Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 354 (2007) 8–13
BBRC
www.elsevier.com/locate/ybbrc

Role of the cytoskeleton in extracellular calcium-regulated PTH release

Stephen J. Quinn *, Olga Kifor, Imre Kifor, Robert R. Butters Jr., Edward M. Brown

Division of Endocrinology, Diabetes and Hypertension and the Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

Received 1 December 2006
Available online 28 December 2006

Abstract

The calcium-sensing receptor (CaR) mediates the effects of extracellular calcium ($[Ca^{2+}]_o$) on PTH release, such that increasing levels of $[Ca^{2+}]_o$ inhibit PTH secretion through poorly defined mechanisms. In the present studies, immunocytochemical analysis demonstrated that F-actin, PTH, CaR, and caveolin-1 are colocalized at the apical secretory pole of PT cells, and subcellular fractionation of PT cells showed these proteins to be present within the secretory granule fraction. High $[Ca^{2+}]_o$ caused F-actin, PTH, and caveolin-1 to move to the apical pole of the cells. Depolymerization of F-actin by cytochalasin reduced the actin network and induced redistribution of actin/caveolin-1 to a dispersed pattern within the cell. The F-actin-severing compounds, latrunculin and cytochalasin, significantly increased PTH secretion, while the actin polymerizing agent, jasplakinolide, substantially inhibited PTH secretion. We have demonstrated that in polarized PT cells, the F-actin cytoskeleton is involved in the regulation of PTH secretion and is critical for inhibition of PTH secretion by high calcium.

Keywords: Calcium-sensing receptor; PTH; Calcium; Cytoskeleton; Parathyroid cells

The parathyroid (PT) glands play a central role in calcium homeostasis by modulating parathyroid hormone (PTH) secretion in response to small changes in the extracellular free Ca^{2+}concentration ($[Ca^{2+}]_o$) [1]. Interestingly, CaR agonists activate transduction pathways that typically increase hormone secretion but result in inhibition of PTH secretion in PT cells [2]. Regulated exocytosis is the process by which a physiologic stimulus leads to the fusion of storage vesicles with the plasma membrane and the subsequent release of secretory products into the extracellular space [8]. In most secretory cells, the cytoplasmic face of the apical membrane is closely associated with a dense network of actin filaments and actin-binding proteins [9,10]. During cell activation, this dense F-actin network is depolymerized to allow fusion of secretory vesicles with the plasma membrane, often associated with a rise in the cytosolic calcium concentration $[Ca^{2+}]_i$. Caveolae are flask-like invaginations of the plasma membrane that serve as repositories for signaling molecules, tend to collect in actin-rich regions of secretory cells, and contain a number of actin-binding proteins, including filamin-A, which can interact directly with both caveolin-1 and actin [11,12]. We previously showed that PT cells express caveolin-1, that caveolae are the major site of localization of the cell surface CaR, and that filamin-A is a binding partner of the CaR [15–17]. In this paper we show that actin polymerization and depolymerization appear to play a critical role in the regulation of PTH secretion. These findings demonstrate that actin, caveolin-1, filamin A, CaR, and PTH colocalize at the apical secretory pole of PT cells, where the secretory vesicles interact with the actin cytoskeleton to play a key role in $[Ca^{2+}]_o$-regulated PTH release.

Materials and methods

Materials. Protease-free bovine serum albumin (BSA) and protease inhibitors were from Roche (Indianapolis, IN). We obtained the Renaissance ECL system from Perkin-Elmer Life Science Products

* Corresponding author. Fax: +1 617 732 5764.
E-mail address: squinn@rics.bwh.harvard.edu (S.J. Quinn).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.12.160

(Boston, MA). Cell culture medium (DMEM Ham's F-12), 5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt (BCIP), and nitroblue tetrazolium chloride (NBT) were purchased from Gibco-BRL (Gaithersburg, MD). Monoclonal antibodies included those to caveolin-1, caveolin-2, and phospho-caveolin from BD Biosciences Transduction Laboratories (Lexington, KY). A polyclonal antibody to caveolin-1 was from Santa Cruz Biochemicals (San Francisco, CA). A rat PTH antibody (directed to the N-terminus) and a monoclonal antibody to chromogranin A were from NeoMarkers (Fremont, CA), and a goat antibody to the PTH C-terminus was from CHEMICON (Temecula, CA). A monoclonal anti-filamin-A antibody was a gift of Drs. John Hartwig and Thomas Stossel (Brigham and Women's Hospital, Boston MA). Biotinylated anti-mouse IgG and Vectashield mounting medium were from Vector Lab. (Burlingame, CA). CaR-specific polyclonal antisera to peptides based on the CaR sequence were raised in rabbits. Antiserum 4637 produced to amino acids 345–359 was a generous gift of NPS Pharmaceuticals (Salt Lake City, UT). Goat anti-mouse IgG coupled to Alexa 568 and goat anti-rabbit IgG coupled to Alexa 488 were purchased from Molecular Probes (Eugene, OR). The TSA™ Cyanine 5 system was from Perkin-Elmer (Boston, MA).

Cell preparation and incubations. Dispersed bovine PT cells were prepared by collagenase and deoxyribonuclease digestion of glands as described previously [17]. For determinations of PTH release, PT cells were used immediately as acutely dispersed cells. The cells were washed with Eagle's minimal essential medium (EMEM) containing 0.5 mM $[Ca^{2+}]_o$, 0.5 mM $[Mg^{2+}]_o$, and 0.2% BSA, and were subsequently incubated at 37 °C with various concentrations of $[Ca^{2+}]_o$ or other additives as detailed in Results.

Immunocytochemistry. Dispersed PT cells were allowed to attach to glass coverslips coated with or without Matrigel (Becton–Dickinson, Bedford, MA), and the adherent cells were incubated at 37 °C. The cells were fixed, permeabilized, and incubated with blocking solution as described previously [16,17]. For triple immunofluorescence staining, the slides were incubated with a mixture of primary antibodies in blocking solution overnight at 4 °C in a humidified chamber. Fluorescence images were viewed using the 100× objective of a Bio-Rad MRC 1024/2P multiphoton microscope equipped with krypton and argon lasers at the Brigham and Women's Hospital Confocal Microscopy Core facility.

Purification of caveolin-rich membrane fractions. Fractions enriched in caveolin were purified as described previously [16,17]. Fractions (1.3 ml) were removed sequentially from the top of the gradient and designated as fractions 1–9 (fractions 2–3 and 6–9 are considered to be of Triton X-100-insoluble caveolar and Triton X-100-soluble non-caveolar origin, respectively). Fractions were subjected to SDS–PAGE and immunoblotting or to PTH determination.

Electrophoresis and immunoblots. Western blot analysis was performed essentially as described previously [15–17]. After preparation of fractions enriched in caveolin-1, equal amounts of protein were separated by SDS–PAGE and transferred to nitrocellulose filters (Schleicher and Schuell, Keene, NH). The blots were subsequently incubated with blocking solution (PBS with 0.25% Triton X-100 and 5% non-fat dry milk) for 1 h at room temperature. The membranes were incubated overnight with primary antiserum and, after washing, with secondary antibodies. The bands were visualized by chemiluminescence. Protein concentration was measured with Micro BCA protein reagent kit (Pierce Chemical Co., Rockford, IL).

Determination of Ca^{2+}-regulated PTH release. Dispersed BPT cells were incubated with 0.5 mM $[Ca^{2+}]_o$ and varying concentration of $[Ca^{2+}]_o$ (0.5–2.0 or 3.0 mM) for 1 h at 37 °C. PTH released into the medium from BPT cells was quantified using a whole rat PTH immunoradiometric assay kit (ALPCO, Salem, NH).

Statistics. The data are presented as means ± SEM of the indicated number of experiments. Statistical software was used to analyze the results by one-way analysis of variance (ANOVA) with the Student–Newman–Keuls Method. A p value of <0.05 was considered to indicate a statistically significant difference.

Results

The regulated secretory pathway in PT cells

Our previous work showed localization of the CaR in the caveolin-1-rich fraction of the plasma membrane of bovine parathyroid cells (BPT) [15–17], suggesting a close association with the secretory pathway in PT cells. Here we analyzed the localization of the CaR relative to that of PTH in BPT cells incubated with low or high $[Ca^{2+}]_o$ (Fig. 1). At low $[Ca^{2+}]_o$, the immunostaining of the CaR is distributed both in the plasma membrane, particularly

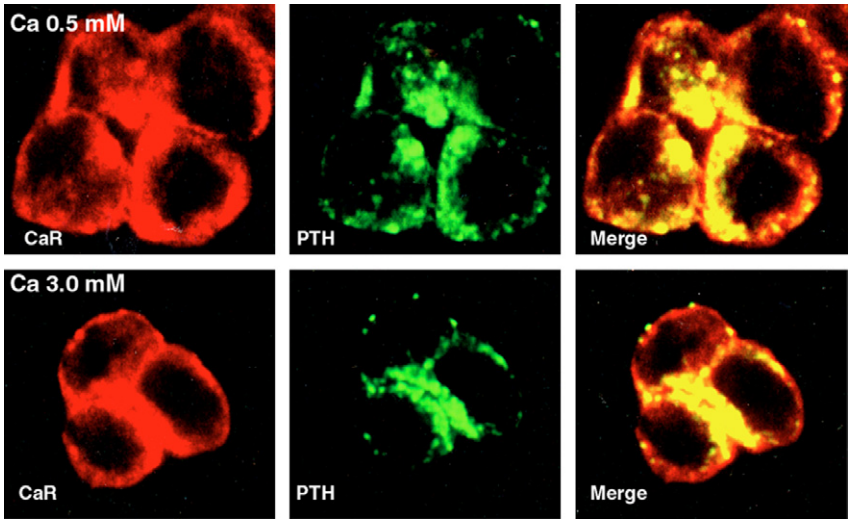

Fig. 1. Confocal analysis detects apical surface expression of CaR and PTH. PT cells were incubated with 0.5 or 3.0 mM $[Ca^{2+}]_o$ in standard medium for 10 min, fixed and stained for the CaR (red) and PTH (green). Yellow color in Merge indicates colocalization of CaR and PTH. Magnification is 1000×. The data are representative of 3 separate experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)