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Neuroendocrine Peptides in Milk

Eli Hazum

Milk, which is a mammal-specific biologic fluid, contains several neuroendocrine peptides at concentrations higher than those found in plasma. These neuroendocrine peptides can be synthesized or processed in the mammary gland or excreted into milk through various pathways. In addition, certain milk proteins, notably casein, can be enzymatically processed to release "exorphins," peptides with opioid activities. In suckling mammals, hormones and neuropeptides are absorbed through the gastrointestinal tract and appear intact in the plasma. This absorption is age dependent and could have physiologic significance in neonatal development.

Lactation is the final phase of the complete reproductive cycle of mammals. The process of lactation may be divided into at least three stages: mammogenesis, that is, mammary growth and development; lactogenesis, milk formation and secretion; and milk ejection. Secretion of milk is a hormonally regulated process that reflects the highly coordinated actions of endocrine, neuroendocrine, and behavioral mechanisms.

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In almost all mammalian species, newborns are dependent upon maternal milk during the neonatal period. The neonatal phase is characterized by the rapid growth and development of several organ systems and endocrine glands that are not fully formed at birth. Therefore, constituents of milk are of great importance and have evolved differently to meet the specific requirements of neonatal development in various mammals. The composition of milk changes with age, stage of lactation (colostrum, transitional, and mature milk), environment, and so on. Basically, milk is an emulsion of fat in water, providing caloric energy of ~70 kcal/100 mL. Colostrum contains ~8% protein, including lactalbumin and lactoglobulin, but it contains little or no casein, fat, or lactose. Mature human milk contains 87.9% water, 0.9% protein (of which 40% is casein), 4.0% fat, 7% lactose, and 0.2% ash; whereas mature cow milk contains more (3.3%) protein (of which 82% is casein) and less (4.7%) lactose.

There are three possible origins of milk constituents. They can be synthesized in the mammary tissues; they can be transferred directly from blood to milk through the cell membranes of the glandular tissue, or they may be of mixed origin, that is, they are both synthesized in the mammary glands and transferred from blood. Hormones and hormonelike substances enter the milk through the alveolar epithelium of the mammary gland by passive diffusion, by being bound to carrier proteins, or by active transport. In some cases (for example, β-casomorphin and morphiceptin), peptides resembling certain neuroendocrine ones, both in structure and activity, are fragments of casein. Hormones and neuroendocrine peptides in milk are absorbed from the gastrointestinal tract of the suckling mammals and in many instances retain their biologic activities. This absorption is age dependent and does not occur in weaned animals.

The presence of hormones in milk was described 50 years ago, but only in the last decade has this subject been explored, owing to the development of highly efficient procedures for the isolation of peptides and the methodologic advances in hormone assays. This approach generally involves extraction of peptides from milk and concentration procedure, followed by purification by high-performance liquid chromatography (HPLC)(Hazum 1989). Purification is usually monitored by reliable and sensitive hormone assays such as biologic activity, binding assay, and radioimmunoassay. The list of hormones and neuropeptides in milk from humans and other mammals is quite extensive and has been previously reviewed (Koldovsky 1980; Sack 1980; Hazum 1983; Schams and Karg 1986; Koldovsky and Thornburg 1987). The main emphasis of this review is on recently discovered neuroendocrine peptides in milk and their role in neonatal development.

> ONLY RECENTLY HAS THE PRESENCE OF HORMONES IN MILK BEEN EXPLORED, OWING TO NEW PROCEDURES FOR THE ISOLATION OF PEPTIDES AND METHODOLOGIC ADVANCES IN HORMONE ASSAYS.

• Hypothalamo–Pituitary Axis

Hypothalamic Hormones

Gonadotropin-releasing hormone (GnRH) and thyrotropin-releasing hormone (TRH) have been found in human milk and bovine colostrum (Baram et al. 1977; Amarant et al. 1982; Smith and Ojeda 1986). Radioimmunoassays of HPLC eluates indicate a significant amount of GnRH (3.9–11.8 ng/mL) and a lower concentration of TRH (0.16–0.34 ng/mL). These concentrations exceed those found in plasma by at least fivefold. Milk GnRH may have a physiologic role in elevating plasma gonadotropin levels and inhibiting ovarian development of human and rat neonates. These changes are prevented by intravenous administration of GnRH antiserum. Moreover, the prevention of suckling by separating rat pups from the mothers reduces luteinizing hormone serum levels and increases ovarian GnRH receptors in the pups. These results suggest that biologically active milk GnRH is absorbed from the gastrointestinal tract of the neonate rat and interacts directly with the neonate pituitary and ovary. Similarly, injections of TRH into lactating rats induce thyroid-stimulating hormone (TSH) release from pituitaries of suckling pups, thus showing that TRH can be transported to milk in a biologically active form and that the plasma TRH-degrading system does not operate in neonates. The concentrations of GnRH and TRH in milk, which greatly exceed those in serum, imply either an active concentration mechanism in the mammary gland or an additional extrahypothalamic origin for these peptides. Studies with rats indicate a one-way transport of [^{3}H]TRH from plasma to milk, which has low TRH-degrading activity and, thereafter, passage of unaltered neurohormone to the gastrointestinal tract of the suckling pups.

Recently, growth hormone-releasing hormone (GHRH) has been found in human milk at concentrations exceeding those detected in plasma (Werner et al. 1986). Studies with ^{125}I-labeled GHRH indicate that the peptide is concentrated from the blood to the milk at the mammary gland, and that it reaches the stomach of rat pup in an intact form. Other studies have shown high plasma growth hormone (GH) levels during the neonatal period. Taken together, these findings suggest that milk GHRH is involved in the regulation of GH secretion from the pituitary of the neonate. In addition, maternal GH in milk may contribute to the plasma GH levels in the neonate.

Prolactin and Thyroid-Stimulating Hormone (TSH)

Prolactin has been quantified by radioimmunoassay in milk from various mammals (Malven 1977) and shown to retain biologic activity following isolation (Gala et al. 1980). Prolactin concentrations in milk samples obtained following the completion of lactogenesis approximate concentrations of the hormone in blood plasma or serum (50 ng/mL). However, concentrations of prolactin in prepartum mammary secretions are much higher (up to 300 ng/mL). This observation is consistent with the hypothesis that during mammary lactogenesis endogenous milk prolactin in the alveolar lumen acts locally as well as parenterally, thereby maximizing its action on the mammary epithelial cells. The fact that suckling rats absorb prolactin has been demonstrated in pups that sucked milk from rats previously injected with labeled prolactin (Whitworth and Grosvenor 1978).

TSH is present in rat and human milk (Tenore et al. 1981). TSH is apparently absorbed by the suckling rats in a biologically active form and results in increased plasma triiodothyronine and thyroxine levels.

• Peptide Hormones Regulating Calcium Homeostasis

Parathyroid hormone (PTH), PTH-like protein (PLP), and calcitonin are considered to be the major peptide regulators of extracellular calcium homeostasis in humans. High concentrations of immunoreactive as well as biologically active PLP have been found in human, rat, and cow milk (Budayr et al. 1989). Similarly, high levels of calcitonin and precursors of calcitonin have been demonstrated in human milk by radioimmunoassay (Bucht and Sjoberg 1987). These findings suggest that these hormones in milk play an important role in regulating neonatal calcium homeostasis.

• Growth Factors

Epidermal Growth Factor (EGF)

Human milk stimulates DNA synthesis and cell division of fibroblasts in vitro. This can be neutralized by the addition of antibody to human EGF (Carpenter 1980). In concert with this finding, other studies have shown that the mitogenic component of human milk has identical biologic and biochemical properties as human EGF (Shing et al. 1987).

EGF apparently survives passage through the neonatal gastrointestinal tract, where specific receptors for it have been demonstrated in the epithelium. This suggests that EGF can produce biologic effects in the newborn. These effects include eyelid opening, enhanced proliferation and differentiation of the epidermis, increased growth, and maturation and stimulation of DNA synthesis. In fact, oral administration of [^{125}I]EGF to suckling rats resulted in passage of the intact polypeptide across the intestinal wall and distribution to a variety of peripheral organs, notably skin, lung, and liver (Koldovsky and Thornburg 1987). Recently, the precursor form of EGF has been found in alveolar cells of lactating mammary gland (Brown et al. 1989). It has been proposed that milk EGF originates from the processing of the alveolar cell membrane-bound precursor.

Insulin and Insulinlike Growth Factors (IGF)

Insulin has been found in human milk at concentrations close to those in plasma (Cevreska et al. 1975), and in cow colostrum at levels exceeding those in blood (Ballard et al. 1982). In the suckling rats, insulin is probably absorbed in an active form, since there is a significant decrease in blood glucose levels (Mosinger et al. 1959). IGF-I has been demonstrated in human milk, with ~80% being complexed with binding proteins (Baxter et al. 1984). These growth factors may contribute to the mitogenic activity of milk and could play a role in neonatal growth.

Other Growth Factors

Several other known polypeptide growth factors have been detected in breast milk. Included among these are: nerve growth factor (Hirata and Orth 1979), transforming growth factors (Zwiebel et al. 1986), and platelet-derived growth factor (Shing et al. 1987).

• Opioid Peptides

Opioid activity has been detected in a commercially available enzymatic digest of cow casein and shown to be due to a heptapeptide Tyr-Pro-Phe-Pro-Gly-Pro-Ile, β-casomorphin (Henschen et al. 1979). At about the same time, it has been shown (Zioudrou and Klee 1979) that pepsin could release peptides with opioid activity, "exorphins," from cow casein and wheat gluten. The high proline-residue content of β-casomorphin confers resistance to many degradative enzymes. Thus, once formed, it can survive longer than most peptides in the gastrointestinal tract and may reach physiologic levels in plasma. Following the discovery of β-casomorphin, the synthetic peptide Tyr-Pro-Phe-Pro-NH_2 (morphiceptin), which is the amide of the N-terminal tetrapeptide of β-casomorphin, has been shown to have morphinelike activity (Chang et al. 1981). It has high specificity for the morphine (μ) receptor subtype and has similar activity to morphine in the guinea-pig ileum. Morphiceptin is probably a milk constituent, because a peptide isolated from enzymic digests of β-casein behaves similarly to morphiceptin on high-performance liquid chromatography and cross-reacts with antibodies directed against morphiceptin. Authentic morphine, the alkaloid, has been found in cow's and human milk at concentrations of 200–500 ng/L (Hazum et al. 1981).

Studies have shown that, after experimental morphine administration, morphine can be detected in milk in significant amounts over 12 h, and infants of addicted mothers often show opiate withdrawal symptoms during weaning. In addition, it has been shown that milk infusion induces analgesia and comforts the infant. These effects are fully blocked by the opiate antagonist naltrexone. Thus, under certain circumstances (for example, during breast feeding), morphine, β-casomorphin, and morphiceptin may interact physiologically with opiate receptors of the brain and gastrointestinal tract. Morphine and the opioid peptides may also have an important role in the mother–infant bonding mechanism, because the infants may be "addicted" to their own mother's milk.

[redacted]

Miscellaneous Hormones

Other hormones found in milk include: somatostatin (Werner et al. 1985a), oxytocin (Leake et al. 1981), melatonin (Reppert and Klein 1978), bombesin (Jahnke and Lazarus 1984), bradykinin (Wilson et al. 1989), vasoactive intestinal peptide (Werner et al. 1985b), δ sleep-inducing peptide (Banks et al. 1983), relaxin (Lippert et al. 1981), gastrin-releasing peptide (Takeyama et al. 1989), and neurotensin and pro-γ-melanotropin (Ekman et al. 1985).

• Summary and Future Directions

The most obvious function of milk is to provide the infant with nutrients and fluid. As indicated in this review, neuroendocrine peptides are natural constituents of milk and are found at concentrations generally exceeding those in serum. This suggests an active concentration mechanism or biosynthesis of these peptides in the mammary gland. The development of highly efficient procedures for the purification of peptides implies that milk may prove to be an excellent source for identifying as yet unknown hormones and neuroendocrine peptides. The precise functional role of these milkborne hormones in neonatal development remains to be determined. They may provide the infant with protection against aggressions from the new environment, facilitate the establishment of a strong bonding between mother and infant, reduce the likelihood of the infant's developing allergies to foreign antigens, and may exercise a degree of control over the infant's metabolism.

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