Praca oryginalna *Original paper*

Detection of gonadotropin-releasing hormone and somatostatin-14 containing cells in the mammary gland of guinea pigs

BERRİN GENCER TARAKCI, MİNE YAMAN, ALİ BAYRAKDAR

Department of Histology, Faculty of Veterinary Science, University of Firat, 23119 Elazig, Turkey

Gencer Tarakcı B., Yaman M., Bayrakdar A.

Detection of gonadotropin-releasing hormone and somatostatin-14 containing cells in the mammary gland of guinea pigs

Summary

The existence of numerous neuropeptides in milk is well established. It is still unclear whether these neuropeptides are produced by the mammary gland or that the gland concentrates them from the general circulation. The aim of the study was to examine the possible localization of these neuropeptides in the mammary gland of guinea pigs at different physiological states by immunohistochemistry. Specific primers have been used for the somatostatin, vasoactive intestinal polypeptide, neurotensin, cholecystocinin, oxytocin, gonadotropin-releasing hormone and growth hormone. Among all the neuropeptides that have been examined gonadotropin-releasing hormone and somatostatin immunoreactivity were found in the mammary gland of lactating guinea pigs, but not in virgin and pregnant guinea pigs. Immunoreactivity was observed in the epithelial cells that compose the secretory alveoli and in the secretory material.

Keywords: neuropeptides, mammary gland, guinea pig

The knowledge of the expression of several neuropeptides in the mammary gland is very limited. Although there are a few papers dealing with the existence of neuropeptides in the gland, the presence of neuropeptides in the milk of several species, in concentrations that exceed those in maternal plasma, has been reported by many studies. Gonadotropin-releasing hormone (GnRH) was initially identified in milk (3), and since then numerous other neuropeptides such as oxytocin (12), neurotensin (29), vasoactive intestinal polypeptide (VIP) (28), somatostatin (SOM)-14 (26, 27), growth hormone-releasing hormone (GHRH) (25, 27), cholecystokinin (CCK) (4, 5) and melotonin (8) have been found to be present in the milk of several species. These findings imply that milk is not just a nutrient source but also a carrier of substances that can play a role in the development physiology of the neonate.

It is still unclear whether these neuropeptides are produced by the mammary gland or that the gland concentrates them from the general circulation. Recently, the expression of GnRH gene in the mammary gland of pregnant and lactating rats (14) and, in addition to GnRH, the expression of SOM in the mammary gland of lactating rats have been demonstrated (5).

Immunolocalization of relaxin in guinea pig mammary glands has been demonstrated (16). Except for this function, the identification of several neuropeptides in guinea pig mammary glands is not fully know. The aim of the study was to examine the possible localization of several neuropeptides in the mammary gland of guinea pigs at different physiological states using immunohistochemical staining methods.

Material and methods

Six female guinea pigs at different physiological states (2 each of pregnant, lactating and virgin guinea pigs) were studied. After anesthesia with ether, mammary glands were taken. Samples were fixed in 10% neutral buffered formaldehyde for 24 hour, and then dehydrated through graded ethanol and embedded in paraffin. 5 μm thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemical staining was carried out by using the peroxidase-antiperoxidase (PAP) method. Blocking of endogenous peroxidase was carried out with 0.0025% hydrogen peroxide in methanol for 10 minutes (22). In order to block unspecific binding, an incubation with (1 : 10) normal goat serum in 0.1 M phosphate buffered saline (PBS), pH 7.2 was performed.

PAP technique. Sections were incubated for 16-20 hours at 4°C in rabbit anti-luteinizing hormone releasing

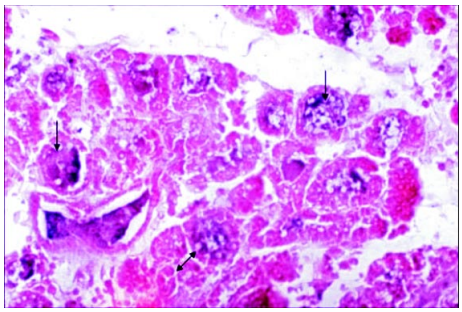

Fig. 1. Immunohistochemical localization of the gonadotropin releasing hormone in the mammary gland (MG) of lactating guinea pigs. The guinea pig MG shows intense staining for gonadotropin releasing hormone throughout the cytoplasm of the epithelial cells (⇔). Some of the alveoli are packed with secretory material (arrows) showing intense staining (× 200)

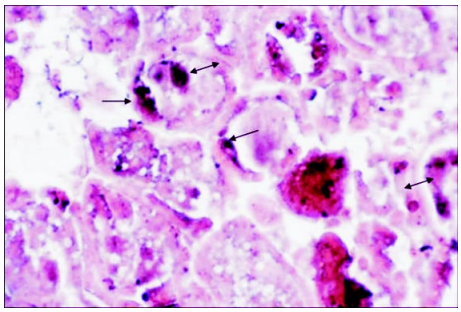

Fig. 2. Immunohistochemical localization of somatostatin-14 in the mammary gland of lactating guinea pig. Somatostatin immunoreactivity was seen throughout the cytoplasm of the epithelial cells (arrows). Some of the alveoli are packed with secretory material (⇔) (× 400)

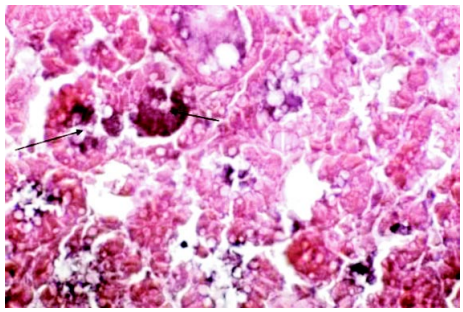

Fig. 3. Somatostatin immunoreactivity in the secretory materials of lactating guinea pig mammary gland (arrows) (× 400)

hormone (AB1567 Chemicon), rabbit anti-somatostatin-14 IgG (AB1976 Chemicon), rabbit anti-somatostatin-28 IgG (AB1752 Chemicon), rabbit anti-vasoactive intestinal polypeptide IgG (AB982 Chemicon), rabbit anti-neurotensin (AB5496 Chemicon), rabbit anti-growth hormone releasing hormone (AB1751 Chemicon), rabbit anti-oxytocin (AB911 Chemicon) and rabbit anti-cholecystokinin (AB1973 Chemicon). Antibodies were diluted to 1 : 20, 1 : 20, 1 : 20, 1 : 50, 1 : 50, 1 : 50 and 1 : 50 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin respectively. Sections were then incubated in goat anti-rabbit IgG (Dako, Z0421, Denmark), followed by rabbit peroxidase anti-peroxidase complex (Zymed Lab., 61.2003, San Francisco), both at a dilution of 1 : 50 in PBS, for 1 hour at room temperature. Sections were washed in PBS for 30 minutes after each incubation and finally immersed in glucose oxidise-DAB-nickel ammonium sulphate substrate (20) for 10 minutes. After washing in distilled water and counterstaining with eosin, sections were dehydrated and coverslips mounted with DPX. The specificity of each immunohistochemical reaction was determined as recommended by Sternberger (23) including the replacement of specific antiserum preincubated with its corresponding antigen. Sections were examined with light microscope and photographs were taken.

Results and discussion

Gonadotropin-releasing hormone and somatostatin--14 immunoreactivity were found in mammary glands of lactating guinea pigs. No immunoreactivity was observed in mammary glands of virgin and pregnant guinea pigs.The immunocytochemical localization of GnRH and SOM-14 in mammary glands shows intense staining for GnRH and SOM-14 throughout the epithelial cells cytoplasm and in the secretory material (fig. 1, 2, 3).

The presence of numerous neuropeptides in mammalian milk and the expression of GnRH gene in pregnant and lactating rat mammary glands (14) as well as identification of somatostatin gene in rat mammary glands during lactation (5) raises the question concerning the origin of the other neuropeptides that are present in the milk.

The present study has demonstrated the immunohistochemical localization of GnRH and somatostatin-14 in guinea pig mammary glands during the lactation period. All other neuropeptides were not found in mammary glands in any periods.

GnRH is the primary regulator of the reproductive system. This neurohormone is synthesized in hypothalamic neurosecretory cells and triggers the secretion of gonadotropic hormones luteinizing hormones and follicle-stimulating hormones from the anterior pituitary (10). GnRH is also synthesized by many non-hypothalamic tissues such as placenta, (19, 24), gonads (2, 13) and mammary glands (1) of different kinds of mammals. GnRH that is synthesized by a lactating mammary gland could have multiple physiological roles and may assume biological activities that are still unknown. GnRH that is synthesized by the mammary gland may exert its bioactivity on the suckling pups as well as on the mother. When suckling was prevented, serum LH concentrations dropped to about 30.6%, and when suckling was allowed to resume, the pup's serum LH concentrations were restored within 1 h (3). These observations suggest that at least part of the milk GnRH is absorbed from the gastrointestinal tract of the suckling pup in a biologically active form. Indeed, the gastrointestinal tract of the neonatal mammals is largely permeable and allows the transport of peptides and proteins across the intestinal epithelium (11). Similar results were also obtained in another study that demonstrated that milk GnRH may have a modulatory role on the development of the infantile rat ovary (21). GnRH synthesized by the mammary gland may function as a paracrine agent within the mammary gland or on the anterior pituitary of the mother. It seems that the mammary glands serve as complementary organs in a mechanism through which the mother exercises control over the infants development and metobolism. Milk provides the mechanism by which regulatory

information is transferred from the mother to the progeny.

Somatostatin is probably the most known neuropeptide and has been reported in neurons of the hypothalamus, limbic system, brain stem, spinal cord and throughout the cerebral cortex (17). Somatostatin is also secreted from different parts of the gastrointestinal tract and from the endocrine pancreas (18). Somatostatin was first discovered in human and sheep milk (26). In the later studies, somatostatin levels in milk were found several fold higher than those present in the maternal plasm (7, 9, 26).

The mammalian somatostatin precursor, prosomatostatin, is a 92 aminoacide protein that is processed posttranslationally by proteinases to produce somatostatin 14 and somatostatin 28. The production of the two somatostatins is independent of each other and somatostatin 14 cannot be processed from somatostatin 28 (15). We have found SOM-14 immunoreactivity in the mammary glands of lactating guine pigs whereas SOM-28 is not present. These finding are compatible with previous findings (7, 26, 27) that have reported the existance of SOM-14, but not of SOM-28, in milk.

SOM-28, vasoactive intestinal polypeptide, neurotensin, cholecystocinin, oxytocin and growth hormone immunoreactivity was not detected in the mammary glands of virgin, lactating and pregnant guinea pigs.

The present study demonstrated that GnRH and somatosatatin is produced by the mammary gland tissue during the lactation period. Evidence for a possible physiological role for somatostatin and GnRH were presented recently by Gama et al. (6) who reported that GnRH and SOM exert inhibitory effects on cell proliferation of the gastric epithelium in suckling rats. The fact that SOM and GnRH are localized in mammary glands of lactating guinea pigs may imply that regulatory peptides produced by the breast can play a major role in the developmental physiology of neonate and or on the growth and differentiation of the mammary gland itself. Furthermore the fact that GnRH and SOM, only two out of seven neuropeptides that have been studied so far, are expressed in the mammary gland of the lactating guinea pig suggest that these neuropeptides may have important physiological roles that have still to be elucidated. These results also imply that most of the peptides that are present in milk are actively concentrated from general circulation.

References

1. *Amarant T., Fridkin M., Koch Y.*: Luteinizing hormone-releasing hormone and thyrotropin-releasing hormone in human and bovine milk. Eur. J. Biochem. 1982, 127, 647-650.
2. *Aten R. F., Williams A. T., Behrman H. R.*: Ovarian gonadotropin-releasing hormone-like protein(s): demonstration and characterization. Endocrinology 1986, 118, 961-967.
3. *Baram T., Koch Y., Hazum E., Fridkin M.*: Gonadotropin-releasing hormone in milk. Science 1977, 198, 300-302.
4. *Berseth C. L., Michener S. R., Nordyke C. K., Go V. L.*: Postpartum changes in pattern of gastrointestinal regulatory peptides in human milk. Am. J. Clin. Nutr. 1990, 51, 985-990.
5. *Chen A., Laskar-Levy O., Koch Y.*: Selective expression of neuropeptides in the rat mammary gland: somatostatin gene is expressed during lactation. Endocrinology 1999, 140, 5915-5921.
6. *Gama P., Alvares E. P.*: LHRH and somatostatin effects on the cell proliferation of the gastric epithelium of suckling and weaning rats. Regul. Pept. 1996, 63, 73-78.
7. *Holst N., Jenssen T. G., Burhol P. G.*: A characterization of immunoreactive somatostatin in human milk. J. Pediatr. Gastroenterol. Nutr. 1990, 10, 47-52.
8. *Illnerova H., Buresova M., Presl J.*: Melatonin rhythm in human milk. J. Clin. Endocrinol. Metab. 1993, 77, 838-841.
9. *Kacsoh B., Terry L. C., Meyers J. S., Crowley W. R., Grosvenor C. E.*: Maternal modulation of growth hormone secretion in the neonatal rat. I. Involvement of milk factors. Endocrinology 1989, 125, 1326-1336.
10. *Koch Y., Chobsieng P., Zor U., Fridkin M., Lindner H. R.*: Suppression of gonadotropin secretion and prevention of ovulation in the rat by antiserum to synthetic gonadotropin-releasing hormone. Biochem. Biophys. Res. Commun. 1973, 55, 623-629.
11. *Koch Y., Werner H., Fridkin M.*: Hypothalamic hormones in milk. Endocr. Regul. 1991, 25, 128-133.
12. *Leake R. D., Weitzman R. E., Fisher D. A.*: Oxytocin concentrations during the neonatal period. Biol. Neonate. 1981, 39, 127-131.
13. *Oikawa M., Dargan C., Ny T., Hsueh A. J.*: Expression of gonadotropin--releasing hormone and prothymosin-alpha messenger ribonucleic acid in the ovary. Endocrinology 1990, 127, 2350-2356.
14. *Palmon A., Ben Aroya N., Tel-Or S., Burstein Y., Fridkin M., Koch Y.*: The gene for the neuropeptide gonadotropin-releasing hormone is expressed in the mammary gland of lactating rats. Proc. Natl. Acad. Sci. USA 1994, 91, 4994-4996.
15. *Patel Y. C., Galanopoulou A. S., Rabbani S. N., Liu J. L., Ravazzola M., Amherdt M.*: Somatostatin-14, somatostatin-28, and prosomatostatin[1-10] are independently and efficiently processed from prosomatostatin in the constitutive secretory pathway in islet somatostatin tumor cells (1027B2). Mol. Cell. Endocrinol. 1997, 131, 183-194.
16. *Peaker M., Taylor E., Tashima L., Redman T. L., Greenwood F. C., Bryant--Greenwood G. D.*: Relaxin detected by immunocytochemistry and northern analysis in the mammary gland of the guinea pig. Endocrinology 1989, 125, 693-698.
17. *Reichlin S.*: Medical progress: somatostatin. N. Engl. J. Med. 1983, 309, 1495-1501.
18. *Reichlin S.*: Medical progress: somatostatin. N. Engl. J. Med. 1983, 309, 1556-1563.
19. *Seeburg P. H., Adelman J. P.*: Characterization of cDNA for precursor of human luteinizing hormone releasing hormone. Nature 1984, 18-24, 311, 666-668.
20. *Shu S., Ju G., Fan L.*: The glucose oxidase-DAB-nikel method in peroxidase histochemistry of the nervous system. Neurosci. Lett. 1988, 85, 169-171.
21. *Smith S. S., Ojeda S. R.*: Maternal modulation of infantile ovarian development and available ovarian luteinizing hormone-releasing hormone (LHRH) receptors via milk LHRH. Endocrinology. 1984, 115, 1973-1983.
22. *Sternberger L. A.*: Immunocytochemistry John Wiley, New York 1986.
23. *Sternberger L. A.*: The Unlabelled Antibody Peroxidase-antiperoxidase (PAP) method, [in:] Sternberger L. A. (Ed.): Immunocytochemistry. John Wiley, New York 1979, p. 104-169.
24. *Tan L., Rousseau P.*: The chemical identity of the immunoreactive LHRH--like peptide biosynthesized in the human placenta. Biochem. Biophys. Res. Commun. 1982, 109, 1061-1071.
25. *Werner H., Amarant T., Fridkin M., Koch Y.*: Growth hormone releasing factor-like immunoreactivity in human milk. Biochem. Biophys. Res. Commun. 1986, 135, 1084-1089.
26. *Werner H., Amarant T., Millar R. P., Fridkin M., Koch Y.*: Immunoreactive and biologically active somatostatin in human and sheep milk. Eur. J. Biochem. 1985, 148, 353-357.
27. *Werner H., Katz P., Fridkin M., Koch Y., Levin S.*: Growth hormone releasing factor and somatostatin concentrations in the milk of lactating woman. Eur. J. Pediatr. 1988, 147, 252-256.
28. *Werner H., Koch Y., Fridkin M., Fahrenkrug J., Gozes I.*: High levels of vasoactive intestinal peptide in human milk. Biochem. Biophys. Res. Commun. 1985, 133, 228-232.
29. *Werner S., Widstrom A. M., Wahlberg V., Eneroth P., Winberg J.*: Immunoreactive calcitonin in maternal milk and serum in relation to prolactin and neurotensin. Early Hum. Dev. 1982, 6, 77-82.

Author's address: Berrin Gencer Tarakcı, Department of Histology, Faculty of Veterinary Science, University of Firat, 23119 Elazig, Turkey; e-mail: btarakci@firat.edu.tr