New Findings on Casomorphins
Relationship between mother & Child

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- 1973  Discovery of opioid receptors

- 1974  Discovery of endogenous opioids

- 1975  Discovery of enkephalins

- 1976  Identification of β-endorphin

- 1979  Discovery of dynorphin

- 1979  Discovery of casomorphins

Dr Hans Kosterlitz (1903-1996)
The brain is not a static organ. It displays plasticity. It changes over the life span but also in its response to various factors. In particular it react in many exciting ways in its response to opioids, the endogenous entities not excluded.
The brain is the source for many events:

Prolactin (PRL) $\rightarrow$ ß-casein $\rightarrow$ ß-casomorphins $\rightarrow$
$\rightarrow$ opioid effects

A pathway, where brain derived hormones affect peripheral tissues to produce peptides essential in the interplay between mother and child…?

In some cases this may be a risky pathway…?
Already in the early history both exogenous and endogenous opioids induced pleasure at all ages of life. Smoking opium has a long tradition for generating pleasure in human but also endogenous opioid can induce pleasure. Lactating and suckling induce pleasure both in mother and child.
A mother’s and child’s pleasure of lactation and suckling may be explained by the release of milk opioids, the β-casomorphins.

Lactating and suckling has been suggested to involve endogenous opioids. The milk protein β-casein is enzymatically degraded to generate peptides with opioid activity. These peptides are known as the β-casomorphins.
β-casein and β-casomorphins
(Past and present)

1. β-casein and formation of β-casomorphins

2. Biological effects of β-casomorphins

3. Detection and quantitation

4. Physiological and pathophysiological aspects

http://www.annaskipper.se/sida40.html
Beta-casomorphins
atypical opioid peptides

1. β-casomorphins activate opioid μ-receptors

2. β-casomorphin are about 1000 times less potent than morphine

3. β-casomorphins induce opioid-like effects (pain relieve, respiratory depression, obstipation, pleasure)

4. β-casomorphins display increased levels in plasma and CSF at term pregnancy and during the puerperium

5. β-casomorphins are relatively metabolic stable
Biosynthesis of atypical opioid peptides
Release of a second signal from functional proteins

β-casein

β-casomorphin

Hemoglobin

Hemorphin
Amino acid sequences of β-casomorphins, enkephalins and endomorphins.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Amino acid sequence</th>
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<tbody>
<tr>
<td>β-Casomorphin-5 (bovine)</td>
<td>H-Tyr-Pro-Phe-Pro-Gly-OH</td>
</tr>
<tr>
<td>β-Casomorphin-8 (bovine)</td>
<td>H-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-OH</td>
</tr>
<tr>
<td>β-Casomorphin-5 (human)</td>
<td>H-Tyr-Pro-Phe-Val-Glu-OH</td>
</tr>
<tr>
<td>β-Casomorphin-8 (human)</td>
<td>H-Tyr-Pro-Phe-Val-Glu-Pro-Ile-Pro-OH</td>
</tr>
<tr>
<td>Met-Enkephalin</td>
<td>H-Tyr-Gly-Gly-Phe-Met-OH</td>
</tr>
<tr>
<td>Leu-Enkephalin</td>
<td>H-Tyr-Gly-Gly-Phe-Leu-OH</td>
</tr>
<tr>
<td>Endomorphin 1</td>
<td>H-Tyr-Pro-Trp-Phe-NH2</td>
</tr>
<tr>
<td>Endomorphin 2</td>
<td>H-Tyr-Pro-Phe-Phe-NH2</td>
</tr>
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What do we know about human β-casomorphins compared to bovine β-casomorphins?

• Human and bovine β-casomorphin differ in structure

• Human β-casein vs bovine β-casein: also differ in structure

• Human milk vs bovine milk: differ in their contents
Milk from cows is quite different in composition than human breast milk and, therefore is suggested not be fed to human infants before one year of age.

Human milk, which is designed specifically for promoting infant health, is much lower in protein, calcium, and sodium, and higher in mono- and polyunsaturated fats, carbohydrates, folate, and vitamin C.

However, both human and bovine milk can give rise to the opioid active β-casomorphin.
Human milk vs bovine milk

Figure 2.0 A comparison of the carbohydrate, protein and fat components of whole cow’s milk and human milk.
Bovine β-casein and β-casomorphin-7

Different mutations in bovine beta casein gene have led to 12 genetic variants and out of these A1 and A2 are the most common. The β-casomorphin-7 has been discussed a risk factor for human health hazards as it can affect brain neurons.
Formation of human $\beta$-casomorphin-8

$\beta$-kasein $\rightarrow$ $\beta$-casein-fragment $\rightarrow$ $\beta$-casein-fragment

Chymotrypsin-like enzymes are involved in a step-wise cleavage process

$\beta$-casomorphin-8

Tyr-Pro-Phe-Pro-Glu-Ile-Pro-Ile
β-casomorphins are detectable in mothers milk, plasma and CSF

Nyberg F¹, Lieberman H, Lindström LH, Lyrenäs S, Koch G, Terenius L.

J Clin Endocrinol Metab.
CSF and Plasma levels of β-casomorphin-8 in controls and during pregnancy and in the puerperium

CSF and plasma immunoreactive as measured by RIA (ir) /β-casomorphin-8 from non-pregnant, pregnant (38 weeks), and lactating women. Samples were pre-separated on a Sep-Pak cartridge before RIA. The horizontal lines indicate mean
β-casomorphin immunoreactivity displays a size difference in mothers milk, plasma and CSF reflecting a cleavage procedure


Size difference in body fluids

Size exclusion chromatography of β-casomorphin-8 (β-CM-8) immunoreactivity in human CSF

This results indicate that the Immunoreactive material in milk is mainly due to large sized protein, whereas that in plasma represents more smaller sized peptides indicating a fragmentation yielding peptides sized as the active β-CM-8.

If milk is pre-separated on SepPak cartidge an active peak eluting in conformity with β-CM-8 was seen.
CSF levels vs Plasma levels of β-casomorphin-8 during pregnancy

A positive correlation between plasma and CSF levels suggest that the peptide may cross the BBB
Reversed phase HPLC of β-casomorphin-8 immunoreactivity in human CSF also reveals molecular heterogeneity.

It appears that the authentic peptide is only present in CSF from pregnant and puerperal subjects.
Reversed phase HPLC of β-casomorphin-8 immunoreactivity in CSF, Plasma and milk

The immunoreactivity in CSF was separated into peaks, one co-eluted with authentic β-casomorphin-8.

In Plasma and milk the major part of the immunoreactive material appeared as a larger peptide, most likely a prestage of the octapeptide.

→ A partial hydrolysis of β-casein
What is known about the physiological significance of the β-casomorphins in mothers milk, plasma and CSF?

- In the child ➔ Stimultion of immune system
- In the child ➔ Sedation during lactation
- In the interplay mother and child
- Essential for mother & child bonding
The breastfeeding rate in Sweden

In Sweden, there were strict routines on maternity wards in the 1950s and sixties. Babies were taken care of in separate baby-rooms and were given to the mothers every 4 hours, just for breast-feeding. Babies belonged to the hospital and not until the day of departure they were given to the parents, who often did not know how to take care of them.

This created problems. The breastfeeding rates fell to the lowest values ever in Sweden. Data from the National Board of Health and Welfare in Sweden show that at the age of 2 months just 20% were breastfed in 1973.

In about 1985 rooming-in was allowed at night as well. Data from the Swedish register showed an increase in breastfeeding rates for 2-month old babies to about 80%.

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The breastfeeding rate in Sweden

- 2 months old babies 20% in 1973
- 2 months old babies 80% in 1985
- 2 months old babies 90% in 1994
- 4 months old babies 80% in 1994
- 6 months old babies 70% in 1994
- 2 months old babies 90% in 2010

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During 1960-ies and 1970-ies many women had problems with their breastfeeding. One problem was that many of them developed mastitis.

Early stages of mastitis can present with local pain, redness, swelling, and warmth.

Later stages with systemic symptoms like fever and flu-like symptoms and in rare cases an abscess can develop.
In Swedish studies *(Dr Lennart Righard)* it was found that the reasons for this was that their babies did not suckle in a correct way.

In the nineties when the knowledge about correct and incorrect suckling had reached all nurses and mothers in Malmö, mastitis problems became rare.

http://www.viforaldrar.se/
A correct and an incorrect suckling

If the healthy newborn infant is allowed to actively search for the breast and latch on, the inborn reflexes work. This leads to a correct suckling technique with a wide-open mouth, the tongue under the areola and using slow deep suckles. Manipulating the nipple into the baby’s mouth may lead to an incorrect, superficial suckling.
A correct suckling decreases the risk for mastitis

β-casomorphin-8-like immunoreactivity in milk from lactating women.

Levels of β-casomorphin-8-like immunoreactivity in milk from lactating women. Each bar represents the average±S.E.M from 14 individuals (mastitis group) or 10 individuals (control group). ***P < 0.001, **P < 0.01 versus acute mastitis group.
Levels of β-casomorphin-8-like immunoreactivity in blood plasma from lactating women. Each bar represents the average ± S.E.M from 10 individuals.
Postpartum depression and postpartum psychosis
POSTPARTUM PSYCHOSIS!

- Very uncommon but severe (1-2 per 1000 births)

- Includes agitation, paranoia, delusions, disorganized thinking and impulsivity

- Often appears as “organic”

- Thoughts of harming the baby are frequently driven by delusions – Child must be saved from harm, child is malevolent and dangerous.

- Rates if infanticide associated with untreated psychosis have been estimated to be as high as 4%
Hippocrates in his ‘Third book on Epidemics’: He called it a mania and indicated that the underlying reason was milk congestion, which in turn caused the symptoms.

The first to give a more detailed description of postpartum psychosis was the French doctor Louis Marcé who in 1858 published a survey of 310 cases. His careful description of symptoms is still valid. He claimed that this psychosis was a single entity, separated from other psychosis.

He had 44 cases of his own and most of them had problems producing milk. He was convinced that this psychosis had organic origin.
Incidence of Psychoses among Swedish First-Time Mothers

Dashed line: all maternal psychoses; solid line: psychoses in mothers without any previous psychiatric diagnoses.
Opioid receptor-active components in the CSF of women with postpartum psychosis

11 women with postpartum psychosis,
11 healthy lactating women,

Opioid activity was significantly higher in CSF of psychotic women in the puerperium than in healthy lactating women.

Very high levels were seen in four psychotic patients. Chromatographic characterization of this material in plasma and CSF suggested that the detected activity emerged from the milk-derived opioid peptide β-casomorphin.

SDS electrophoresis of milk samples from mothers milk (β-casein decreases in postpartum psychosis)

a) and c) are milk from healthy lactating woman, whereas b) and d) are from women with postpartum psychosis.

The arrow ➔ indicates the position of intact β-casein.
Does a correct suckling decreases the risk for the development of postpartum psychosis?

http://www.annaskipper.se/sida40.html
Decreased number of psychosis during the past decades!

The Swedish National Board of Health and Welfare has recorded the incidence of psychosis among puerperal women over the past decades.

1973 – 1983: average of 25-30 per year

1985 - 1995: average of 10-15 per year

1995 – 2005: average of 5-8 per year

2005 – 2015: average of < 5 per year
Beta-casomorphins in infants on different type of feeding and different levels of psychomotor development

Casomorphins are the most important during the first year of life, when postnatal formation is most active and milk is the main source of both nutritive and biologically active material for infants.

Kost NV et al., V Peptides 2009; 30(10) 1854-1860.
Beta-casomorphins in infants on different type of feeding and different levels of psychomotor development

A study on a total of 90 infants, of which 37 were fed with breast milk and 53 were fed with formula containing cow milk. Elevation of β-casomorphins (β-CM) levels after feeding was detected mainly in infants in the first 3 months of life. The highest basal human β-CM was observed in breast-fed infants with normal psychomotor development and muscle tone. In contrast, elevated basal β-CM was found in formula-fed infants showing delay in psychomotor development and heightened muscle tone.

Kost NV et al., V Peptides 2009; 30(10) 1854-1860.
Beta-casomorphins in infants on different type of feeding and different levels of psychomotor development

The data indicate that breast feeding has an advantage over artificial feeding for infants' development during the first year of life and support the hypothesis for deterioration of bovine casomorphin elimination as a risk factor for delay in psychomotor development and other diseases such as autism.

Kost NV et al., V Peptides 2009; 30(10) 1854-1860.
Oxytocin appears to be of crucial importance for understanding mother-infant relationships.

Oxytocin is a chemical messenger released in the brain chiefly in response to social contact, but its release is especially pronounced with skin-to-skin contact. In addition to providing health benefits, this hormone-like substance promotes bonding patterns and creates desire for further contact with the individuals inciting its release.
Oxytocin appears to be of crucial importance for understanding mother-infant relationships. Very recent studies suggest that β-casomorphin may interact with oxytocin-producing neurons and thereby affect the “mother & child bonding peptide.”

A CHALLENGE FOR THE FUTURE…