Examination of PACAP-38 in different milk and infant formula samples and PAC1-receptors in mammary gland

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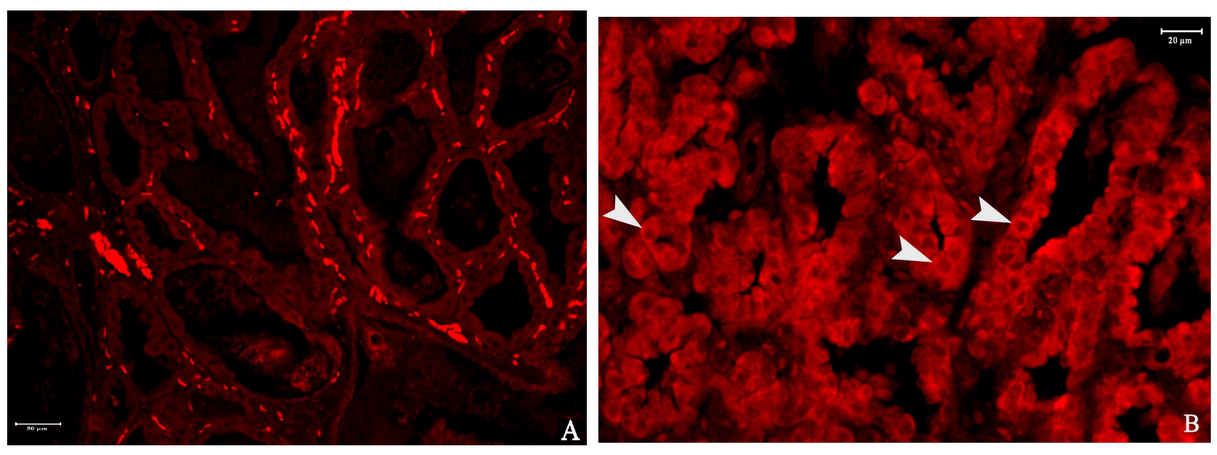
Introduction

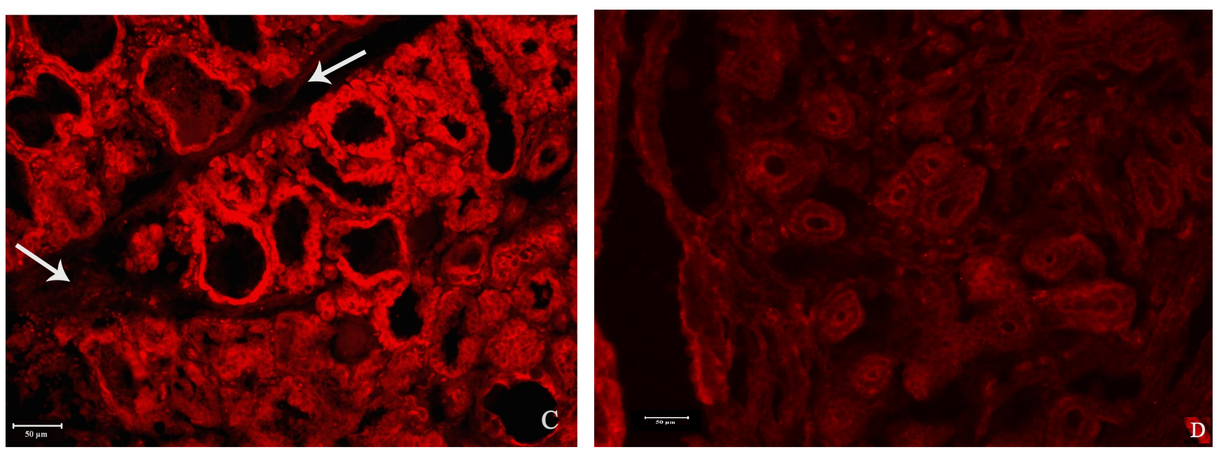
Pituitary adenylate cyclase activating polypeptide (PACAP) is a pleiotropic and multifunctional neuropeptide. It belongs to the vasoactive intestinal peptide (VIP)/secretin/glucagon peptide family. Recently, we have provided evidence that PACAP is present in human placenta, seminal fluid, follicular fluid and cerebrospinal fluid samples. We have identified the peptide in the milk in much higher concentrations than in the respective plasma samples. We showed PACAP-38-like immunoreactivity (LI) in sheep mammary gland samples with radioimmunoassay (RIA) and we detected PAC1-receptor expression in the lactating udder biopsies by immunohistochemistry.

The aim of the first part of the study was to investigate the changes in PACAP-38-LI in human milk during lactation with RIA. In the second part of the study we examined the presence of PACAP-38 in cow milk and cow milk-based commercial infant formulas by mass spectrometry and RIA. Finally, in the third part of the study we compared the presence of PAC1-receptors in lactating and non-lactating udder biopsies of sheep and normal human mammary gland samples with immunohistochemistry.

Material and methods

Human milk was obtained from voluntary mothers at different stages of lactation. Bovine milk was collected on different postpartum days. Infant formula samples were purchased on the Hungarian market. Udder biopsy samples were taken from lactating ewes and the PAC-1 receptor expression was investigated by immunohistochemistry. PACAP-38-LI was measured by RIA, for identify PACAP-38 in the infant formulas MALDI TOF/TOF mass spectrometry was used.





The glandular epithelial cells express clear and intensive immunopositivity of the PAC1-receptor, particularly in the membrane of these cells (arrowheads, B), but granular immunostaining could also be detected in the cytoplasmic region. Panel C represents the typical segmental structure of the mammary gland where the main part of the tissue shows remarkable PAC1-receptor labeling, but the connective tissue sections between the segments are negative (arrows). Decreased PAC1-receptor immunpositivity was detected from non-lactating mammary gland (A). The human breast samples showed the same PAC1-receptor expression as the non lactating samples (D). Scale bars: 50 µm in A, C, D and 20 µm in B.

Results

No significant difference could be observed in the PACAP-38-LI of milk samples during the first 7 months of lactation (Fig. 1).

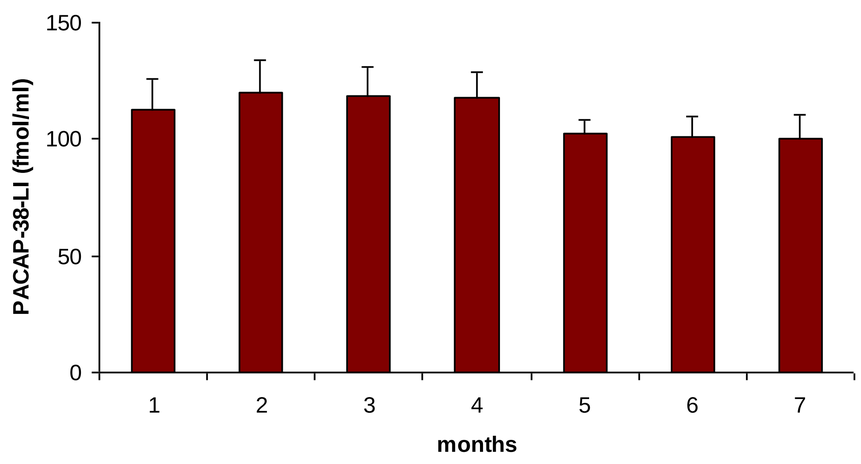


Figure 1.: PACAP-38-LI of humna milk samples during the first 7 months of lactation.

PACAP-38-LI did not show significant changes within the examined 10-month-period of lactation after delivery, but a significant increase was detected after that period compared to the levels of the first 3 months.*p<0,05; ***p<0,001 vs PACAP-38 LI in 1-3 months samples (Fig. 2).

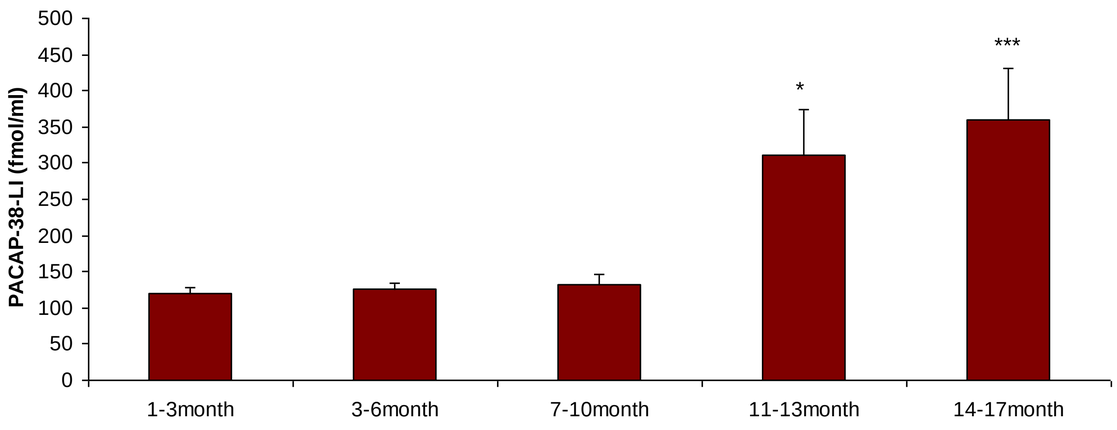


Figure 2.: PACAP-38-LI of human milk samples during the first 17 months of lactation.

MALDI TOF spectrum of a hypoallergenic infant formula sample in positive ion mode using linear detection indicating the average peak of protonated quasimolecular ion [M+H+] of PACAP-38. The result of mass spectrometry could prove the presence of PACAP-38 in the infant formula (Fig. 3).

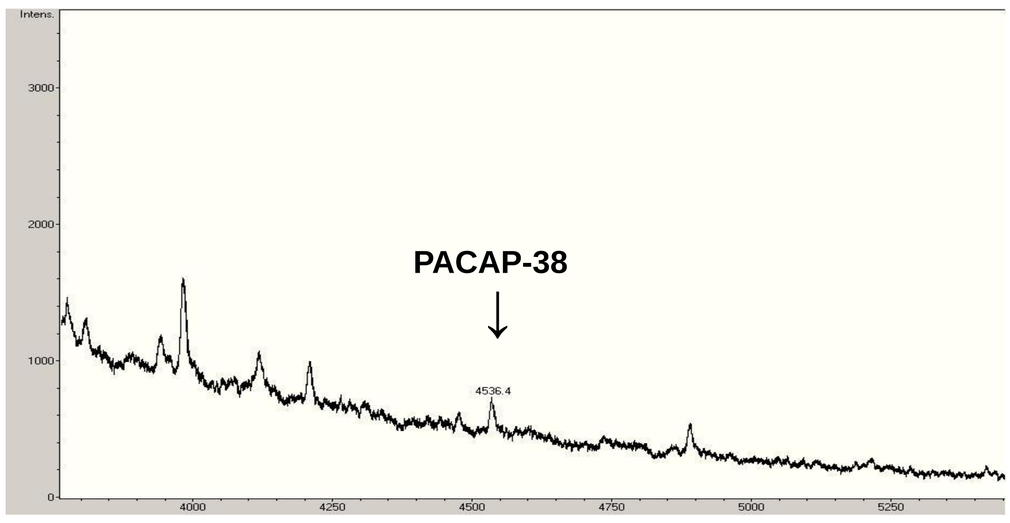


Figure 3. MALDI TOF spectrum of a hypoallergenic infant formula sample.

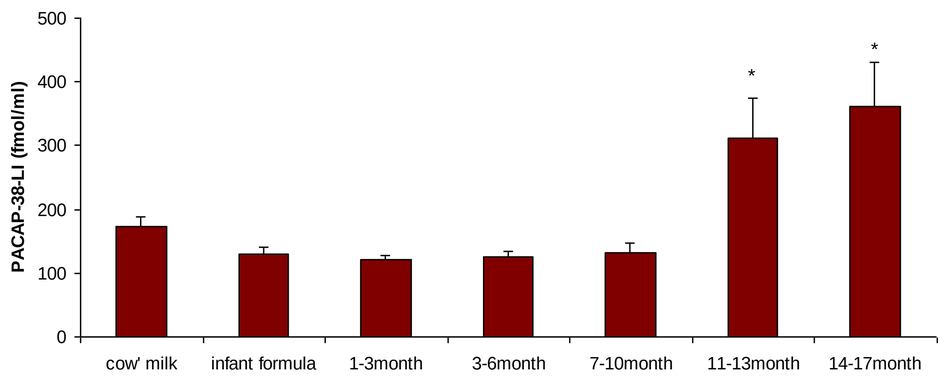


Figure 4.: PACAP-38-LI of humnan milk samples during the first 17 months of lactation, cow milk samples and infant formula samples.

The cow milk, infant formula and human milk in the first 1-10 months of lactation showed similar PACAP-38-LI with RIA. The PACAP-38-LI of the human milk in the 11-17 month of lactation was significantly elevated compared to the infant formulas. *p<0,05 vs PACAP-38 LI in the infant formula samples.

Our preliminary results showed that bovine foremilk contains increased PACAP-38-LI compared to normal bovine milk.(Fig. 4)

Discussion

The composition of the milk is not constant during lactation, it varies continually according to the physiological demand of the newborn. PACAP-38-LI shows similar values in human milk samples during the first 6 months of lactation. With the progress of lactation the decreased amount of milk production is compensated by the increased level of PACAP-38 in the milk after the 10th month. The composition of bovine milk differs from the human milk, however the PACAP-38-LI is the same. The majority of available infant formulas are made of cow milk. We determined the presence of PACAP-38 and measured its concentration in industrially modified infant formulas, which concentrations were the same as in cow milk samples. The precence of PACAP-38 in different milk samples and increased expression of PAC1-receptors in lactating mammary gland samples also indicates the important roles of PACAP in the lactation and might be required for the growth and development of the newborn. (OTKA K72592, 73044, CNK78480, TAMOP 4.2.2/B-10/1-2010-0029, Bolyai Scholarship, Richter Foundation, PTE ÁOK Grant SROP-4.2.2/B-10/1-2010-0029).