Chromogranin A-derived parastatin peptides as autocrine inhibitors of endocrine secretion.

Brigitte H. Fasciotto Dunn and David V. Cohn

Department of Molecular, Cellular and Craniofacial Biology, University of Louisville Health Sciences Center, Kentucky, USA.

Correspondence: Dr. Brigitte H. Fasciotto Dunn, ElectroOptics Research Institute & Nanotechnology Center, Lutz Hall, Room 441, University of Louisville, Louisville, KY 40292, USA.
Phone: 502-852-7308; **Fax:** 502-852-1577; **Email:** bhfasc01@louisville.edu

Cell Biology of the Chromaffin Cell
R. Borges & L. Gandía Eds.
Instituto Teófilo Hernando, Spain, 2004

Parathyroid hormone (PTH) and chromogranin A (CgA) represent the two major calcium-regulated secretory proteins of the parathyroid gland. Secretion of PTH and CgA in the parathyroid occurs from a "storage/mature granule" pool and from a "new/immature granule" pool[1]. Upon stimulation by hypocalcaemia, parathyroid cells secrete preferentially newly synthesized PTH and CgA without prior equilibration with the mature granule pool.

CgA is a highly acidic, glycosylated protein, widely distributed in the endocrine and neuroendocrine system. CgA is a precursor of bioactive peptides including parastatin (PARA, $pCgA_{347-419}$) that inhibits parathyroid cell secretion[2]. We recently reported that porcine parathyroid cells produce and secrete bioactive PARA-related peptides, suggesting that these peptides may act as autocrine inhibitors[3].

Several mammalian subtilisin-like serine proteases have been described that process proproteins to biologically active hormones. It has been reported that furin and PC7, but not PC1 and PC2, are candidates for processing of pro-PTH in the parathyroid. Porcine CgA contains one consensus sequence RRGWR at residues 364-368 that may be a potential furin/PC7 cleavage site. To determine if furin participates in CgA processing, purified pCgA was incubated *in vitro* with purified furin for 48 hours at 37C. Furin converted pCgA to small peptides within 24 to 48 hours (not shown). Without furin, pCgA remained unprocessed.

If PARA peptides act as physiological autocrine inhibitors of parathyroid secretion, blockage of CgA processing should release cells from autoinhibition, resulting in an enhanced PTH secretion, as we previously showed with CgA antiserum[4]. To test this, we used the cell-permeant furin inhibitor decanoyl-RVKR-chloromethylketone (dec-RVKR-CMK). Porcine parathyroid cells were stimulated at 0.5 mM Ca for 7 h in presence or absence of 100 μM dec-RVKR-CMK. Dec-RVKR-CMK potentiated secretion of both immunoactive PTH (iPTH, Fig. 1-A, left panel) and immunoactive CgA (iCgA, Fig. 1-A, right panel) 3-fold or more. Palm-FAKR-CMK that does not contain the furin recognition site was only one-tenth as potent as dec-RVKR-CMK (not shown).

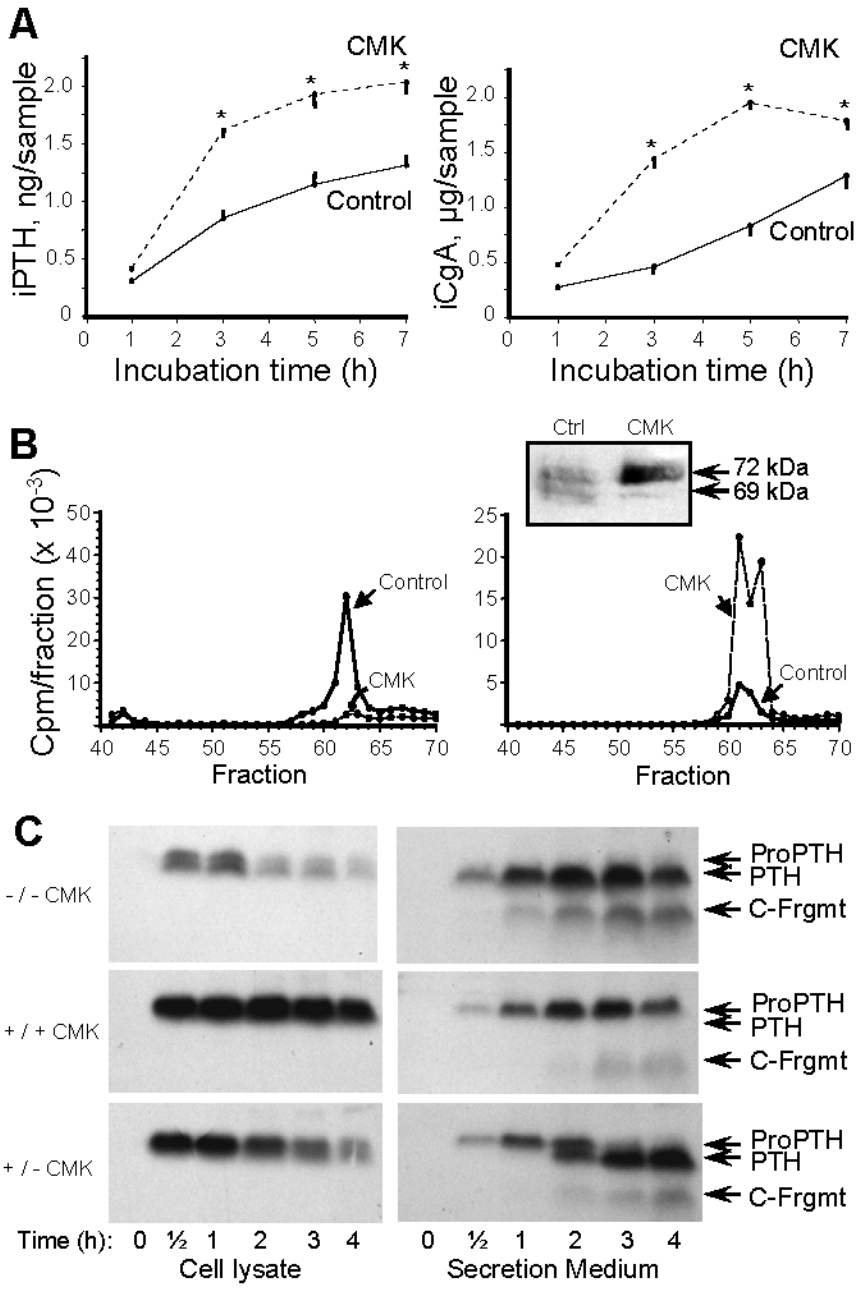
A
CMK
Control
iPTH, ng/sample
Incubation time (h)
CMK
Control
iCgA, µg/sample
Incubation time (h)
B
Cpm/fraction (x 10-3)
Control
CMK
Fraction
Ctrl
CMK
72 kDa
69 kDa
CMK
Control
Fraction
C
- / - CMK
+ / + CMK
+ / - CMK
ProPTH
PTH
C-Frgmt
Time (h): 0 ½ 1 2 3 4
Cell lysate
0 ½ 1 2 3 4
Secretion Medium

Figure 1. A) Parathyroid cells were incubated at 0.5 mM Ca in absence (Control) or presence of 100 μM dec-RVKR-CMK (CMK). Secreted iPTH (left panel) was measured using the C-mid molecule RIA kit from Nichols Institute, and iCgA (right panel) was measured by RIA using bCgA antiserum generated in our laboratory. * $p<0.001$ vs Control. **B)** Parathyroid cells were labeled at 0.5 mM Ca for 6 h in absence (Control) or presence of 100 μM dec-RVKR-CMK (CMK). Secretion media (left panel) and cell lysates (right panel) were immunoprecipitated using bCgA antiserum. Precipitates were separated by HPLC and fractions were analyzed for radioactivity. **C)** Parathyroid cells were either preincubated then labeled in absence (-/- CMK), or preincubated then labeled in presence (+/+ CMK), or preincubated in presence then labeled in absence (+/- CMK) of 100 μM dec-RVKR-CMK. Cell lysates (left panels) and secretion media (right panels) were immunoprecipitated using PTH antiserum. Precipitates were analyzed by SDS-PAGE and autoradiography. Positions of pro-PTH, PTH and PTH C-fragment are indicated.

We then studied the effect of dec-RVKR-CMK on the secretion of newly synthesized, radioactive CgA (Fig. 1-B) and PTH (Fig. 1-C). Parathyroid cells were stimulated in medium containing ^{3}[H]-Leu. Dec-RVKR-CMK decreased secretion of newly synthesized CgA at 0.5 mM Ca (Fig. 1-B, left panel). This is in contrast with the stimulation of immunoactive CgA observed in Fig 1-A. The decreased secretion of CgA was accompanied by an intracellular build-up of a 72 kDa CgA form (Fig. 1-B, right panel). Immunoprecipitation with PTH antiserum shows that dec-RVKR-CMK blocked the conversion of pro-PTH to PTH (Fig. 1-C, +/+ CMK). Newly synthesized pro-PTH accumulated intracellularly and no PTH or C-fragment was detected. No PTH was detected in the secretion medium. The effect of dec-RVKR-CMK was reversible (Fig. 1-C, +/- CMK). After removal of dec-RVKR-CMK at the beginning of the labeling period, pro-PTH was converted to PTH and then secreted, with a time lag of 2 h, the time required for synthesis and activation of new furin.

The data show that furin is involved in the conversion of pro-PTH to PTH and in the processing of CgA in parathyroid cells. The intracellular accumulation of newly synthesized pro-PTH and CgA in the presence of dec-RVKR-CMK suggests that inhibition of furin, a TGN resident protease, may affect the intracellular trafficking of secretory proteins in parathyroid cells. For proteins that have passed into the storage secretory granules, as measured for secretion of iPTH and iCgA, inhibition of furin affects the regulation of secretion. We

propose that inhibition of CgA processing, decreases the amount of PARA available to inhibit secretion. This results in increased secretion of proteins that are stored in secretory granules. These results agree with the hypothesis that CgA-derived peptides serve as autocrine inhibitors of parathyroid secretion. Autocrine regulation of parathyroid secretion could explain the physiological pulsatile mode of PTH secretion *in vivo*[5].

REFERENCES

1. Morrissey, J.J. and D.V. Cohn, *Secretion and degradation of parathormone as a function of intracellular maturation of hormone pools*. *Modulation by calcium and dibutyryl cyclic AMP.* J Cell Biol, 1979. **83**:521-528.
2. Fasciotto, B.H., et al., *Parastatin (porcine chromogranin A347-419), a novel chromogranin A-derived peptide, inhibits parathyroid cell secretion.* Endocrinology, 1993. **133**:461-466.
3. Fasciotto, B.H., et al., *Processing of chromogranin A in the parathyroid: generation of parastatin-related peptides.* Peptides, 2000. **21**:1389-1401.
4. Fasciotto, B.H., et al., *Autocrine regulation of parathyroid secretion: inhibition of secretion by chromogranin-A (secretory protein-I) and potentiation of secretion by chromogranin-A and pancreastatin antibodies*. Endocrinology, 1990. **127**:1329-1335.
5. Harms, H.M., et al., *Pulse amplitude and frequency modulation of parathyroid hormone in plasma.* J Clin Endocrinol Metab, 1989. **69**:843-851.