New Findings on Casomorphins

Relationship between mother & Child

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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…?
A mother and child pleasure of lactation and suckling may be explained by the release of a milk opioid, the $\beta$-casomorphin.

Lactating and suckling has also been suggested to involve endogenous opioids. In this case it has been postulated that the milk itself may produce opioid-like substances that may reach the brain both in mother and child. The milk protein $\beta$-casein may be enzymatically degraded to generate a peptide fragment with opioid activity. This active fragments is known as $\beta$-casomorphin.
β-casein and β-casomorphins

- β-casein and formation of β-casomorphins
- Biological effects of β-casomorphins
- Formation of β-casomorphins
- Detection and quantitation
- Physiological and pathophysiological aspects

http://www.annaskipper.se/sida40.html
Atypical endogenous opioids (atypical endorphins)

- **Endomorphin-1**
  - Tyr-Pro-Trp-Phe

- **Endomorphin-2**
  - Tyr-Pro-Phe-Phe

- **ß-kasomorphin-8**
  - Tyr-Pro-Phe-Pro-Glu-Ile-Pro-Ile

- **Hemorphin-7**
  - Tyr-Pro-Trp-Thr-Val-Arg-Phe

- **Enkephalin**
  - Tyr-Gly-Gly-Phe-Met
Biosynthesis of atypical opioid peptides

β-kasein

β-kasomorfin

Hemoglobin

Hemorfin
Entire amino acid sequence of the milk protein β-casein (β-casomorphin included)

NYBERG ET AL.

1  ARG-GLU-THR-ILE-GLU-SER-LEU-SER-SER-SER-GLU-GLU-SER-ILE-PRO-
16 -GLU-TYR-LYS-GLN-LYS-VAL-GLU-LYS-VAL-LYS-HIS-GLU-ASP-GLN-GLN-
31 -GLN-GLY-THR-ASP-GLN-HIS-GLN-ASP-GLN-ILE-TYR-PRO-SER-PHE-GLN-
46 -PRO-GLN-PRO-LEU-ILE-TYR-PRO-PHE-VAL-GLU-PRO-ILE-PRO-TYR-GLY-
61 -PHE-LEU-PRO-GLN-ASN-ILE-LEU-PRO-LEU-ALA-GLN-PRO-ALA-VAL-VAL-
76 -LEU-PRO-VAL-PRO-GLN-PRO-GLU-ILE-MET-GLU-VAL-PRO-LYS-ALA-LYS-
91 -ASP-THR-VAL-TYR-THR-LYS-GLY-ARG-VAL-MET-PRO-VAL-LEU-LYS-GLN-
106 -PRO-THR-ILE-PRO-PHE-PHE-ASP-PRO-GLN-ILE-PRO-LYS-LEU-THR-ASP-
121 -LEU-GLU-ASN-LEU-HIS-LEU-PRO-LEU-PRO-LEU-GLN-PRO-SER-MET-
136 -GLN-GLN-VAL-PRO-GLN-PRO-ILE-PRO-GLN-THR-LEU-ALA-LEU-PRO-PRO-
151 -GLN-PRO-LEU-TRP-SER-VAL-PRO-GLU-PRO-LYS-VAL-LEU-PRO-ILE-PRO-
166 -GLN-GLN-VAL-LEU-PRO-TYR-PRO-VAL-ARG-ALA-VAL-PRO-VAL-GLN-ALA-
181 -LEU-LEU-LEU-ASN-GLN-GLU-LEU-LEU-LEU-ASN-PRO-PRO-HIS-GLN-Ile-
196 -TYR-PRO-VAL-PRO-GLU-PRO-SER-THR-THR-GLX-ALA-ASX-HIS-PRO-ILE-
211 -SER-VAL
Amino acid sequences of $\beta$-casomorphins, enkephalins and endomorphins.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Amino acid sequence</th>
</tr>
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<tbody>
<tr>
<td>$\beta$-Casomorphin-5 (bovine)</td>
<td>H-Tyr-Pro-Phe-Pro-Gly-OH</td>
</tr>
<tr>
<td>$\beta$-Casomorphin-8 (bovine)</td>
<td>H-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-OH</td>
</tr>
<tr>
<td>$\beta$-Casomorphin-5 (human)</td>
<td>H-Tyr-Pro-Phe-Val-Glu-OH</td>
</tr>
<tr>
<td>$\beta$-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>
</tbody>
</table>
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

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

5. β-casomorphins are relatively metabolically stable
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 A1 and A2 variants of beta casein differ at amino acid position 67 with histidine (CAT) in A1 and proline (CCT) in A2 milk as a result of single nucleotide difference.
A1 and A2 and bovine β-casein

A relationship between disease risk and intake of a specific bovine β-casein fraction with either A1 or A2 genetic variants has been identified. Bovine β-casomorphin-7 is suggested as a risk factor for human health hazards as it can potentially affect numerous opioid receptors in the nervous, endocrine and immune system.

Protein chain showing amino acids in A1 and A2 beta-casein

One amino acid difference at position 67 in the protein chain
Bovine β-casomorphin-7
Formation of human β-casomorphin-8

β-kasein

β-casein-fragment

β-casein-fragment

β-casomorphin-8
CSF and Plasma levels of β-casomorphin-8 in controls and during pregnancy

CSF and plasma immunoreactive (ir) β-casomorphin-8 from non-pregnant, pregnant, and lactating women. Samples were pre-separated on a Sep-Pak cartridge before RIA. The horizontal lines indicate mean values.
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 axcrive peak eluting in conformity with β-CM-8 was seen
CSF levels vs Plasma levels of β-casomorphin-8 during pregnancy

Concentrations were highly correlated, suggesting that the CSF peptide(s) originates in plasma. However, the apparent penetrance was surprisingly high, particularly if one considers individual molecular species. In CSF, there was a 1:1 ratio between a β₉-casomorphin-8-sized peptide and a 1500- to 2000-dalton component. In plasma the corresponding ratio was about 0.1. Assuming that the β-casomorphin-8 sized peptide is the same in CSF and plasma, this would indicate practically no blood-brain barrier penetration of the smaller molecule. Nonetheless, the fragments as large as

and CSF revealed a more in approximately 10-

the 12,000-dalton component into plasma or also of this size has been introduced by treatment with
Reversed phase HPLC of β-casomorphin-8 immunoreactivity in human CSF
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.
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 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%.
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
During 1960-ies and 1970-ies many women had problems with their breastfeeding. One problem was that many of them developed mastitis.

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.
A correct suckling

http://www.viforaldrar.se/

http://www.viforaldrar.se/

http://www.annaskipper.se/sida40.html
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 mastisis

http://www.viforaldrar.se/

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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%)

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

- Often appears “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 in healthy lactating and woman with postpartum psychosis

Levels of β-casein in milk samples from three healthy lactating women (a,c, e) and three women with postpartum psychosis (b, d, f). The second peak from the right (marked with an arrow) corresponds to the fraction of β-casein. Samples were analyzed on SDS-polyacrylamide gel electrophoresis, Lindström et al., Lakartidningen 1987;84:755–8].
SDS electrophoresis of milk samples from mothers milk

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

The arrow $\Rightarrow$ indicates the position of intact $\beta$-casein
β-casomorphin-8 in milk from woman with postpartum psychosis

Immunoreactive material with the same chromatographic properties as synthetic human beta-casomorphin-8 was determined by amino acid sequence analysis to be Tyr-Pro-Phe-Val-Glu-Pro-Ile-Pro. Its molecular mass was determined by fast atom bombardment-mass spectrometry to be 962.3 Da. These determinations, which ultimately identify the immunoreactive material as human beta-casomorphin-8, represent the first structural identification of a beta-casomorphin peptide from a human body fluid.

Does a correct suckling decreases the risk for the development of postpartum psychosis?

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

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-7 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-7 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-7 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.
β-casomorphin in brain pathology


Autistic children display elevated urine levels of bovine casomorphin-7 immunoreactivity. Sokolov O¹, Kost N², Andreeva O², Korneeva E³, Meshavkin V², Tarakanova Y², Dadayan A⁴, Zolotarev Y⁴, Grachev S⁵, Mikheeva I³, Varlamov O⁶, Zozulya A². Peptides. 2014 Jun;56:68-71.
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.
β-casomorphin and oxytocin

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”