTH-related peptide (PTHrP) has been shown to be responsible for the hormonal hypercalcemia of malignancy. Previously, we demonstrated that both 1,25(OH)$_2$-vitamin D_3 and the N-terminal fragment of PTH (1-34) stimulates the rapid transport of Ca^{2+} (transcaltachia) in the perfused chick intestine. Since there is a sequence homology between these two hormones in the n-terminal fragment, in this study we examined the effect of PTHrP(1-40) on stimulation of transcaltachia in the perfused chick duodenum. The results indicate that the maximal stimulation of transcaltachia occurs at 50 pM PTHrP(1-40), and that the dose-response curve is biphasic in nature. Perfusion with 25 pM, 50 pM, 100 pM or 200 pM PTHrP(1-40) for 40 min increases the transport of Ca^{2+} in perfused intestine 1.8-, 3.0-, 2.4- and 1.6- fold, respectively. The response is rapid, occurring within 10 min of introduction of the PTHrP. The Ca^{2+} channel inhibitor nifedipine, which is known to abolish the transcaltachic effect elicited by 1,25(OH)$_2$ vitamin D_3, also inhibited the rapid transport of Ca^{2+} stimulated by PTHrP(1-40). The transcaltachic effect of PTHrP(1-40) may be mediated by a signal transduction pathway in which Ca^{2+} channels are activated.

PTH-related peptide (PTHrP) is elaborated from certain tumors and is thought to play a role in the etiology of the hormonal hypercalcemia of malignancy (1,2). PTHrP shares considerable sequence homology with bovine PTH (bPTH) within the first 13 amino acids, 8 of which are identical to those in PTHrP. Most studies have found PTHrP to have bioactivity closely resembling that of PTH, including the ability to stimulate cAMP formation in bone and kidney (3,4), to enhance the adenylate cyclase activity in membranes from canine and bovine kidney (5,6), to increase the cytosolic calcium in osteoblast-like cells (7) and to stimulate the release of $^{45}Ca^{2+}$ from fetal rat long bones in organ culture (8,9). In addition, PTHrP and PTH have been shown to bind with varying affinities to the same receptor not only in the classical PTH target cells of kidney and bone (10-11), but also in a number of other cells, including dermal fibroblasts (12), embryonal carcinoma cells (13), vascular smooth muscle cells (14) and human keratinocytes (15). In analogy to PTH, the active site of PTHrP for the induction of biological effects is believed to reside within the first 34 amino acids (3,7).

Previous work from this laboratory has demonstrated that 1,25(OH)$_2$$D_3$ stimulates the rapid transport of Ca^{2+} (transcaltachia) from the lumen to the venus effluent in the

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perfused intestine of vitamin D-replete chicks (16). Using the same intestinal perfusion system, b-PTH(1-34) has also shown to stimulate the transcaltachic effect in vitamin D-replete chicks (17). Considering the structural and biological similarities shared by PTHrP and PTH, we studied the effect of PTHrP(1-40) on initiating the transcaltachic response in the perfused chick duodenum. In addition, the involvement of Ca^{2+} channels in the process of transcaltachia initiated by PTHrP was evaluated by using the Ca^{2+} channel antagonist nifedipine. The results indicated that PTHrP(1-40) can induce the rapid transport of Ca^{2+} in perfused chick intestine, and Ca^{2+} channels may be activated in the transcaltachic process.

MATERIALS AND METHODS

Chemicals

$^{45}CaCl_2$ (1 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Nifedipine was from Sigma Chemical CO., St. Louis, MO. The PTHrP peptide or hypercalcemia of malignancy factor (1-40) was obtained from Bachem Inc, Torrance, CA.

Animals

White leghorn cockerels (Lakeview Farms, Lakeview, CA) were obtained on the day of hatch and maintained on a vitamin D-supplemented diet (1.0% calcium and 1.0% phosphorus; O. H. Kruse Grain and Milling, Ontario, CA) for 5-6 weeks to prepare normal vitamin D_3-replete chicks. All experiments employing animals were approved by the local UC-Riverside Chancellor's Committee for use of animals in research.

Intestinal Ca^{2+} transport measurements

Measurements of Ca^{2+} transport were carried out in perfused chick duodena as previously described (16). Normal vitamin D-replete chicks, weighing approximately 500 g were anesthetized with chloropent (0.3ml/100 g), and the duodenal loop was surgically exposed. Three pairs of blood vessels branching off from the celiac artery were ligated before cannulation of the celiac artery itself. The duodenal loop was then excised, and after cannulation of the celiac vein, placed between layers of saline-moistened cheesecloth at 24#C. The arterial perfusion was initiated at the time of cannulation with modified Gey's balanced salt solution (GBSS) containing 0.9 mM $CaCl_2$, at a flow rate of 2ml/min. This bicarbonate-containing buffer was oxygenated with 95% 0_2/ 5% CO_2. An auxiliary pump was used for the introduction of albumin (0.125% wt/v g final concentration) and vehicle (0.004 mM actic acid) or test substances to the vascular perfusate at a rate of 0.25ml/min. The intestinal lumen was then flushed and filled with GBSS containing $^{45}CaCl_2$ (5 μCi/ml) but lacking bicarbonate and glucose. A basal transport rate was established by perfusion with control medium for 20 min after the lumen was filled with $^{45}Ca^{2+}$; the total Ca^{2+} concentration in the lumen of the intestine was 0.9 mM. The tissue was then exposed to PTHrP(1-40) or re-exposed to control medium for an additional 40 min. The vascular perfusate from the celiac vein was collected at 2 min intervals during both the basal and treatment periods. Duplicate 100 μl aliquots were taken for quantitation of the $^{45}Ca^{2+}$ by liquid scintillation spectrometry. Results are expressed as the ratio of the $^{45}Ca^{2+}$ appearing in the 40 min test period divided by the average initial basal transport period.

Statistics

Statistical evaluation of the data was performed by Students' t test for unpaired observations.

RESULTS

The effect of PTHrP(1-40) on the transport of $^{45}Ca^{2+}$ in perfused chick intestine was investigated (Fig. 1A). A 40 min perfusion with 50 pM PTHrP, at which the maximal response was achieved, resulted in a 3-fold increase in the transport of $^{45}Ca^{2+}$ in chick

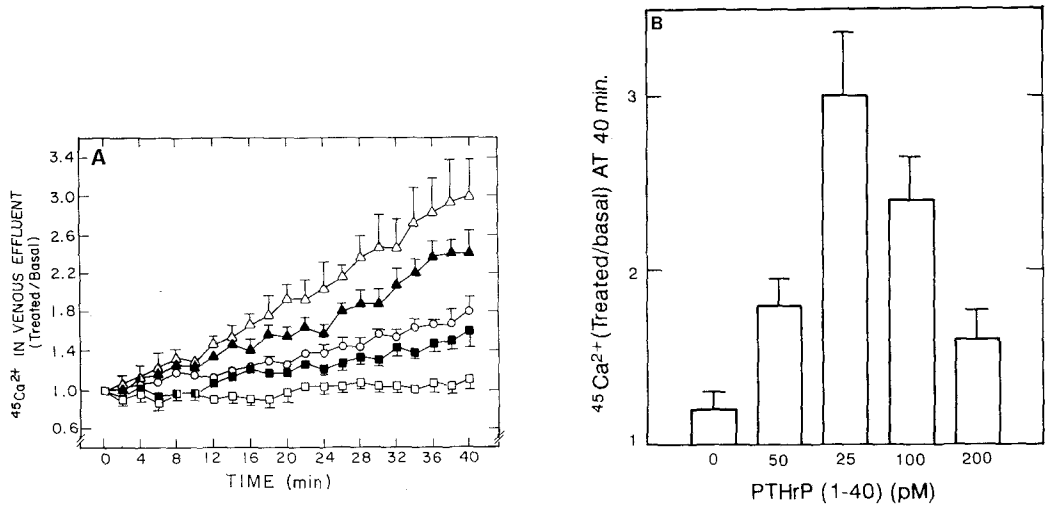

Figure 1A
Effect of PTHrP(1-40) on the appearance of $^{45}Ca^{2+}$ in the venous effluent of perfused duodena from vitamin D-replete chicks. Each duodenum, filled with $^{45}CaCl_2$ (5μCi/ml) in GBSS, was vascularly perfused (25°C) for the first 20 min with control medium (GBSS containing 0.125% BSA and 0.004 mM acetic acid) and then with 25 pM (O), 50 pM (△), 100 pM (▲) and 200 pM (■) PTHrP(1-40) or control medium (□). Values are the mean ± SEM for three duodena within each experimental group.

Figure 1B
Dose response analysis of PTHrP(1-40) on $^{45}Ca^{2+}$ transport in perfused duodena. Experimental conditions were the same as in Fig. 1A. The reported values are the treated/basal ratios observed at 40 min. The values are the mean ± SEM for three duodena within each experimental group.

intestine. The response was rapid, and perfusion with 50 pM PTHrP for 14 min increased the Ca^{2+} transport level 1.5-fold. This effect was virtually identical to that induced by equimolar concentrations of bPTH(1-34) (17). Perfusion with 25 pM, 100 pM and 200 pM PTHrP for 40 min gave a ratio of treated to basal of approximately 1.8, 2.4 and 1.6, respectively. The biphasic nature of the PTHrP dose-response curve can clearly be seen in Fig. 1B.

It has been previously shown that Ca^{2+}-channels are involved in the process of 1,25-dihydroxyvitamin D_3-mediated transcaltachic process (18). To evaluate the possible involvement of Ca^{2+}-channels in PTHrP(1-40) stimulated transcaltachia, nifedipine, the Ca^{2+}-channel blocker, was used in the perfusion experiment. As shown in Fig. 2, the presence of 2 μM nifedipine in the vascular perfusate blocked the stimulatory effect of PTHrP(1-40) on transcaltachia.

DISCUSSION

The results obtained in this study indicate that PTHrP is capable of initiating the rapid transport of Ca^{2+} from the lumen to the venous effluent in the perfused chick intestine. The transcaltachic effect induced by PTHrP(1-40) closely resembles that observed with the 1-34 fragment of bPTH; the magnitude and duration of the response to either of the two peptide agonists is almost identical, and the dose-response relationship of

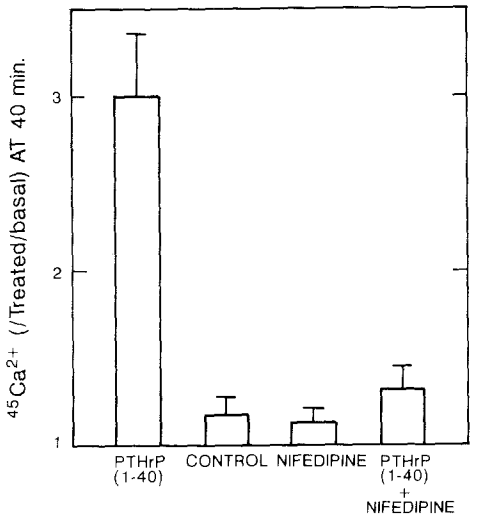

Figure 2
Suppression of PTHrP(1-40)-induced duodenal $^{45}Ca^{2+}$ transport by the calcium-channel blocker nifedipine. Experimental conditions were as described in Fig. 1A. During the treatment phase, duodena were vascularly perfused with 50 pM PTHrP; 0.004 mM acetic acid, 0.004 mM acetic acid + 2 μM nifedipine or 50pM PTHrP + 2 μM nifedipine. Values are the mean $\pm$ SEM of three duodena for each treatment.

PTHrP(1-40) is equivalent to that of bPTH(1-34). These findings are consistent with observations that the N-terminal fragments of bPTH and PTHrP have a high sequence homology and that they bind to the same receptor on the membrane surface in different cell lines to produce the biological effects (4,15).

The mechanism of $1,25(OH)_2D_3$-mediated transcaltachia has been extensively studied in our laboratory. It has been proposed that $1,25(OH)_2D_3$ increases intestinal Ca^{2+} transport through a vesicular pathway (19). In such a model, the absorbed Ca^{2+} is internalized in endocytic vesicles, followed by fusion of the vesicles with lysosomes and movement of the lysosomes to the basal lateral membrane, where exocytosis of the contents completes the transport process. The stimulatory response is observed only when the secosteroid is introduced into the basal lateral membrane surface but not when $1,25(OH)_2D_3$ is introduced to the brush border membrane surface (16). This sidedness may indicate the presence of a putative receptor for $1,25(OH)_2D_3$ at the basal lateral membrane. There is evidence indicating that $1,25(OH)_2D_3$ is involved in the activation of basal lateral membrane voltage-dependent Ca^{2+} channels as an early event in the transcaltachic response, since the stimulatory response of $1,25(OH)_2D_3$ can be mimicked by the Ca^{2+}-channel agonist Bay K-8644 and abolished by the antagonist nifedipine (18,21). Additionally, the second messange system such as protein kinase C and cAMP-dependent protein kinase has been implicated as a mediator of the rapid effects of $1,25(OH)_2D_3$; TPA and forskolin were found to stimulate Ca^{2+} transport in the perfused intestine of normal chicks to the same extent as physiological concentration of $1,25(OH)_2D_3$. The potentiating effects of both agents could be suppressed by specific protein kinase inhibitors (20).

It is likely that the binding of $1,25(OH)_2D_3$ with a putative membrane receptor could activate a second messenger system, such as protein kinase C and/or cAMP-dependent protein kinase. Then the phosphorylation-dependent activation of voltage-gated Ca^{2+} channels might cause the transient increase in intracellular Ca^{2+} concentration, which may trigger the vesicular pathway for intestinal Ca^{2+} transport.

Similarly, we postulate the existence of receptors for both PTH and PTHrP on the basal lateral membrane of the intestinal epithelial cell. Accordingly, we envision that these peptide receptors, and upon ligand binding, initiate the transcaltachic response. Studies are in progress to examine the details of the PTHrP stimulated transcaltachia.

The results of this study suggest that one contributing mechanism for the hormonal hypercalcemia of malignancy may be inappropriate stimulation of intestinal Ca^{2+} transport by PTHrP.

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