Prolactin-stimulated transepithelial calcium transport in duodenum and Caco-2 monolayer are mediated by the phosphoinositide 3-kinase pathway

Walailuk Jantarajit,1* Narongrit Thongon,1* Jantarima Pandaranandaka,2,3 Jarinthorn Teerapornpuntakit,1 Nateeep Krishnamra,1,2 and Naraftphol Charoenphandhu1,2

1Department of Physiology and 2Consortium for Calcium and Bone Research, Faculty of Science, Mahidol University, Bangkok; and 3Faculty of Medicine, Thammasat University, Pathumthani, Thailand

Submitted 3 March 2007; accepted in final form 18 April 2007

Jantarajit W, Thongon N, Pandaranandaka J, Teerapornpuntakit J, Krishnamra N, Charoenphandhu N. Prolactin-stimulated transepithelial calcium transport in duodenum and Caco-2 monolayer are mediated by the phosphoinositide 3-kinase pathway. Am J Physiol Endocrinol Metab 293: E372–E384, 2007. First published May 8, 2007; doi:10.1152/ajpendo.00142.2007.—Prolactin (PRL) has been shown to stimulate intestinal calcium absorption but the mechanism was still unknown. This study aimed to investigate the mechanism and signaling pathway by which PRL enhanced calcium transport in the rat duodenum and Caco-2 monolayer. Both epithelia strongly expressed mRNAs and proteins of PRL receptors. Ussing chamber technique showed that the duodenal active calcium fluxes were increased by PRL in a dose-response manner with the maximal effective dose of 800 ng/ml. This response diminished after exposure to LY-294002, a phosphoinositide 3-kinase (PI3K) inhibitor. Caco-2 monolayer gave similar response to PRL with the maximal effective dose of 600 ng/ml. By nullifying the transepithelial potential difference, we showed that the voltage-dependent paracellular calcium transport did not contribute to the PRL-enhanced flux in Caco-2 monolayer. In contrast, the calcium gradient-dependent paracellular transport and calcium permeability were increased by PRL. Effects of PRL on Caco-2 monolayer were abolished by PI3K inhibitors (LY-294002 and wortmannin), but not by inhibitors of MEK (U-0126) or JAK2 (AG-490). To investigate whether the PRL-enhanced paracellular transport was linked to changes in the epithelial charge selectivity, the permeability ratio of sodium and chloride (PNa/Pc) was determined. We found that PRL elevated the PNa/Pc in both epithelia, and the effects were blocked by PI3K inhibitors. In conclusion, PRL directly and rapidly stimulated the active and passive calcium transport in the rat duodenum and Caco-2 monolayer via the nongenomic PI3K-signaling pathway. This PRL-enhanced paracellular calcium transport could have resulted from altered charge selectivity.

charge selectivity; dilution potential; paracellular transport; prolactin receptor; tight junction; transepithelial transport

AS ONE OF THE CALCIUM-REGULATING HORMONES during pregnancy and lactation, prolactin (PRL) has been shown to stimulate intestinal calcium absorption (38), thereby protecting against development of negative calcium balance during these reproductive periods. Further investigations in nonmated female rats also revealed stimulatory actions of PRL on intestinal calcium absorption (11, 54), especially in the duodenum which was the most efficient site for calcium transport (21, 32). Although the presence of PRL receptor (PRLR) proteins in duodenal enterocytes of rats was controversial, expression of rat PRLR (rPRLR) transcripts in the duodenal mucosa, demonstrated by

* W. I. and N. T. contributed equally to this study.
Address for reprint requests and other correspondence: N. Charoenphandhu, Dept. of Physiology, Faculty of Science, Mahidol Univ., Rama VI Road, Bangkok 10400, Thailand (e-mail: narat@naratsys.com).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
naling pathways of PRL in several tissues, including mammary epithelia (1, 47, 51). Since increased calcium uptake into the duodenal absorptive cells was seen in 8 min and enhanced calcium absorption occurred within 60 min after PRL exposure (12), it was likely that PRL stimulated the intestinal calcium absorption by a nongenomic pathway.

In the present study, we determined the mechanism and signaling pathway of PRL in the duodenal tissue of rat and in human colorectal adenocarcinoma cell line (Caco-2). Despite deriving from the colon, confluent Caco-2 monolayer has been widely used in the studies of calcium and drug absorption because it has functional similarity to the small intestine, including the presence of the brush border, expression of sucrase-isomaltase enzymes, expression of the transcellular calcium transporters, and responses to vitamin D (16, 20, 63, 64). Although the presence of human PRLR (hPRLR) in Caco-2 cells had not been reported, Otte et al. (44) demonstrated mitogenic effects of PRL on Caco-2 cells, suggesting the presence of functional hPRLRs in this cell line. We thus planned to study PRL-enhanced calcium transport in Caco-2 monolayer and, if found suitable, to use it as a cellular model for further investigations of the molecular signaling of PRL.

The objectives of this study were 1) to demonstrate the expression of PRLR mRNA and proteins in duodenal epithelial and Caco-2 cells, 2) to show that PRL directly stimulated active and passive calcium transport in duodenum and Caco-2 monolayer, 3) to investigate PRL-induced changes in passive calcium transport and charge-selective property in the intestinal epithelium, and 4) to identify the possible signaling pathway of PRL in duodenum and Caco-2 monolayer.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats, weighing 180–200 g (10 wk old), were obtained from the National Laboratory Animal Center, Salaya, Thailand. They were housed in the laboratory animal husbandry unit for ≥7 days prior to the experiments under 12:12 h dark-light cycle and were fed regular pellets containing 1.0% calcium and 0.9% phosphorus (CP, Bangkok, Thailand) and distilled water ad libitum. The room had a temperature of 20–25°C and humidity of 50–60%.

Cell Culture

Caco-2 cells (ATCC no. HTB-37) were grown in Dulbecco’s modified Eagle’s medium (DMEM; Sigma, St. Louis, MO) supplemented with 15% fetal bovine serum (FBS; Gibco, Grand Island, NY), 1% l-glutamine (GIBCO), 1% nonessential amino acid (Sigma), 100 U/ml penicillin-streptomycin, and 0.25 μg/ml amphotericin B (Sigma). Cells were propagated in a 75-cm² T flask (Corning, Corning, NY) under a humidified atmosphere containing 5% CO₂ at 37°C and subcultured as described in the ATCC protocol. For the calcium flux study, confluent Caco-2 monolayers were prepared by seeding cells (5.0 × 10⁵ cells/cm²) on polyester Snapwell inserts with 12-mm diameter and 0.4-μm pore size (Corning). Culture medium was changed daily after 48 h of seeding. Monolayers were incubated at 37°C for 14 days in a humidified atmosphere containing 5% CO₂. Electrical parameters of the monolayer were consistent with the previous report (10).

Tissue Preparation

A median laparotomy was performed under pentobarbital sodium (50 mg/kg ip; Abbott, North Chicago, IL) anesthesia. Intestinal segments, including duodenum (10 cm), proximal and distal jejunum (10 cm), ileum (8 cm), cecum (4 cm), and proximal and distal colon (8 cm), were removed, rinsed in an ice-cold bathing solution, and cut longitudinally along the radix mesenterii to expose the mucosa. The duodenal segment was then mounted in a modified Ussing chamber with an exposed surface area of 0.69 cm² to measure calcium fluxes, as described previously (13). The tissue was incubated for 20 min in the chamber before the 60-min experiment was carried out. For mRNA and protein expression studies, intestinal epithelial cells were collected by scraping the mucosal surface of intestinal segments with an ice-cold glass slide (13).

Bathing Solution

The bathing solution, continuously gassed with humidified 5% CO₂ in 95% O₂, contained (in mmol/l) 118 NaCl, 4.7 KCl, 1.1 MgCl₂, 1.25 CaCl₂, 23 NaHCO₃, 12 d-glucose, and 2 mannitol (all purchased from Sigma). The solution was maintained at 37°C, pH 7.4, and had an osmolality of 290–295 mmol/kg water as measured by a freezing point-based osmometer (model 3320; Advanced Instruments, Norwood, MA). Water used in the present work had a resistance higher than 18.3 MΩ·cm and free-ionized calcium concentration <2.5 mmol/l.

mRNA Isolation, PCR, and Sequencing

By using TRIzol reagent (Invitrogen, Carlsbad, CA), total RNA was prepared from mucosal scrapings or Caco-2 homogenate according to the methods of Charoenphandhu and colleagues (13, 15). One microgram of the total RNA extract was reverse-transcribed with the oligo(dT18) primer and the iScript kit (Bio-Rad, Hercules, CA) to cDNA by using a thermal cycler (model MyCycler, Bio-Rad). Rat or human glyceraldehyde-3-phosphate dehydrogenase (rGAPDH or hGAPDH), a housekeeping gene, served as a control gene to check the consistency of reverse transcription (%coefficient of variation <1, n = 20). Sense and antisense primers of PRLR and GAPDH were designed by OLIigo 6 (Molecular Biology Insights, Cascade, CO) and Primer Validator 1.4 (Naratt Software, Bangkok, Thailand), as shown in Table 1. The amplification reaction using conventional thermal cycler was performed with the GoTaq Green Master Mix (Promega, Madison, WI), and relative quantitation (qRT-PCR) using real-time PCR (model MiniOpticon, Bio-Rad) was performed with the iQ SYBR Green SuperMix (Bio-Rad) according to the manufacturers’ instructions. Relative expression of PRLR over GAPDH was calculated from the threshold cycle (Ct) values by using 2⁻ΔΔCt method. After conventional PCR or qRT-PCR, the PCR products were visualized on a 2% agarose gel stained with 1.0 μg/mL ethidium bromide under a UV transilluminator (Alpha Innotech, San Leandro, CA). After electrophoresis, all PCR products were extracted by the HiYield Gel/PCR DNA Extraction Kit (Real Biotech, Taipei, Taiwan) and were sequenced by the ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Western Blot Analysis

Scraped intestinal cells or Caco-2 cells were lysed in lysis buffer (0.5 mmol/l Tris pH 7.5, 1.5 mol/l NaCl, 10% NP-40, 5% DOC, 10 mmol/l Na-EDTA, 1 mmol/l PMSF, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 1 μg/ml pepstatin A; all purchased from Sigma). After a 20-min incubation at 4°C, lysates were sonicated, centrifuged at 20,000 g for 10 min, and then heated for 5 min at 95°C before being loaded onto a gel. Proteins (100 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (PVDF; Amersham, Buckinghamshire, UK) by electroblotting. Membranes were blocked at 25°C.
for 4 h with 5% nonfat milk and probed overnight at 4°C with 1:500 rabbit anti-rPRL or hPRL polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) raised against the conserved extracellular domain. Membranes were later reprobed with 1:5,000 mouse anti-r or human β-actin monoclonal antibodies (Santa Cruz Biotechnology). After a 2-h incubation at 25°C with 1:20,000 goat anti-rabbit or anti-mouse secondary antibodies (Santa Cruz Biotechnology), blots were visualized by enhanced chemiluminescence (Amersham).

Confocal Laser-Scanning Microscopy

As previously described (15), a 10-cm duodenal segment was fixed in 4% paraformaldehyde (Sigma) overnight at 4°C and embedded in Tissue-Tek optimum cutting temperature (OCT) compound (Sakura Finetek, Torrance, CA) in liquid nitrogen. Frozen tissue was sectioned with a microtome (Leica, Wetzlar, Germany) at −23°C to obtain 10-μm-thick tissue slices. The sections were then incubated overnight at 20°C with 1:200 rabbit anti-rPRL antibody (Santa Cruz Biotechnology). Thereafter, they were incubated for 3 h at 20°C with 1:200 Alexa flour 488-conjugated mouse anti-rabbit secondary antibody (green signal; Molecular Probes, Eugene, OR). Nuclei were stained with 1:500 TO-PRO-3 (red signal; Molecular Probes). Images were captured with a confocal laser-scanning microscope.

Electrical Measurement

Three electrical parameters, i.e., potential difference (PD), short-circuit current (Isc), and transepithelial resistance (TER), were determined as previously described (11). A pair of Ag/AgCl electrodes connected to agar bridges (3.0 mol/l KCl per 4 g% agar) located near each surface of the mounted tissue or Snapwell for measurement of PD. The other ends of the PD-sensing electrodes were connected to a preamplifier (model EVC-4000; World Precision Instruments, Sarasota, FL) and finally to a PowerLab/4SP operated with the software Chart 5.2.2 for Mac OS X (ADInstruments, Colorado Springs, CO). Another pair of Ag/AgCl electrodes was placed at the end of each hemichamber to supply fsc, which was also measured by a PowerLab/4SP connected in series to the EVC-4000 current-generating unit (World Precision Instruments). TER and conductance (G; G = 1/TER) were calculated by the Ohm’s equation.

Calcium Flux Measurement

Duodenal and Caco-2 calcium fluxes were determined by the modified methods of Karbach (32) and Charoenphandhu et al. (13). After a 20-min incubation, the Ussing chamber was filled with new, fresh bathing solution. One side was 45CaCl2-containing bathing solution (initial amount of 5 mCi/ml; final specific activity of ~450–500 mCi/ml; Amersham). As described previously (13), seven samples were collected per each setup to calculate the unidirectional flux (JH−→C, nmol·h−1·cm−2) from the hot side (H) to the cold side (C) with Eqs. 1 and 2.

\[ J_{H\rightarrow C} = R_{H\rightarrow C}(S_H \times A) \] (1)
where $R_{\text{MT-C}}$ was the rate of tracer appearance in the cold side (cpm/h); $S_{\text{NH}}$ was the specific activity in the hot side (cpm/mmol); $A$ was the surface area of the tissue or Snapwell (cm$^2$); and $C_{\text{Na}}$ was a mean of radioactivity in the hot side (cpm); and $C_T$ was the total calcium in the hot side (nmol). Radioactivity of $^{45}\text{Ca}$ was analyzed by liquid scintillation spectrophotometry (model 1219; LKB Wallac, Turku, Finland). Total calcium concentration of the bathing solution was analyzed by atomic absorption spectrophotometry (model SpectraAA-300; Varian Techtron, Springvale, Australia).

Calcium fluxes in the absence of a calcium concentration gradient (i.e., bathing solution in both hemichambers contained equal calcium concentration) represented the active calcium transport (11). The calcium gradient-dependent paracellular passive fluxes were measured by determining the calcium fluxes in the presence of varying apical calcium concentrations (13), i.e., 1.25, 2.5, 5, 10, 20, 40, and 80 mmol/l.

**Permeability Measurement**

Permeability of sodium ($P_{\text{Na}}$) and chloride ($P_{\text{Cl}}$) were measured by the dilution potential technique, modified from the methods of Kahle et al. (31) and Hou et al. (27). In brief, Caco-2 monolayer was equilibrated for 20 min in normal bathing solution containing 145 mmol/l NaCl before the apical solution was substituted with 72.5 mmol/l NaCl-containing solution. Osmolality was maintained by an equivalent amount of mannitol. Changes in the electrical parameters before and after fluid substitution were recorded every 10 s until stable. The ion permeability ratio ($P_{\text{Na}}/P_{\text{Cl}}$) was calculated from the dilution potential ($V_b$) by using the Goldman-Hodgkin-Katz equation (50)

$$V_b = RT / F \ln \left( P_{\text{Na}} C_a / P_{\text{Cl}} C_b \right)$$

(3)

where $P_{\text{Na}}$ was the absolute permeability of sodium; $P_{\text{Cl}}$ was the absolute permeability of chloride; $C_a$ was the apical NaCl concentration; $C_b$ was the basolateral NaCl concentration; $R$, $T$, and $F$ had their conventional meanings.

When given $\rho = P_{\text{Na}}/P_{\text{Cl}}$, $\phi = C_b/C_a$, and $v = F V_b/RT$, Eq. 3 could be rewritten as

$$\rho = (\phi - e^v)/(\phi e^v - 1)$$

(4)

$P_{\text{Na}}$ and $P_{\text{Cl}}$ were calculated from the conductance ($G$) and $P_{\text{Na}}/P_{\text{Cl}}$ by using the simplified Kimizuka-Koketsu equation (27, 31), as follows:

$$P_{\text{Na}} = \frac{G R T}{C_a F} \times \frac{\rho}{1 + \rho}$$

(5)

$$P_{\text{Cl}} = \frac{P_{\text{Na}}}{\rho}$$

(6)

The Caco-2 permeability of calcium (P$_{ca}$) via the passive paracellular pathway was calculated from Eq. 7 (53):

$$P_{\text{Ca}} = J_{\text{Ca}}/\Delta C$$

(7)

where $J_{\text{Ca}}$ was the paracellular passive calcium flux; and $\Delta C$ was the difference between the apical and basolateral calcium concentrations.

**Experimental Protocols**

**Protocol 1.** The objective of this protocol was to demonstrate that the rat intestine and Caco-2 cells expressed PRLR. Eleven-week-old adult female rats and Caco-2 cells cultured in 75-cm$^2$ T flask were used. Expressions of PRLR mRNAs in seven intestinal segments were determined qualitatively by conventional PCR technique, whereas those in duodenum and Caco-2 cells were determined quantitatively by qRT-PCR. Expressions of PRLR proteins in duodenal epithelial and Caco-2 cells were demonstrated by Western blot analysis. Localization of PRLRs was confirmed by confocal microscopic technique. All samples were performed in triplicate.

**Protocol 2.** The objectives of this protocol were to investigate the acute effect of PRL on transepithelial calcium flux in duodenum and Caco-2 monolayer, the dose-response pattern of PRL, and signaling pathways of PRL. In an Ussing chamber, the duodenal segment was directly exposed on the serosal side to 200, 400, 600, 800, or 1,000 ng/ml PRL (purified from ovine pituitary gland; catalog no. L6520, Sigma) during an 80-min experiment, while Snapwell-cultured Caco-2 monolayer was directly exposed to similar concentrations of recombinant human (rh)PRL (purity >97%; catalog no. 682-PL; R&D Systems, Minneapolis, MN). Before calcium fluxes and electrical parameters were determined, the mounted duodenum was incubated for 20 min with PRL to allow it to diffuse from the bathing solution and bind to PRLRs on the basolateral membrane of the absorptive cells. Measured calcium fluxes were the active calcium fluxes, as both sides of the mounted epithelium were exposed to bathing solution containing equal calcium concentration. The dilution potential technique, modified from the methods of Kahle et al. (31) and Hou et al. (27), was used. Expressions of PRLR mRNAs in seven intestinal segments were determined quantitatively.

**Protocol 3.** Electrochemical gradient was a major determinant of paracellular calcium transport in several epithelia (41). This study was to investigate effects of transepithelial PD and calcium concentration gradient on calcium fluxes in Caco-2 monolayer incubated with 600 ng/ml rhPRL. Under open-circuit condition, $I_{sc}$ was applied for 1 s every 10 min to determine electrical parameters, whereas under short-circuit condition $I_{sc}$ was continuously applied to the mounted monolayer to nullify PD. To study the effect of PRL on the gradient-dependent paracellular passive calcium transport, the apical calcium concentration was varied from 1.25 to 80 mmol/l in the presence of 600 ng/ml rhPRL or 600 ng/ml rhPRL plus 75 $\mu$mol/l LY-294002. This experiment was performed on 105 independent Snapwell setups (n = 5 per each value of the apical calcium concentration). Transepithelial calcium fluxes and calcium permeability were measured as mentioned earlier.

**Protocol 4.** Because earlier work showed that the effects of PRL on paracellular calcium transport in rat duodenum was not related to the widening of tight junction (54), changes in the gradient-dependent paracellular calcium fluxes may be due to altered charge-selective property of the epithelium. Thus, permeability ratio ($P_{\text{Na}}/P_{\text{Cl}}$) and absolute permeability of sodium and chloride, which reflected charge selectivity of the tight junction (19, 27), were determined by the dilution potential technique. In this protocol, mounted duodenal sheet was incubated with 800 ng/ml PRL or 800 ng/ml plus 75 $\mu$mol/l LY-294002, whereas Caco-2 monolayer was incubated with 600 ng/ml rhPRL or 600 ng/ml rhPRL plus inhibitors, including 50 $\mu$mol/l AG-490, 75 $\mu$mol/l LY-294002, and 200 nmol/l wortmannin.

**Statistical Analyses**

Results were expressed as means ± SE. Two sets of data were compared using the unpaired Student’s t-test. One-way ANOVA with Dunnett’s multiple comparison test was used for multiple sets of data. Linear regression with slope analysis was performed to obtain the apical calcium concentration-calcium flux relationship. Nonlinear regression was performed to demonstrate $\Delta$calcium-calcium permeability relationship, as previously described (13, 53). The level of

$S_{\text{NH}} = C_{\text{NH}} / C_T$

(2)
significance for all statistical tests was \( P < 0.05 \). Data were analyzed with GraphPad Prism 4.0 for Mac OS X (GraphPad Software, San Diego, CA).

**RESULTS**

**Duodenal Epithelial and Caco-2 Cells Strongly Expressed PRLRs**

By using five pairs of primers matched to different regions of rPRLR mRNA, both short and long isoforms of rPRLR mRNAs were identified in all intestinal segments, i.e., duodenum, proximal jejunum, distal jejunum, ileum, cecum, and proximal and distal colon (Fig. 1A). qRT-PCR revealed strong expressions of short and long rPRLRs with equal amounts in duodenum (Fig. 1B). rPRLR proteins migrated as multiple bands in the molecular mass range of 30–50 kDa (Fig. 1C). The short, intermediate, and long isoforms of hPRLR mRNAs as well as several isoforms of hPRLR proteins with sizes ranging between 30 and 105 kDa were present in Caco-2 cells (Fig. 2, A–C). In addition, as seen in Fig. 2B, qRT-PCR showed an equally intense expression of the inter-
mediate and long isoforms and a ~2.4-fold weaker expression of the short isoform of hPRLRs. Fluorescent images captured from a confocal laser-scanning microscope also revealed expressions of rPRLR proteins in the duodenal absorptive cells (Fig. 1D) and expression of hPRLR proteins in Caco-2 cells (Fig. 2D). Sequencing of amplicons confirmed correct rPRLR and hPRLR nucleotide sequences. These results indicated that duodenal epithelial and Caco-2 cells were targets of PRL.

**PRL Directly Stimulated Active Calcium Fluxes in Duodenum and Caco-2 Monolayer**

These series of experiments were performed to demonstrate a direct stimulatory effect of PRL on the duodenal and Caco-2 active calcium fluxes by the Ussing chamber technique. We found that, in duodenum (Fig. 3A), 600 and 800 ng/ml PRL enhanced the active calcium fluxes from a control value of 36.87 ± 2.51 (n = 10) to 53.40 ± 5.13 nmol·h⁻¹·cm⁻² (n = 8, P < 0.05) and to 58.08 ± 4.70 nmol·h⁻¹·cm⁻² (n = 8, P < 0.01), respectively. In Snapwell-cultured Caco-2 monolayer (Fig. 3B), 400 and 600 ng/ml rhPRL significantly stimulated the active calcium fluxes from a control value of 9.47 ± 0.57 (n = 8) to 13.39 ± 0.45 (n = 7, P < 0.01) and to 17.98 ± 0.71 nmol·h⁻¹·cm⁻² (n = 8, P < 0.01), respectively. Biphasic response of PRL was demonstrated in both cellular models by an absence of effects of 1,000 ng/ml PRL on calcium fluxes. Duodenal electrical parameters were not altered by 200–800 ng/ml PRL. However, 1,000 ng/ml PRL applied to duodenum led to a slight increase in I_{sc} and a decrease in TER (Table 2). In Caco-2 monolayer, 400, 600, and 800 ng/ml rhPRL decreased TER but not other electrical parameters (Table 3). These results confirmed that PRL directly stimulated

### Table 2. Epithelial electrical parameters for duodenum

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>PD, mV</th>
<th>I_{sc}, μA/cm²</th>
<th>TER, Ω cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>4.73±0.40</td>
<td>35.64±3.52</td>
<td>135.26±17.79</td>
</tr>
<tr>
<td>PRL treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 ng/ml</td>
<td>6</td>
<td>4.92±0.54</td>
<td>52.78±4.14</td>
<td>94.74±6.37</td>
</tr>
<tr>
<td>400 ng/ml</td>
<td>5</td>
<td>4.84±0.93</td>
<td>41.22±6.01</td>
<td>120.07±14.44</td>
</tr>
<tr>
<td>600 ng/ml</td>
<td>5</td>
<td>4.32±0.55</td>
<td>32.78±5.96</td>
<td>140.26±9.17</td>
</tr>
<tr>
<td>800 ng/ml</td>
<td>5</td>
<td>6.25±0.49</td>
<td>54.58±5.04</td>
<td>122.55±12.91</td>
</tr>
<tr>
<td>1,000 ng/ml</td>
<td>5</td>
<td>5.33±0.37</td>
<td>65.11±6.67**</td>
<td>86.01±5.32*</td>
</tr>
<tr>
<td>DMSO</td>
<td>6</td>
<td>5.42±0.33</td>
<td>53.51±3.79</td>
<td>105.85±8.58</td>
</tr>
<tr>
<td>Inhibitor without PRL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG-490</td>
<td>6</td>
<td>4.47±0.35</td>
<td>47.01±2.68</td>
<td>105.56±10.98</td>
</tr>
<tr>
<td>LY-294002</td>
<td>5</td>
<td>4.12±0.25</td>
<td>45.80±9.58</td>
<td>126.93±11.01</td>
</tr>
<tr>
<td>800 ng/ml PRL + AG-490</td>
<td>8</td>
<td>5.40±0.46</td>
<td>46.10±3.85</td>
<td>122.51±8.34</td>
</tr>
<tr>
<td>LY-294002</td>
<td>8</td>
<td>3.68±1.00</td>
<td>36.51±9.85</td>
<td>126.93±12.72</td>
</tr>
</tbody>
</table>

Values are means ± SE. Epithelial electrical parameters consisted of transepithelial potential difference (PD), short-circuit current (I_{sc}), and transepithelial resistance (TER) in duodenum directly exposed to various concentrations of PRL. 800 ng/ml PRL + JAK2 inhibitor (50 μmol/l AG-490), 800 ng/ml PRL + PI3K inhibitor (75 μmol/l LY-294002), or inhibitors without PRL. DMSO was vehicle for inhibitor preparation. The apical side was negative with respect to the basolateral side. *P < 0.05, **P < 0.01 vs. control group.

### Table 3. Epithelial electrical parameters for Caco-2 cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>PD, mV</th>
<th>I_{sc}, μA/cm²</th>
<th>TER, Ω cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>0.93±0.20</td>
<td>2.63±0.68</td>
<td>373.09±14.42</td>
</tr>
<tr>
<td>rhPRL treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 ng/ml</td>
<td>6</td>
<td>0.97±0.13</td>
<td>2.83±0.40</td>
<td>349.16±11.00</td>
</tr>
<tr>
<td>400 ng/ml</td>
<td>7</td>
<td>0.97±0.11</td>
<td>3.71±0.42</td>
<td>301.17±15.07**</td>
</tr>
<tr>
<td>600 ng/ml</td>
<td>8</td>
<td>0.96±0.11</td>
<td>3.75±0.45</td>
<td>259.17±9.61***</td>
</tr>
<tr>
<td>800 ng/ml</td>
<td>6</td>
<td>0.82±0.11</td>
<td>2.67±0.42</td>
<td>302.33±14.25**</td>
</tr>
<tr>
<td>1,000 ng/ml</td>
<td>6</td>
<td>0.88±0.16</td>
<td>2.50±0.50</td>
<td>349.17±13.97</td>
</tr>
<tr>
<td>DMSO</td>
<td>8</td>
<td>1.00±0.15</td>
<td>2.50±0.38</td>
<td>383.33±10.45</td>
</tr>
<tr>
<td>Inhibitor without rhPRL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U-0126</td>
<td>5</td>
<td>0.92±0.32</td>
<td>2.40±0.75</td>
<td>376.67±33.22</td>
</tr>
<tr>
<td>AG-490</td>
<td>5</td>
<td>0.86±0.17</td>
<td>2.40±0.40</td>
<td>356.67±27.69</td>
</tr>
<tr>
<td>LY-294002</td>
<td>5</td>
<td>1.04±0.19</td>
<td>2.80±0.49</td>
<td>375.00±19.36</td>
</tr>
<tr>
<td>Wortmannin</td>
<td>5</td>
<td>0.88±0.22</td>
<td>2.40±0.60</td>
<td>366.67±27.89</td>
</tr>
<tr>
<td>600 ng/ml rhPRL + U-0126</td>
<td>6</td>
<td>1.03±0.23</td>
<td>3.50±0.56</td>
<td>288.89±24.97*</td>
</tr>
<tr>
<td>AG-490</td>
<td>8</td>
<td>0.70±0.10</td>
<td>3.00±0.46</td>
<td>262.29±18.80***</td>
</tr>
<tr>
<td>LY-294002</td>
<td>8</td>
<td>0.96±0.10</td>
<td>3.25±0.37</td>
<td>320.92±18.53*</td>
</tr>
<tr>
<td>Wortmannin</td>
<td>8</td>
<td>1.04±0.13</td>
<td>3.00±0.38</td>
<td>355.42±25.15</td>
</tr>
</tbody>
</table>

Values are means ± SE. Epithelial electrical parameters consisted of PD, I_{sc}, and TER in Caco-2 monolayer directly exposed to various concentrations of rhPRL, rhPRL + MEK inhibitor (10 μmol/l U-0126), rhPRL + JAK2 inhibitor (50 μmol/l AG-490), rhPRL + PI3K inhibitors (75 μmol/l LY-294002 or 200 nmol/l wortmannin), or inhibitors without rhPRL. DMSO was vehicle for inhibitor preparation. The apical side was negative with respect to the basolateral side. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control group.

---

**AJP-Endocrinol Metab Volume 293 July 2007 www.ajpendo.org**
the active calcium transport in duodenum and Caco-2 monolayer in a dose-response manner. The two most effective doses of PRL, i.e., 800 and 600 ng/ml, were used in duodenum and Caco-2 cells, respectively, in the following experiments.

**PRL Stimulated Duodenal and Caco-2 Active Calcium Fluxes via the PI3K Pathway**

To demonstrate the signaling pathways of PRL, we exposed duodenal tissues and Caco-2 monolayers to various inhibitors. In duodenum (Fig. 4A), 800 ng/ml PRL plus JAK2 inhibitor (50 μmol/l AG-490) manifested comparable calcium fluxes to 800 ng/ml PRL alone, suggesting that the acute action of PRL was not mediated by the JAK2 pathway. Interestingly, 75 μmol/l LY-294002, a specific PI3K inhibitor, completely abolished PRL-enhanced duodenal active calcium fluxes, thus indicating the PI3K pathway as a possible signaling pathway of PRL. Similar to the results in the duodenum, JAK2 inhibitor failed to abolish PRL-enhanced active calcium fluxes in Caco-2 monolayer, whereas 75 μmol/l LY-294002 and 200 nmol/l wortmannin totally abolished the effects of PRL on Caco-2 calcium fluxes (Fig. 4B). MEK inhibitor (10 μmol/l U-0126) also had no effect on PRL-enhanced active calcium fluxes in Caco-2 monolayer.

As for the electrical parameters, AG-490 and LY-294002 had no effect on duodenal PD, Isc, or TER (Table 2). Although TER in the wortmannin-treated Caco-2 monolayer was comparable to that in the control group, LY-294002 did not completely reverse TER to the control level (Table 3). U-0126 and AG-490 did not prevent rhPRL-induced reduction of TER in Caco-2 monolayer. Neither the inhibitors alone nor 0.3% vol/vol DMSO (vehicle) affected the calcium flux (Fig. 4), TER (Tables 2 and 3), or dilution potential (data not shown) in the duodenum and Caco-2 cells. The above evidence indicates that the rapid stimulatory actions of PRL on the duodenal and Caco-2 active calcium fluxes occurred via the PI3K pathway.

**PRL Increased Paracellular Passive Calcium Fluxes and Calcium Permeability via the PI3K Pathway**

Since PRL has been suggested to stimulate the paracellular calcium transport in the small intestine (34, 54), we further investigated the effect of PRL on two major components of the paracellular transport in Caco-2 monolayer, i.e., voltage-dependent active and gradient-dependent passive calcium fluxes. As seen in Fig. 5, 600 ng/ml rhPRL significantly stimulated Caco-2 active calcium fluxes under an open-circuit condition from 9.53 ± 0.81 (n = 5) to 16.89 ± 0.60 nmol·h⁻¹·cm⁻²·1⁸ (n = 5, P < 0.001). However, under a short-circuit condition, the same dose of PRL still increased Caco-2 calcium fluxes to a level comparable to that in open-circuit condition, i.e., from
a control value of 9.41 ± 0.70 (n = 5) to 17.54 ± 1.03 nmol·h⁻¹·cm⁻² (n = 5, P < 0.001). Therefore, the voltage-dependent active calcium transport did not contribute to the PRL-enhanced calcium flux in Caco-2 monolayer.

Figure 6A showed that varying the apical calcium concentrations from 1.25 to 80 mmol/l increased the paracellular passive calcium fluxes in Caco-2 monolayer with a linear correlation in control group [y = (3.76 ± 0.12)x + (1.34 ± 3.99)], r² = 0.97], as well as in 600 ng/ml rhPRL-treated group [y = (6.39 ± 0.22)x + (5.18 ± 7.50)], r² = 0.96]. Interestingly, the slope of the regression line was increased from 3.76 ± 0.12 in control to 6.39 ± 0.22 × 10⁻³ cm/h in the 600 ng/ml rhPRL-treated group (P < 0.001). Interestingly, 75 μmol/l LY-294002 decreased the slope of PRL-treated monolayer to the control value. At all apical calcium concentrations, calcium flux in the PRL-treated group was significantly greater than that in the control group, and LY-294002 completely abolished the PRL-enhanced passive calcium fluxes. Calcium permeability via the paracellular pathway was also increased by 600 ng/ml rhPRL (Fig. 6B). Consistent with the passive calcium fluxes, PRL-enhanced calcium permeability diminished after exposure to LY-294002. Our results suggested that PRL not only stimulated the active calcium transport but also increased the gradient-dependent paracellular passive calcium transport and calcium permeability via the PI3K signaling pathway.

PRL Altered Charge Selectivity in Duodenum and Caco-2 Monolayer

Since PRL stimulated the paracellular calcium transport in both duodenal epithelium and Caco-2 monolayer, we determined the epithelial charge selectivity, which was known to influence transport of several ions through the paracellular pathway (19, 57). In the duodenum, 800 ng/ml PRL increased PNa/PCl from a control value of 2.40 ± 0.06 (n = 8) to 3.16 ± 0.23 (n = 8, P < 0.01; Fig. 7A). An increase in duodenal PNa/PCl in the PRL-treated group was due to an increase in PNa from 16.01 ± 1.19 (n = 8) to 24.19 ± 2.68 × 10⁻⁶ cm/s (n = 8, P < 0.01; Fig. 7B) and a concurrent decrease in PCl from 12.96 ± 1.09 (n = 8) to 7.82 ± 0.95 × 10⁻⁶ cm/s (n = 8, P < 0.01; Fig. 7C). LY-294002 diminished the PRL-induced changes in PNa/PCl and PNa to control levels. However, PCl in the LY-294002-treated tissue was still lower than that in the control tissue.

In Caco-2 monolayer, 600 ng/ml rhPRL significantly increased PNa/PCl from 3.70 ± 0.31 (n = 6) to 7.05 ± 0.11 (n = 5, P < 0.05; Fig. 8A) and PNa from 8.15 ± 0.30 (n = 6) to 10.94 ± 0.68 × 10⁻⁶ cm/s (n = 5, P < 0.01; Fig. 8B). PRL was without any effect on PCl in Caco-2 monolayer (Fig. 8C). In the presence of 75 μmol/l LY-294002 or 200 nmol/l wortmannin, PRL-induced changes in PNa/PCl and PNa were diminished to their control levels. AG-490, as expected, did not inhibit effects of PRL on PNa/PCl and PNa in Caco-2 monolayer. The present results implied that the stimulatory effect of PRL on paracellular calcium transport involved alteration of the charge-selective property of the duodenal epithelium and Caco-2 monolayer.

DISCUSSION

Intestinal calcium absorption is the sole source of calcium supply to the body. When calcium demand is increased, such as during pregnancy and lactation, several hormones, including PRL, act in concert to provide adequate calcium gain and alleviate negative calcium balance (59). Herein, we demonstrated that duodenum and human intestinal cell line (Caco-2), which strongly expressed PRLR under normal condition, responded to the acute actions of PRL by increasing the transepithelial calcium fluxes through the PI3K signaling pathway. High physiological PRL in vivo not only stimulated intestinal calcium absorption but also enhanced bone turnover and reduced urinary calcium excretion, thus raising circulating calcium for fetal growth and development (14, 38, 48).

Although several in vivo and in vitro investigations provided convincing evidence that PRL stimulated duodenal calcium transport, it was not known whether PRL exerted its actions directly on the absorptive cells. In the present study, we demonstrated strong expressions of short- and long-form

---

![Figure 6](http://ajpendo.physiology.org/)

**Figure 6.** Effects of 600 ng/ml rhPRL and 600 ng/ml rhPRL + PI3K inhibitor (75 μmol/l LY-294002) on passive paracellular calcium flux (A) and calcium permeability (B) in Caco-2 monolayer. The basolateral solution had a constant calcium concentration of 1.25 mmol/l, whereas the apical solution had calcium concentration of 1.25, 2.5, 5, 10, 20, 40, or 80 mmol/l. ΔCalcium is difference between calcium concentrations in the apical and basolateral solution. Each point is mean ± SE calculated from 5 independent Snapwell setups (n = 5 per each value of the apical calcium concentration). *P < 0.05, **P < 0.01 vs. control value at the same apical calcium or Δcalcium concentration.
PRLR mRNA and proteins in duodenal epithelial cells, evidence of duodenal epithelium as a target site of PRL. In addition, both short- and long-form mRNA transcripts were found in mucosal scrapings of jejunum, ileum, cecum, and colon. Our findings agreed with the previous reports of duodenum-expressed PRLR mRNAs identified by in situ hybridization and Northern blot analysis techniques in female Wistar rats and rabbits, respectively (18, 45). Although hPRLR was expressed in the duodenal epithelial cells of fetus (12–14 wk of gestation) (22) and several human colorectal cancers, including colonic adenocarcinoma (44), expressions of hPRLR in the duodenum of adult human as well as in Caco-2 cells were controversial. The present study thus provides, for the first time, evidence of the strong expression of various isoforms of hPRLR in Caco-2 cells with predominant intermediate and long isoforms.

Generally, PRL exerted its actions through the long- and/or intermediate-form receptors, whereas the short isoform seemed to have fine-tuning or counterbalancing actions (5, 30). However, the significance of each intestine-expressed isoform has not been demonstrated. It was known that PRL regulated sodium and water transport in both the small and large intestine, whereas PRL-stimulated calcium absorption was seen only in the duodenum and proximal jejunum and not in the more distal segments (33, 34, 39, 40). Effects of PRL on calcium transport in Caco-2 monolayer had never been reported before. Since we found that Caco-2 cells, which have functional properties resembling cells in the small intestine rather than colonocytes (63, 64), responded to PRL in a similar manner to that of the duodenal tissue, e.g., increases in calcium fluxes and changes in the chloride selectivity, it could be a suitable model for further studies of PRL signaling and PRL-enhanced transepithelial calcium transport.

Interestingly, we found herein that rapid actions of PRL on calcium absorption were seen at PRL concentrations of 400–600 ng/ml, which were comparable to high physiological levels of 400–650 ng/ml in lactating animals during suckling (4, 17, 61). Indeed, the plasma concentration of PRL of ~7–10 ng/ml in normal rats was required to maintain the basal rate of duodenal calcium absorption (3); however, anterior pituitary transplantation-induced chronic hyperprolactinemia of ~90–100 ng/ml (comparable to levels during pregnancy) was found to result in a sustained increase in calcium absorption (56). Lactating animals with plasma PRL of 200–300 ng/ml also exhibited an increase in the duodenal calcium transport (8). It was thus postulated that chronic PRL exposure was responsible for maintaining the duodenal calcium absorption at a higher rate during lactation, and the transient surge of PRL level during suckling rapidly enhanced more transepithelial calcium transport to match calcium loss in milk.

Transepithelial calcium movement in the duodenum occurred through the transcellular and paracellular pathways. Transcellular active calcium transport is a metabolically energized process consisting of three important steps, i.e., apical calcium uptake via the transient receptor potential vanilloid family channels (TRPV) 5 and 6, cytoplasmic translocation in a Ca\(^{2+}\)-calbindin-D\(_{9K}\)-bound form, and basolateral extrusion by plasma membrane Ca\(^{2+}\)-ATPase (PMCA) 1b (26). We (12) have previously shown that PRL directly stimulated the transepithelial active calcium transport in the duodenum by enhancing the apical calcium uptake and PMCA\(_{1b}\) activity. However, the physiological significance of the transepithelial active calcium transport in nonmated animals was doubtful, because the transcellular transport contributed less than 15% of the total active calcium transport (11). In addition, calbindin-D\(_{9K}\) knockout mice exhibited normal plasma calcium concentration (35). However, under certain conditions, such as pregnancy and lactation, the transcellular calcium transport may play a more significant role in the extraction of extra calcium from luminal contents, perhaps under the regulation of PRL.

The paracellular calcium transport consisted of three components, namely the solvent drag-induced, voltage-dependent, and gradient-dependent transport (11, 54). The first two components were considered active process and could be abolished by inhibition of Na\(^{+}\)-K\(^{+}\)-ATPase activity, which created a paracellular hyperosmotic milieu and generated PD (voltage) for the solvent drag-induced and voltage-dependent transport, respectively (11). The present results show that PRL directly stimulated the total active calcium fluxes in a dose-response manner in both duodenal tissue and Caco-2 monolayer. The results obtained from Caco-2 cells under a short-circuit condition confirmed previous reports in duodenum that the...
voltage-dependent transport was negligible and was not affected by PRL (11). Due to the small contribution of the transcellular active calcium transport, PRL-enhanced active calcium transport was likely due to the solvent drag-induced mechanism.

The gradient-dependent paracellular calcium transport was a passive process. Under physiological condition with apical calcium concentration greater than ~5 mmol/l, such as in animals fed normal or calcium-supplemented diet (13, 60), this mode of calcium transport became predominant. According to Fig. 6A, the very low y-intercept value, representing the non-gradient-dependent calcium transport (i.e., active transport) in the control group, confirmed a greater contribution of the paracellular passive fluxes to the total calcium absorption. We also found that PRL directly stimulated the gradient-dependent calcium transport, as well as the paracellular calcium permeability, thus supporting the previous reports from in vivo experiments (34). Indeed, the paracellular calcium transport, either via the solvent drag-induced active or gradient-dependent passive mechanisms, was determined by two properties of the tight junction (or epithelia), namely size and charge selectivity (58). Widening of the tight junction by contraction of the perijunctional actomyosin ring complex regulated ion and nutrient absorption in a size-selective manner (43). However, Tanrattana et al. (54) clearly showed that widening of the tight junction did not affect PRL-enhanced paracellular calcium transport in the duodenum of rats, indicating that it was the charge selectivity, not the size selectivity, that determined paracellular calcium transport.

The charge-selective property of the tight junction could be demonstrated by $P_{Na}/P_{Cl}$, and their ratio (27, 31, 57). Normally, the paracellular channels in the small intestine intrinsically favored positively charged molecules and ions, i.e., high $P_{Na}/P_{Cl}$ ratio (24, 57), as confirmed by $P_{Na}/P_{Cl}$ of 2.4 and 3.7 in the duodenum and Caco-2 monolayer, respectively. After a direct exposure to PRL, $P_{Na}/P_{Cl}$ in both duodenal tissue and Caco-2 monolayer was elevated due to an increase in $P_{Na}$ in both cell types. PRL-treated duodenum also showed increased restriction of negatively charged molecules (decreased $P_{Cl}$), whereas in Caco-2 cells such a change was statistically insignificant. Nevertheless, the higher $P_{Na}/P_{Cl}$ together with a decrease in TER of Caco-2 monolayer after exposure to 600 ng/ml rhPRL indicated that cations, e.g., sodium and calcium, traversed the epithelial sheet more readily than anions. However, in more leaky epithelia (lower TER) such as the duodenum, changes in electrical parameters may be absent, as previously reported (11), or may be seen only with higher doses of PRL, i.e., 1,000 ng/ml. Recently, the expression, distribution or phosphorylation of the integral membrane proteins of the claudin family were found to be responsible for altering the charge selectivity without widening of the tight junction, thus leading to changes in the paracellular ion transport (37, 58). Claudins possess several charged amino acids on their extracellular loops which aided ion movement through the tight junction in a channel-like manner (58). It has been widely accepted that the cation-selective paracel- lulin-1, a member of the claudin family, regulated paracellular calcium and magnesium reabsorption in the thick ascending limb of the loop of Henle (52). In the duodenum, claudin-3 expression was under the control of 1,25-(OH)$_2$D$_3$, a major regulator of transcellular and paracellular calcium transport.

![Figure 8](http://ajpendo.physiology.org/)
(36). Moreover, a number of investigations have revealed that changes in claudin expression in the intestinal absorptive cells were closely related to the enhanced paracellular calcium transport (49) and/or conditions that could enhance calcium absorption, e.g., chronic metabolic acidosis (13). Therefore, it is possible that PRL enhanced the paracellular calcium transport in the duodenum and Caco-2 monolayer by altering the epithelial charge selectivity through changing the expression or function of Claudins.

Despite available evidence of PRL-enhanced intestinal calcium absorption, nothing was known regarding PRL signal transduction in the intestinal epithelia. In mammary gland, PRL binding to PRLRs triggered dimerization of PRLRs (ratio of PRL to PRLRs was 1:2), and activation of the JAK2-STAT5 cascade. In addition, PRL-bound PRLR dimer also activated the PI3K and MEK pathways in the liver, pancreas, and T-lymphoma Nb2 cells (2, 7, 62). The typical biphasic action of PRL was also demonstrated in both duodenum and Caco-2 cells; i.e., in contrast to the lower doses, 1,000 ng/mL PRL were unable to stimulate calcium transport. This phenomenon has been explained in terms of dimerization of PRLRs. At very high concentrations of PRL, most available PRLRs were occupied as nonfunctional 1:1 complexes instead of the 1:2 functional complexes needed to induce signal transduction (23, 28).

Although most reported signal transduction of PRL had been genomic pathways involving translocation of the signaling proteins to the nucleus to activate transcription of PRL-responsive genes, we previously reported a rapid, presumably nonnongenic effect of PRL on the duodenal calcium transport which was usually seen within 60 min after PRL exposure (11). PRL-enhanced apical calcium uptake occurred as rapidly as 8 min, whereas PRL-activated PMCA1b and Na\(^{+}\)-K\(^{+}\)-ATPase activities were detected in 30 min (12). Although the non-nongenic signal transductions of PRL via JAK2, PI3K, and MEK pathways had been reported in several cellular models (1, 47, 51), we found the PI3K pathway to be predominant in both duodenum and Caco-2 monolayer. Inhibition of the PI3K pathway by LY-294002 or wortmannin completely abolished PRL-induced changes in calcium fluxes, calcium permeability, electrical parameters, and charge selectivity, thus substantiating PRL signaling via the PI3K pathway. Details of the signaling cascade and the phosphorylation of target proteins, which could be Claudins, TRPV6, or PMCA1b, remained to be investigated.

Finally, it could be concluded that, first, duodenum and Caco-2 cells with strongly expressed PRLRs responded to the direct action of PRL by increasing the transepithelial calcium transport in a dose-response manner. Second, the active and paracellular passive, but not voltage-dependent, components of calcium fluxes, as well as calcium permeability, were significantly enhanced by PRL. Third, the PRL-stimulated paracellular calcium transport was mediated by an increase in \( P_{\text{Na}}/P_{\text{Cl}} \) and a decrease in TER. Last, PRL used the PI3K signaling pathway to acutely and directly increase duodenal and Caco-2 calcium fluxes and alter the epithelial charge selectivity. Further investigations are needed to demonstrate the signaling downstream of PRL-induced PI3K activation as well as modification of its target proteins.

ACKNOWLEDGMENTS

We thank Kukiat Tudpor, Kannikar Wongdee, and Yuvaadee Siriyasab for their excellent technical assistance. We are grateful to Drs. Chureeporn Sitichumroonchokechai and Kittiporn Phanvihtisiri at the Institute of Nutrition, Mahidol University, Thailand, for a kind gift of Caco-2 cells.

GRANTS

This research was supported by grants from the Royal Golden Jubilee program (to N. Thongon), the Thailand Research Fund, and the National Center for Genetic Engineering and Biotechnology.

REFERENCES


E383

PRL STIMULATES INTESTINAL CALCIUM TRANSPORT


