Presence of Atriopeptin-like Immunoreactivity in Human and Rat Milk

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Abstract. Maternal milk is a significant source of hormones and other bioactive substances. They might be involved either in the control of mammary gland function or in the regulation of growth and development of the neonate. Atriopeptin (atrial natriuretic factor, ANF) is a peptide with strong diuretic, natriuretic and vasorelaxant actions, and it has been suggested to play an important role in the circulatory adaptation to extrauterine life. The aim of this study was to determine whether ANF is present in maternal milk, using radioimmunological analysis. The levels of ANF-like substance in human milk were found to be in the range of 0.3–3.0 pg/ml, those in rat milk between 37–117 pg/ml. The measured concentrations of ANF were proportional to the volume of the extracted milk. Serial dilutions of the extracts yielded curves which were not totally parallel to the human α-ANF standard curve. Our data indicate that, during the first days after delivery, ANF levels in human milk are higher than those in later periods of lactation. This pilot study provides the first description of the presence of atriopeptin in milk. Though a detailed characterization of milk ANF-like immunoreactivity is needed, a biological significance of present findings seems possible.

Key words: Atrial natriuretic factor — ANF — Maternal milk

Introduction

In addition to supplying nourishment, the mammary gland is thought to provide regulatory factors to support the growth and development of the neonate (Štrbák 1985). Breast milk contains a variety of hormones, growth factors and other bioactive substances present in the maternal organism. Concentrations of some of the hormones or growth factors in milk exceed those that occur in maternal plasma.

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Other substances appear in milk in a form different from that found in maternal serum, indicating synthesis or posttranslational processing by mammary tissue (Grosvenor et al. 1992).

It is suggested that the bioactive substances in milk possess important physiological functions. These substances, and growth factors in particular, may be involved in the control of growth and secretory functions of maternal mammary tissue, such as the maintenance and cessation of lactation (Schams 1994). In the neonate, milk hormones may function in the regulation of growth, development, and maturation of the gut, the immune system, and several neuroendocrine systems (Grosvenor et al. 1992).

Of the hormones detected in milk many are of protein or peptide nature, however, no data are available on the presence or absence of atriopeptin (atrial natriuretic peptide or factor, ANF). Atriopeptin is a hormone originally isolated from the heart, with strong diuretic, natriuretic and vasorelaxant actions. Though the major actions of this hormone are related to fluid and electrolyte homeostasis, it has a significant impact on various neuroendocrine functions (Samson 1990). Moreover, atriopeptin is suggested to play an important role during early human development (Semmekrot and Guignard 1991). The present pilot study was aimed at verifying the hypothesis that atriopeptin is present in the breast milk. First positive data obtained by analysis of human milk were published in an abstract form (Ježová et al. 1994b).

Materials and Methods

Human milk

Samples of breast milk were obtained from 6 lactating women aged 21–30 years, at different times after the childbirth (8–44 days). The volume of the individual milk samples varied between 5 and 18 ml. The samples of milk were kept on ice, subjected to ultracentrifugation ($100,000 \times g$) for one hour, and the infranatants were stored frozen at −20 °C until analyzed.

The extraction of atriopeptin from milk infranatants was performed with the use of Sep-Pak C_{18} cartridges which were activated by methanol (25 ml) and washed with distilled water (25 ml). Different volumes of milk infranatants (3–5 ml; 1 part) were applied into disposable syringes placed on the cartridges, immediately followed by the application of 4 parts of ice-cold 4% acetic acid. The cartridges were rinsed with 2 ml of normal saline and 6 ml of water. The elution was performed with 2×2 ml of 75% methanol in 1% trifluoroacetic acid. The eluates were evaporated to dryness in a Speed Vac concentrator, and stored frozen (−20 °C).

At the time of analysis, the extracts were reconstituted in 250 μl of assay buffer and assayed in duplicates. A part of reconstituted extracts was pooled and used to prepare serial dilutions. Concentrations of atriopeptin immunoreactivity were mea-

sured by a radioimmunoassay for human ANF using commercial kits (Amersham, Buckinghamshire, UK).

Rat milk

Milk was obtained from lactating females after intramuscular administration of 5 IU of oxytocin under ether anesthesia, 10–14 days after the delivery. Milk sampled from 2–3 rats was pooled with resulting volumes of 0.8 to 1.6 ml. The samples of rat milk were handled, centrifugated, stored and extracted in the same way as described above for human samples.

To measure atriopeptin immunoreactivity in milk extracts, a radioimmunoassay for rat ANF developed in collaboration with Prof. C. Oliver (Lab. Neuroendocr. Exp., INSERM U 297, Marseille, France) was used. The antiserum was generated in rabbits against synthetic rat ANF coupled to bovine serum albumin with carbodiimide and the assay was performed as reported previously (Ježová et al. 1994a). The radioactive tracer was prepared by labelling rat ANF (1–28) purchased from Peninsula with ^{125}I, obtained from Amersham and purified by Sep-Pak chromatography. The second antibody technique was used for separation of free and bound fractions.

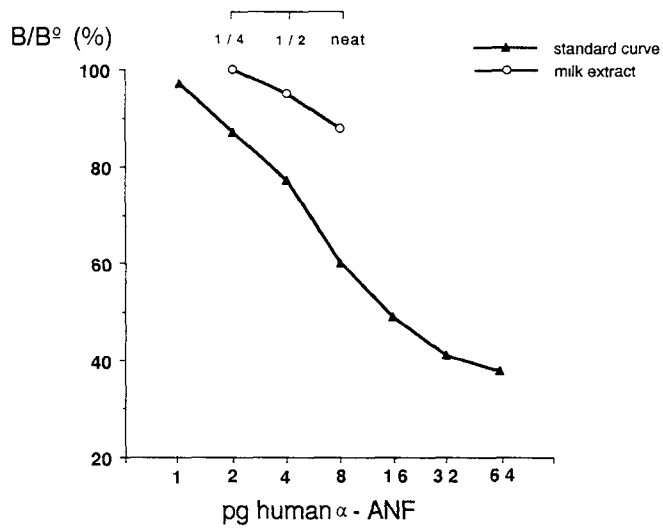

Figure 1. A typical standard curve of human α-ANF (▼) and dilution curve of the extracted human milk (○). The human milk was pooled from several collections.

Results

Analysis of milk extracts showed measurable concentrations of atriopeptin immunoreactivity. The concentrations of the peptide measured were proportional to the volume of milk extracted. As shown in Fig. 1, serial dilution of human milk extracts yielded a curve which was not totally parallel to the human alpha ANF standard curve.

The levels of ANF like substance in human milk were found to be in the range of 0.3–3.0 pg/ml. In the rat the concentrations of atriopeptin like immunoreactivity in milk reached values of 37–117 pg/ml.

Figure 2 Concentrations of human ANF like immunoreactivity in individual milk samples collected on days 8, 12, 19, and 44 after the childbirth. A simple regression analysis indicated a decrease of ANF level in milk over time.

A limited number of human milk samples analyzed individually was related to the duration of lactation (Fig. 2). A simple regression analysis showed a negative correlation between the concentrations of atriopeptin in milk and the time period after delivery ($y = 3.78 - 0.17\ x = 0.909$).

Discussion

The presented data of our pilot study provide the first description of the presence of atriopeptin in milk. Measurable levels of atriopeptin immunoreactivity were ob-

served in both human and rat milk. The precise nature of the atriopeptin-like immunoreactive substance present in milk as well as its biological significance remains to be elucidated.

Atriopeptin belongs to a family of structurally related peptides which can be categorized into three main subtypes (A-, B-, and C-type natriuretic peptides). These peptides share a common internal disulfide ring which appears necessary for the expression of their natriuretic activity (Samson 1992). The major secreted form of ANF is the 28 amino acid peptide, and it is species specific. The rat isoform differs from that present in humans by one amino acid, and the appropriate forms were used in the radioimmunoassay of rat and human milk in these experiments. Serial dilution of extracts from milk inhibited binding of the radioactive tracer almost in parallel with that of synthetic 1–28 ANF used as standard. The difference in parallelity suggests that the atriopeptin-like substance present in human milk is not absolutely identical with human α-ANF, and more detailed studies are needed for its precise characterization.

Some hormones are rapidly transported into milk from the maternal circulation unchanged in structure and activity, others appear to be modified (Grosvenor et al. 1992). Our data do not allow a comparison with the values in maternal blood as maternal plasma was not analyzed. The recovery of atriopeptin extraction from milk infranatant was not evaluated but in the case of plasma, the recovery of our extraction procedure was above 85% (Ježová et al. 1994a).

Atriopeptin has been identified in the human heart as early as during weeks 9–10 of gestation (Kikuchi et al. 1987). Similarly, atriopeptin was found to be present in the rat fetus shortly after the completion of organogenesis (Dolan and Dobrozsi 1987). In full-term healthy neonates, plasma atriopeptin levels are elevated during the early postnatal life. In humans, the peptide concentrations show a peak between days 2 and 4 of life, and then decrease towards adult levels (Rascher et al. 1987). Adult hormone levels are usually reached at the age of 2 months (Semmekrot and Guignard 1991). Interestingly, the results of this study suggest that atriopeptin-like immunoreactivity gradually decreases during the first weeks of lactation.

The exact mechanism and factors contributing to increased atriopeptin release during the postnatal period are not known but they seem to include ductus flow, transient pulmonary hypertension, elevated concentrations of vasoconstrictory agents, and secretion at extra-atrial site (Semmekrot and Guignard 1991). It cannot be excluded that atriopeptin originating from milk also contributes to raised peptide levels during the lactation period.

In the neonate, atriopeptin may play a role in the control of water and electrolyte balance, similar to that established in adults. Elevated levels of atriopeptin during the postnatal period suggest that this hormone is possibly involved in the postnatal volume contraction and may antagonize vasoconstrictor hormones (Rascher et al. 1987). An interesting hypothesis was presented by Semmekrot and

Guignard (1991) indicating an important role of atriopeptin in the circulatory adaptation to extrauterine life.

Our data indicate that the above mentioned processes might be supported by the peptide originating in maternal milk. The finding of the presence of atriopeptin-like immunoreactivity in breast milk and its possible changes during the lactation period are encouraging enough to prompt elucidation of its precise chemical nature and biological significance.

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References

Dolan L. M., Dobrozsi D. J., (1987): Atrial natriuretic polypeptide in the fetal rat: ontogeny and characterization. Pediat. Res. **22,** 115–117

Grosvenor C. E., Picciano M. F., Baumrucker C. R. (1992): Hormones and growth factors in milk. Endocrine Rev. **14,** 710–728

Ježová D., Guillaume V., Juránková E., Carayon P., Oliver C. (1994a): Studies on the physiological role of ANF in ACTH regulation. Endocrine Reg. **28,** 163–169

Ježová D., Tokarev D., Košťálová Ľ., Štrbák V. (1994b): Atrial natriuretic factor (ANF) is present in maternal milk. In: Abstracts 17th Meeting Slovak Czech Endocr. Soc., Košice (in Slovak)

Kikuchi K., Nakao K., Hayashi K., Morii N., Sugawara A., Sakamoto M., Imura H., Mikawa H. (1987): Ontogeny of atrial natriuretic polypeptide in the human heart. Acta Endocrinol. **115,** 211–217

Rascher W., Bald M., Kreis J., Tulassay T., Heinrich U., Schärer K. (1987): Atrial natriuretic peptide in infants and children. Hormone Res. **28,** 58–63

Samson W. K. (1990): Cardiac hormones and neuroendocrine function. In: Circulating Regulatory Factors and Neuroendocrine Function (Eds. J. C. Porter and D. Ježová) pp. 177–190, Plenum Press, New York

Samson W. K. (1992): Natriuretic peptides, a family of hormones. Trends Endocrinol. Metab. **3,** 86–90

Schams D. (1994): Growth factors in milk. Endocrine Reg. **28,** 3–8

Semmekrot B., Guignard J. P. (1991): Atrial natriuretic peptide during early human development. Biol. Neonate **60,** 341–349

Štrbák V. (1985): The Role of Maternal Milk in Endocrine Regulation of Sucklings. VEDA, Bratislava

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