Introduction

Milks of various species contain a number of hormones of a non-peptide and peptide character as well as several hormonally active peptides. A list of these active substances (to be called collectively hormones) present in human milk is given in table I; only selected references are listed here, the reader being referred to preceding reviews for detailed references [22-25].

Because of the use of assay methods with low specificity, concentrations of thyroid and steroid hormones in human colostrum and milk were overestimated in early investigations. Therefore, when older data (before 1980) are given in the literature, the concentration values must be considered cautiously.

In infant formulae some hormones exhibited either very low concentrations or were not detected at all. These are epidermal growth factor (EGF)[26], insulin-like growth factor-I (IGF-I) [18], calcitonin-gene-related peptide [12], substance P [12], and parathyroid hormone-related peptide (PTHRP). Concentrations of PTHRP in infant formulae were about 10% of those in bovine milk; only one formula had higher values (33%); as expected, PTHRP was not detectable in soy-based formulae [13].

The need to explore the significance of hormones and hormonally active substances in human milk is stressed by the fact that they are generally missing in infant formulae.

Table 1: Human milk-borne hormonally active substances.

<table>
<thead>
<tr>
<th>Group</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid</td>
<td>1, 2</td>
</tr>
<tr>
<td>Adrenal cortex</td>
<td>3</td>
</tr>
<tr>
<td>Sex steroids</td>
<td>4, 5, 6</td>
</tr>
<tr>
<td>Hypothalamo-hypophyseal hormones</td>
<td></td>
</tr>
<tr>
<td>Gonadotropin releasing hormone (GnRH)</td>
<td>7</td>
</tr>
<tr>
<td>Growth hormone releasing factor (GRF, GHRH)</td>
<td>8</td>
</tr>
<tr>
<td>Growth hormone (GH)</td>
<td>9</td>
</tr>
<tr>
<td>Prolactin</td>
<td>10</td>
</tr>
<tr>
<td>Thyrotropin releasing hormone (TRH)</td>
<td>7</td>
</tr>
<tr>
<td>Thyroid stimulating hormone (TSH)</td>
<td>11</td>
</tr>
<tr>
<td>Thyro-parathyroid group</td>
<td></td>
</tr>
<tr>
<td>Calcitonin-gene-related peptide</td>
<td>12</td>
</tr>
<tr>
<td>Parathyroid hormone-like protein</td>
<td>13</td>
</tr>
<tr>
<td>Gastrointestinal regulatory peptides</td>
<td></td>
</tr>
<tr>
<td>Gastrin</td>
<td>14</td>
</tr>
<tr>
<td>Gastric inhibitory peptide (GIP)</td>
<td>14</td>
</tr>
<tr>
<td>Gastrin releasing peptide (GRP)</td>
<td>14</td>
</tr>
<tr>
<td>Neureotensin</td>
<td>14</td>
</tr>
<tr>
<td>Peptide histidine methionine (PHM)</td>
<td>14</td>
</tr>
<tr>
<td>Peptide YY (PYY)</td>
<td>14</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>8</td>
</tr>
<tr>
<td>Substance P</td>
<td>12</td>
</tr>
<tr>
<td>Vasoactive intestinal peptide (VIP)</td>
<td>14</td>
</tr>
<tr>
<td>Growth factors</td>
<td></td>
</tr>
<tr>
<td>Epidermal growth factor (EGF)</td>
<td>15</td>
</tr>
<tr>
<td>Insulin-like growth factor-I (IGF-I)</td>
<td>16</td>
</tr>
<tr>
<td>Insulin-like growth factor-II (IGF-II)</td>
<td>17</td>
</tr>
<tr>
<td>Neural growth factor (NGF)</td>
<td>18</td>
</tr>
<tr>
<td>Transforming growth factor α (TGF α)</td>
<td>19</td>
</tr>
<tr>
<td>Transforming growth factor β (TGF β)</td>
<td>20</td>
</tr>
<tr>
<td>Other growth factors</td>
<td>21-25</td>
</tr>
</tbody>
</table>
Do milk-borne hormones have a physiological significance for the neonate?

It is well established (see textbooks of pharmacology) that non-protein hormones are generally well absorbed from the gastrointestinal tract of humans. Several years ago, there was an active discussion in the literature concerning the significance of the intake of milk-borne thyroid hormones for hypothyroid neonates. Some data supported an ameliorating role. Nevertheless, it became clear that replacement treatment in such cases is the only safe approach [1, 2].

Effects of milk-borne hormones of peptide character on neonates is an open question. Approaches to the problem separate into two major groups, namely studies in humans and other mammals. For obvious ethical reasons, there are only a few studies addressing this problem in humans. The other important difference is that the human neonate is, with the exception of preterm infants, born in a more mature state than the majority of other mammals.

The low proteolytic activities in the gastrointestinal tract of newborns and the higher “permeability” for macromolecules in neonates indicate a possible functional role for milk-borne hormonally active peptides. Furthermore, absorption of peptides with preserved biological activity is more likely in a less mature neonate [27]. The possibility that orogastrically delivered hormones are important for development in preterm infants is suggested by the fact that amniotic fluid containing considerable concentrations of various hormones [28, 29] is swallowed continuously [30].

Studies in humans

If an ingested peptide hormone is to function within the gastrointestinal tract (GIT) and beyond, its “survival” in the GIT (i.e. its resistance to proteolytic degradation) is necessary. Britton et al. [31] have shown that in vitro degradation of $^{125}$I-EGF by the gastric juices of preterm infants is negligible; interestingly, degradation of EGF was found to be higher in gastric [32, 33] and intestinal juices [32] from adult subjects. Similar animal studies support these conclusions [34].

The only other effort to address the role of milk-borne hormones in humans was a comparison of urinary EGF output in neonates. Although an early study showed that the urinary EGF output by 2-week-old breast-fed infants was higher than observed in infants fed EGF-poor diets, i.e. either bovine milk-based formulae or total parenteral nutrition [35], a later study did not confirm this observation [36].

Studies in experimental animals

The largest body of data was obtained with EGF and the basic observations on its gastrointestinal handling are summarized in Table II. Other studies have been published that demonstrate effects of gastrointestinally administered EGF on rats during the suckling period. Various published reports are available demonstrating diverse effects of orogastrically administered “supraphysiological” doses of hormones, including EGF, on suckling animals. This led us to define the “physiological” dose of a milk-borne hormone as the amount that corresponds to that taken in milk by the suckling neonate.

Table II: Studies on EGF gastrointestinal handling in suckling rats, mice and rabbits.

<table>
<thead>
<tr>
<th>Study</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present in milk of rats, mice and rabbits</td>
<td>37-39</td>
</tr>
<tr>
<td>EGF receptors in gastrointestinal tract</td>
<td>40-42</td>
</tr>
<tr>
<td>EGF messenger RNA low to absent in the small intestine</td>
<td>43</td>
</tr>
<tr>
<td>$^{125}$I-EGF administered orogastrically or incubated in vitro with gastrointestinal juices is degraded very little</td>
<td>34, 45</td>
</tr>
<tr>
<td>EGF content in the small intestine</td>
<td></td>
</tr>
<tr>
<td>* is several fold higher than in adults</td>
<td>44</td>
</tr>
<tr>
<td>* decreases considerably after fasting for 8 to 18 hours</td>
<td>44</td>
</tr>
<tr>
<td>* returns after suckling or feeding rat milk or rat milk substitute supplemented with EGF in amounts present in milk</td>
<td>44</td>
</tr>
<tr>
<td>* not influenced by feeding rat milk substitute only</td>
<td>44</td>
</tr>
<tr>
<td>Rat milk contains peptidase inhibitors that slow down breakdown of EGF by intestinal juices in vitro</td>
<td>46</td>
</tr>
<tr>
<td>$^{125}$I-EGF is taken up by the small intestine in a receptor active form and small amounts appear in peripheral organs</td>
<td>45</td>
</tr>
</tbody>
</table>
per day as calculated from the known concentration of the hormone in milk and the daily milk intake [24].

**Epidermal growth factor**
The first group of experiments examined effects of EGF administered orogastrically to mother-fed suckling rats. The effects of an excess EGF in milk was by this way tested, but it is my opinion that the support for the hypothesis of the physiological role of milk-borne hormone for the neonate in these studies is less than that of the experiments discussed in the next paragraph. EGF orogastrically instilled (3 \( \mu g/100 \) g bw daily, i.e. three times daily intake) to suckling rats between day 11 and 13 increased the mitotic labeling indices of fundic, antral and ileal mucosal cells and that of the exocrine pancreas [48]. Orogastrian administration of EGF to suckling rabbits (4 \( \mu g/100 \) g bw; daily intake being about 500 ng, i.e. 8 times daily intake) between days 3 and 18 evoked an increase in the wet weight of the stomach and pancreas, in the DNA content of the ileum, and a precocious increase of sucrase activity concomitant with a decrease of lactase activity in the proximal segments of the small intestine [49]. This treatment also caused precocious maturation of liver functions, i.e. the size of the bile salt pool and the biliary secretion and activity of glucokinase [50].

In another study, luminally added EGF stimulated tyrosine phosphorylation of the EGF receptor in the jejunum of suckling rats; the response was rapid (within several minutes) and the EGF doses used were within the physiological range [42].

Another group of studies, possibly more physiological, were performed in artificially fed suckling rats. The elegant experiments of Berseth and Go demonstrated that the intestine of suckling rats fed with pooled rat milk to which antibodies against EGF were added (neutralizing 300 ng of EGF) exhibited decreased development as indicated by a lower wet weight, decreased DNA synthesis and content, as well as lower RNA content [51].

In other experiments, suckling rats were fed artificial milk supplemented and unsupplemented with EGF. The stomach wet weight of newborn rats fed artificial formula to which EGF was added (about 10 \( \mu g/100 \) g bw daily, i.e. 10 times daily intake) was larger than those fed unsupplemented formula [52]. Suckling rats from 11 to 14 days of age fed artificial milk with added EGF in a daily dose of 1.6 \( \mu g/100 \) g bw (i.e. less than two times daily intake) exhibited lower protein content and higher DNA content in the colon than rats fed artificial milk with no added EGF [53]. Supplementation of artificial milk with EGF normalized the development of Kupffer cell functions in suckling rats [54].

Since milk-borne hormones are presented under normal conditions to the suckling in a hormone and growth factor "cocktail" which may have agonist and antagonist interactions, data obtained in studies that use doses exceeding the daily intake, probably indicate the extent of the physiological response. These studies with EGF, therefore, argue strongly that, at least in the immature rodent pup, the milk-borne peptide regulates intestinal and liver development.

**Transforming growth factor beta**
Studies of Letterio et al. suggest an important role for maternal sources of transforming growth factor-beta 1 (TGF-\( \beta 1 \)) during development. These studies detected both TGF-\( \beta 1 \) and TGF-\( \beta 2 \) in mouse milk. Labeled TGF-\( \beta 1 \) was recovered intact from lung, heart and liver 15-30 minutes after oral administration to 5-day-old mice [55].

**Insulin-like growth factor - I**
Interestingly, the relative abundance of insulin-like growth factor-I (IGF-I) mRNA in the small intestine of suckling rats was found to be 3 times lower than that of adult rats [56]. This led us to speculate as to the importance of milk-borne IGF-I. Approximately 40% of orally administered radiolabeled IGF-I and IGF-II were recovered in gastrointestinal tissues and luminal contents of the suckling for at least 30 minutes after ingestion; a significant fraction of recovered radioactivity was eluted in HPLC in a position identical to “native” IGF, suggesting the preservation of its biological activity [57]. IGF-I is relatively resistant to degradation by gastrointestinal juices of the suckling rat and its degradation is slowed by the presence of various protease inhibitors in rat milk [46]. Vacher et al. found only traces of \( ^{125}I \) IGF-I in the mesenteric vein of calves to whom IGF-I was administered directly into the jejunal lumen [58].
Suckling rats fed a rat milk substitute (RMS) supplemented with IGF-I (500 ng/ml; note: concentration about the same as in colostrum, i.e. 10 times higher than in rat milk) gained more weight and increased brain and liver wet weight proportionally to body weight. Moreover, they demonstrated increased serum IGF-I levels, increased IGF binding proteins 2 and 3, and an increased rate of enterocyte migration from crypts in the duodenum and proximal jejunum, when compared to RMS alone [59].

Feeding artificial milk with added IGF-I (750 ng/ml; note: colostrum levels are about 500 ng/ml, in later milk about 50 ng/ml) to newborn calves yielded increased intestinal DNA synthesis, intestinal absorptive capacity as measured by xylose uptake and serum IGF-I concentration. The latter effect was however noted only several days after IGF supplementation [60, 61].

Whether or not these effects are related to the direct absorption of exogenous IGF-I or are related to the enhanced gastrointestinal tract differentiation with subsequent stimulation of nutrient uptake is not clear.

**Prolactin**

Absorption of prolactin was demonstrated in vivo in suckling rats [62].

**Somatostatin**

Studies performed in suckling rats suggest the possibility of local action on the gastrointestinal tract of milk-borne somatostatin. Koch et al. [63] found that labeled sheep somatostatin extracted from the stomach of suckling rats one hour after its oral administration eluted exactly at the same position as the native peptide. In in vitro studies, Rao et al. demonstrated that labeled somatostatin-14 is not degraded by stomach flushings from suckling rats; however, considerable degradation was observed when somatostatin-14 was incubated with juice from the lumen of the small intestine of such rats, but the degradation was substantially inhibited with the addition of rat milk (especially its cytosolic fraction) [64]. These experiments suggest that milk-borne somatostatin remains intact long enough to exert an effect on the gastrointestinal tract. According to Koch et al., only 5% of the total radioactivity present in plasma one hour after oral administration of somatostatin to suckling rats was undegraded peptide [63].

**Growth hormone and luteinizing hormone**

Growth hormone (GH) secretion in vivo was stimulated in 2- or 8-day-old suckling rats after the intragastric administration of rat milk extract containing growth hormone-releasing factor (GRF)-like immunoreactivity [65]. These data are in agreement with a previous report that suckling rats removed from their mothers exhibited a decrease in plasma growth hormone and that GH levels normalized after pups were returned to their mothers [66].

A similar phenomenon has been observed in serum levels of luteinizing hormone (LH). Suckling female rats exhibited a decrease of the normally high serum levels of LH within 3 hours after removal from their mothers and it can be speculated that this phenomenon is related to a lack of milk-borne luteinizing hormone-releasing hormone (LHRH) [67]. Smith-White and Ojeda [68] elegantly explored this issue further and demonstrated that the content of available LHRH receptors in the ovaries of suckling rats was low and increased four-fold after weaning, whereas the total number of binding sites (using MgCl₂ dissociation) was the same, implying the sites were occupied by LHRH in the sucklings. If the pups were removed from the dam for several hours available receptors increased markedly; refeeding (either by suckling or by direct orogastric feeding of milk) led to the return of low normal values within 2 hours. Smith-White and Ojeda also reported the presence of LHRH in the stomach of fed suckling rats as well as its absence in those rats fasted for 6 hours [68].

**Erythropoietin**

The possibility of erythropoietin transfer from lactating mothers to the suckling was tested in laboratory rodents. Grant [69] observed that the blood of rats and mice nursed by dams exposed to anoxia for 6 hours a day in a low pressure chamber exhibited an increased haemoglobin content compared to those nursed by control dams. Moreover, Carmichael et al. [70] found increased erythropoiesis in 2- to 5-day-old rats suckling phlebotomized mothers.
**Should we recommend adding hormones to infant formulae?**

Our brief review of data concerning the present knowledge of hormones and hormone-like substances in milk and amniotic fluid, and their effects on sucklings after orogastric administration, leads us logically to pose the above question. The importance of such a question is underlined when we consider previous studies showing some milk-borne hormones to be missing in infant formulae, while others are not reported.

During the last decades, we have seen many changes in the preparation of infant formulae, made with intent to increase their resemblance to human milk. The composition of energy sources has been changed, vitamin and mineral composition has been modified, substances like taurine and carnitine have been added. Among the candidates for future engineering of infant formulae are endocrine factors.

Thus the first concern to be answered is whether we should consider addition of hormones to infant formulae at all. We know that most babies fed infant formulae without hormonal supplementation are considered to be doing “well”. However, we can question what is “well” and we may question how lives continue once the years of visits to pediatricians are over. From studies about delayed effects of various perinatal manipulations [71-74], we learned the importance of neonatal nutrition as seen to affect adult subjects. Together with others [73] we have questioned the importance of milk as a carrier of endocrin factors [22-24].

A further group of concerns relates to the fact that hormones are present in the milk as a “cocktail” of potentially neutral, agonistic and antagonistic substances, where the addition of any one hormone might disturb the balance. Similarly, some growth factors in excess might stimulate pathological growth.

We cannot neglect such questions, considering them esoteric (Table III). We need more studies in experimental animals. On the one hand, we need to know the immediate effects of milk-borne hormones – given individually or as a mixture of several hormones. On the other hand, we need to follow up the delayed effects of hormonal absence in the formulae of neonates. It is clear that the answers are not easy to come by, but difficulties must not block us in this path. The answer is important for the nutrition of the neonate and for the well-being of adults.

**Conclusions**

Available, but limited data support the hypothesis that milk-borne hormones play a physiological regulatory role in the neonate. The large number of hormones present in milk contrasts with the small number of hormones whose physiological role has been explored. Furthermore, in addition to known hormones and growth factors, other undefined peptides also may play an important physiological role in the neonate. For example, the original studies of Porter et al. [75] showing that the differentiation of pituitary lactotropes in

<table>
<thead>
<tr>
<th>Fact</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk and amniotic fluid contain “hormones”</td>
<td>Infant formulae do not</td>
</tr>
<tr>
<td>Infants fed infant formulae (without hormone supplementation) do “well”</td>
<td>What is “well”? Life does not end after the years of visits to pediatricians</td>
</tr>
<tr>
<td>Experiments in suckling rats show that exogenous maturative hormones can accelerate growth factor production by the suckling</td>
<td>Overdoses bring with them possibility of harmful effects(s) of precocious activation of “biological clock”</td>
</tr>
<tr>
<td>Hormones are present in the milk as a “cocktail” of potentially neutral, agonistic and antagonistic substances</td>
<td>Addition of one of the hormones might disturb the balance</td>
</tr>
<tr>
<td>Some growth factors might stimulate pathological growth</td>
<td>Risk/benefit ratio must be evaluated</td>
</tr>
</tbody>
</table>
neonatal rats is modulated by a maternal signal that is transferred to the neonate by its mother's milk early in lactation was the first definitive demonstration that a component of milk plays a systematic regulatory role.

Furthermore, the effect of milk-borne hormones during the suckling period, both in experimental mammals and human neonates, might be not only physiological (i.e. enabling normal development) but also protective against noxious factors (cytoprotection), a role demonstrated for EGF [76]. In this respect, we can speculate about their significance as "protectors and healers" in the case of necrotizing enterocolitis [22-24, 32]. However, the present state of knowledge is definitively not sufficient to recommend adding hormones to infant formulae.

References


