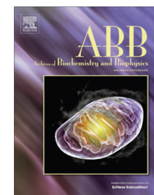
Archives of Biochemistry and Biophysics 536 (2013) 46–52

Contents lists available at SciVerse ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi



Fibroblast growth factor-23 negates $1,25(OH)_2D_3$-induced intestinal calcium transport by reducing the transcellular and paracellular calcium fluxes

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ARTICLE INFO

Article history:
Received 3 February 2013
and in revised form 17 April 2013
Available online 4 June 2013

Keywords:
Arrhenius plot
Calcium permeability
Paracellular transport
Transcellular transport
Ussing chamber

ABSTRACT

The calciotropic hormone 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] has been known to stimulate intestinal calcium transport via both transcellular and paracellular pathways. Recently, we reported that the $1,25(OH)_2D_3$-enhanced calcium transport in the mouse duodenum could be abolished by fibroblast growth factor (FGF)-23, but the targeted calcium transport pathway has been elusive. Herein, the $1,25(OH)_2D_3$-enhanced calcium transport was markedly inhibited by FGF-23 and inhibitors of the basolateral calcium transporters, NCX1 and $PMCA_{1b}$, suggesting the negative effect of FGF-23 on the transcellular calcium transport. Similar results could be observed in the intestinal epithelium-like Caco-2 monolayer. Although the Arrhenius plot indicated that FGF-23 decreased the potential barrier (e.g., activation energy) of the paracellular calcium movement, FGF-23 was found to modestly decrease the $1,25(OH)_2D_3$-enhanced paracellular calcium transport and calcium permeability. Moreover, FGF-23 affected the $1,25(OH)_2D_3$-induced change in duodenal water permeability as determined by tritiated water, but both $1,25(OH)_2D_3$ and FGF-23 were without effects on the transepithelial fluxes of paracellular markers, ^{3}H-mannitol and ^{14}C-polyethylene glycol. It could be concluded that FGF-23 diminished the $1,25(OH)_2D_3$-enhanced calcium absorption through the transcellular and paracellular pathways. Our findings have thus corroborated the presence of a bone–kidney–intestinal axis of FGF-23/vitamin D system in the regulation of calcium homeostasis.

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Introduction

Fibroblast growth factor (FGF[1])-23 is generally recognized as a hypophosphatemic hormone that promotes renal phosphate excretion [1], whereas 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] is a potent hormone that stimulates intestinal calcium and phosphate absorption [2]. Recently, we provided evidence that FGF-23 was able to inhibit the $1,25(OH)_2D_3$-induced calcium absorption despite having no effect on the basal intestinal calcium flux [3]. However, whether FGF-23 modulated the transcellular or paracellular components of the $1,25(OH)_2D_3$-enhanced intestinal calcium transport remained elusive.

Although paracellular calcium transport accounts for a large portion of the intestinal calcium absorption, little is known about $1,25(OH)_2D_3$-induced paracellular calcium transport across the intestinal epithelia. A body of evidence suggested that it required the presence of transepithelial calcium gradient (i.e., high luminal calcium concentration) and/or convective water flow—known as solvent drag in which calcium is transported along with the stream of water [4]. On the other hand, the $1,25(OH)_2D_3$-induced transcellular calcium transport is a three-step cellular energy-driven mechanism comprised of (*i*) apical calcium entry via the transient receptor potential calcium channels subfamily V (TRPV)-5 and TRPV6, (*ii*) calbindin-D_{9k}-mediated cytoplasmic transfer, and (*iii*) basolateral calcium extrusion by plasma membrane Ca^{2+}-ATPase ($PMCA_{1b}$) and Na^{+}/Ca^{2+} exchanger (NCX)-1 [5,6]. Both transcellular and paracellular mechanisms take place with high degree of efficiency in the proximal small intestine, especially the duodenum [4,7]. Therein, $1,25(OH)_2D_3$ markedly upregulated the expressions of transcellular calcium transporters, such as TRPV5, TRPV6 and calbindin-D_{9k}, which were abolished by FGF-23 [3]. We thus hypothesized that FGF-23 diminished $1,25(OH)_2D_3$-induced calcium absorption, at least, via the transcellular pathway.

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[1] *Abbreviations used*: FGF, Fibroblast growth factor; TRPV, Transient receptor potential calcium channels subfamily V; TNF, tumor necrosis factor.

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http://dx.doi.org/10.1016/j.abb.2013.05.009

Therefore, the aims of the present study were (*i*) to investigate whether FGF-23 could abolish the $1,25(OH)_2D_3$-enhanced transcellular and paracellular calcium transport in the mouse duodenum, and (*ii*) to determine whether $1,25(OH)_2D_3$ and FGF-23 altered the epithelial calcium and water permeability.

Materials and methods

Animals

Male mice [Imprinting Control Region (ICR) strain; 7-week-old] were obtained from National Laboratory Animal Center, Thailand. They were placed in solid bottom, open-top plastic cages, fed standard chow containing 1.0% wt/wt calcium, 0.9% wt/wt phosphorus and 4000 IU/kg vitamin D (CP Co. Ltd., Bangkok, Thailand), and reverse osmosis water ad libitum under 12/12-h light/dark cycle. The room had temperature of 20–25 °C, humidity of 50–60% and average illuminance of 150–200 lx in the daytime. This study has been approved by the Institutional Animal Care and Use Committee of the Faculty of Science, Mahidol University.

Cell culture

Caco-2 cells [American Type Culture Collection (ATCC) no. HTB-37] were first propagated in 75 cm^2 T-flask (Corning, NY, USA) containing Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO, USA) supplemented with 15% fetal bovine serum (Gibco, Grand Island, NY, USA), 1% nonessential amino acid (Sigma), 1% L-glutamine (Gibco), and 100 U/ml penicillin-streptomycin (Gibco) in humidified 5% CO_2 atmosphere at 37 °C. Thereafter, the intestinal epithelium-like Caco-2 monolayers were prepared by seeding cells at 250,000 cells/well on polyester Snapwell (12-mm diameter and 0.4-μm pore size; Corning), and maintained for 14 days in humidified 5% CO_2 at 37 °C.

Experimental design

Since we have demonstrated that intravenous FGF-23 injection (140–280 μg/kg) was able to abolish $1,25(OH)_2D_3$-induced calcium absorption, but not the baseline calcium transport in the mouse duodenum [3], prior to the experiment, mice were once-daily injected for 3 days with 1 μg/kg $1,25(OH)_2D_3$ s.c. (catalog no. 71820; Cayman Chemical, Ann Arbor, MI, USA) or 3 ml/kg vehicle (control group; 9:1 propylene glycol–ethanol s.c.) as described previously [3]. Serum levels of total calcium and free-ionized calcium were determined to show the effectiveness of $1,25(OH)_2D_3$ treatment. Thereafter, the duodenal tissue was removed and mounted in Ussing chamber for measurement of the transepithelial calcium transport. Before testing the FGF-23 action in vitro, the serum levels of FGF-23 were first determined in mice treated with a single dose of 280 μg/kg recombinant mouse FGF-23 i.v. (catalog no. 2629-FG; R&D Systems, Minneapolis, MN, USA) at 0–9 h post-injection.

In the active calcium flux study, recombinant mouse FGF-23 was directly added in the basolateral solution of Ussing chamber and was thus present throughout the 70-min experiment. The effect of FGF-23 on the $1,25(OH)_2D_3$-induced active calcium transport was also demonstrated in the epithelium-like Caco-2 monolayer, which was first pre-incubated for 48 h in culture medium with 10 nmol/l $1,25(OH)_2D_3$, followed by a basolateral FGF-23 exposure in Ussing chamber. The active calcium fluxes were determined in the absence of transepithelial calcium gradient (i.e., both apical and basolateral compartments contained equal calcium concentration of 1.25 mmol/l). In some experiments, NCX1 inhibitor (KB-R7943 mesylate; catalog no. 1244; Tocris) and/or PMCA inhibitor [trifluoperazine (TFP); catalog no. T8516; Sigma] were added in the basolateral solution to inhibit the transcellular active calcium flux.

In the subsequent experiments, mice were once-daily injected for 3 days with 1 μg/kg $1,25(OH)_2D_3$ s.c., or 1 μg/kg $1,25(OH)_2D_3$ s.c. plus 140 μg/kg FGF-23 i.v. via the tail vein. Mice in the control group were once-daily injected with 3 ml/kg 9:1 propylene glycol–ethanol s.c. [for $1,25(OH)_2D_3$ preparation] and 1 ml/kg 0.9% NaCl i.v. (for FGF-23 preparation). To determine the paracellular calcium transport, the mounted duodenal tissue was bathed in the presence of transepithelial calcium gradient (i.e., apical calcium concentrations of 5, 10, 20 or 40 mmol/l vs. a relatively low basolateral calcium concentration of 1.25 mmol/l). The duodenal calcium permeability and activation energy of the paracellular calcium movement were shown in the Arrhenius plot. Since widening of the tight junction and solvent drag could contribute to the paracellular calcium movement [4,8], we also measured the transepithelial fluxes of paracellular markers [^{3}H-mannitol and ^{14}C-polyethylene glycol (PEG)-4000] and tritiated water (^3H_2O) as indicators of tight junction widening and solvent drag, respectively. Under normal conditions, the intestinal epithelium is only slightly permeable to mannitol and PEG-4000 with molecular radii of 350 and 2500 pm, respectively [9].

In normal mice, serum FGF-23 levels are very low (<100 pg/ml), but the levels can be elevated several times by $1,25(OH)_2D_3$. Since the present study aimed to investigate the negative effect of FGF-23 on $1,25(OH)_2D_3$ action (as a part of negative feedback loop), it was necessary to use relatively high FGF-23 concentrations.

Blood chemistry and hormone assay

After a median thoracotomy, cardiac puncture was performed for arterial blood collection (~5 ml). Serum total calcium (ionized and complexed forms) was determined by *o*-cresolphthalein complexone method using a Dimension RxL analyzer (Dade Behring, Marburg, Germany). Free-ionized calcium was measured by an ion-selective electrode (model Stat Profile CCX; Nova Biomedical, Walthame, MA) under anaerobic condition. Serum FGF-23 level was determined by a commercial enzyme-linked immunosorbent assay kit (catalog no. 60-6300; Immutopics, San Clemente, CA, USA).

Measurement of transepithelial calcium flux

After 1.5-cm median laparotomy was performed, the duodenal tissue (0–5 cm from the pylorus) was removed and cut longitudinally to expose the mucosa. The tissue was mounted in Ussing chamber with an exposure area of 0.3 cm^2. In some experiments, a Caco-2 monolayer in Snapwell was mounted in Ussing chamber with an exposed surface area of 1.13 cm^2. Viability of the mounted epithelium was continuously monitored by measuring transepithelial potential difference and short circuit current as described previously [9,10]. To investigate the transcellular calcium transport, the duodenal tissue or Caco-2 monolayer was bathed on both sides with 3 ml normal bathing solution containing (in mmol/l) 118 NaCl, 4.7 KCl, 1.25 $CaCl_2$, 1.1 $MgCl_2$, 23 $NaHCO_3$, 12 D-glucose, and 2 mannitol (all purchased from Sigma). The solution was aerated with 5% CO_2 in 95% O_2 at 37 °C, and had osmolality of ~290 mmol/kg H_2O. After 10-min equilibration, the apical (mucosal) chamber was filled with bathing solution containing ^{45}Ca (360 mCi/mol; catalog no. NEZ013; PerkinElmer, Boston, MA, USA). Unidirectional calcium flux ($J_{H\to C}$; nmol h^{-1} cm^{-2}) from the hot side (H; apical side) to the cold side (C; basolateral side) was calculated by Eqs. (1) and (2).

$$J_{H\to C} = R_{H\to C}/(S_H \times A) \quad (1)$$

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