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Review

Bioactive peptides derived from bovine whey proteins: opioid and ace-inhibitory peptides

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Regulatory peptides can be released by enzymatic proteolysis of food proteins and may act as potential physiological modulators of metabolism during the intestinal digestion of the diet. The possible regulatory effects of peptides relate to nutrient uptake, immune defence, opioid and antihypertensive activities. Milk proteins, especially caseins, are an important source of these bioactive peptides. During recent years, major whey protein components, α-lactalbumin and β-lactoglobulin, were also shown to contain bioactive sequences. Peptides showing opioid and angiotensin I-converting enzyme (ACE) inhibitory activity were found in α-lactalbumin and β-lactoglobulin. Opioid peptides, α-lactorphin and β-lactorphin, were liberated during *in vitro* proteolysis of bovine whey proteins, and pharmacological activity was observed at micromolar concentrations. Whey hydrolysates showed ACE-inhibitory activity after proteolysis with different digestive enzymes, and several active peptides were identified. The results demonstrated the existence of several biologically active whey-derived peptides and hydrolysates. The findings of the study can be exploited in the development of foods with special health claims (e.g. treatment of hypertension) as well as in identifying new applications in food.

Introduction

Milk is known to be a complex mixture of molecular species, containing bioactivity, which confers special properties for the support of infant development and growth beyond basic nutrition. It contains modulators of digestive and gastrointestinal functions, hormones and growth factors potentially capable of influencing the development and growth of the gastrointestinal tract, other specific organs, immunoregulation, non-immune disease defence, and modulation of the gut microflora population [1]. Many of the bioactivities of milk are attributable to the proteins and peptides secreted into milk by the mammary gland. The bioactivities of several milk proteins are latent; either absent or incomplete in the native protein. Only during proteolytic digestion of the protein are the active peptide fractions released from the native protein/peptide. These active peptides can be formed also during food processing. Once the bioactive peptides are liberated, they may act as regulatory compounds with hormone-like activity. Bioactive peptides were first mentioned by Mellander in 1950 [2], when he suggested that casein-derived phosphorylated peptides enhanced vitamin D-independent bone calcification in rachitic infants. The knowledge of bioactive peptides has steadily increased since 1979 and, at present, numerous peptides exhibiting various activities—such as opiate, antithrombotic or antihypertension activity, immunomodulation, or mineral utilization properties—have been reported (Fig. 1). The activity is based on the inherent amino acid composition and sequence. Bioactive peptides usually contain 3–20 amino acid residues per molecule. Although animal as well as plant proteins contain potential bioactive sequences, milk proteins are currently the main source of a range of biologically active peptides. Bioactive peptides have been studied by means of the following investigation techniques: establishment of an assay system to determine biological activity, hydrolysis of proteins by digestive enzymes, the isolation of peptides and the determination of the structures and synthesis of peptides for the verification of activity. The investigation strategies have further included the synthesis of peptides within milk proteins, based on sequence similarities of peptides having known biological activity. Most of the studies so far have been conducted *in vitro*. The physiological functions of these peptides, however, remain to be established *in vivo*.

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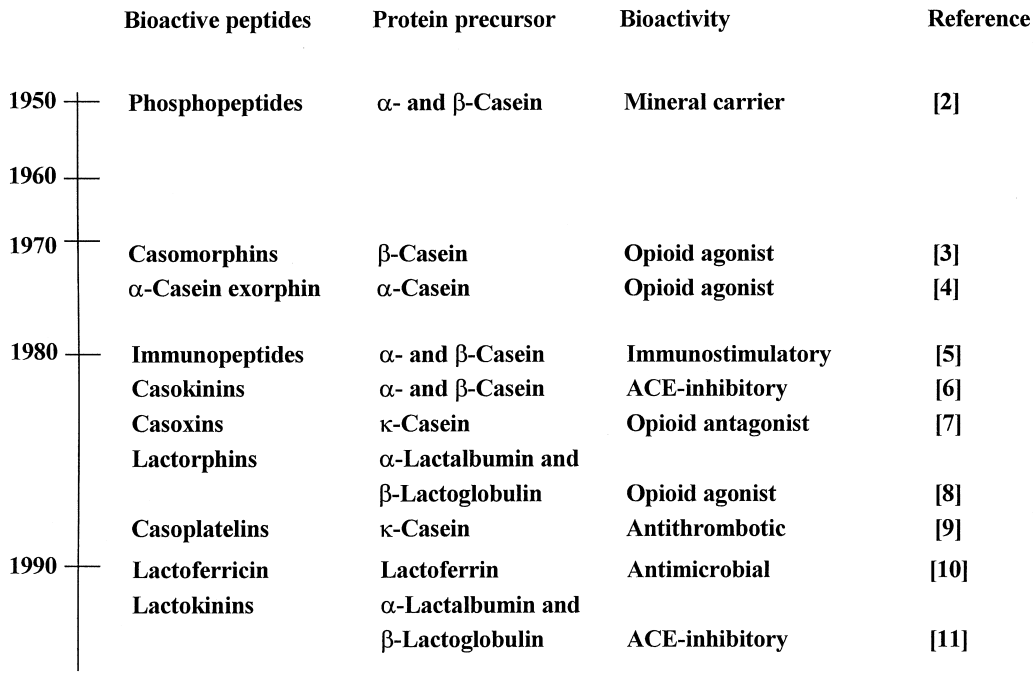

Fig. 1. Chronological overview of identified bioactive peptides derived from milk proteins.

The following review is based on the whey-derived opioid and ACE-inhibitory peptides, their structures, liberation and activity [12–16]. Furthermore, their possible physiological importance will be discussed.

Peptides with opioid activity

Opioid peptides are defined as peptides such as enkephalins, which have an affinity for an opiate receptor as well as opiate-like effects, inhibited by naloxone. Typical opioid peptides all originate from three precursor proteins: proopiomelanocortin (endorphins), proenkephalin (enkephalin) and prodynorphin (dynorphins) [17]. All these typical opioid peptides have the same N-terminal sequence, Tyr-Gly-Gly-Phe. Opioid peptides exert their activity by binding to specific receptors of the target cell. The individual receptors are responsible for specific physiological effects—e.g. the μ-receptor for emotional behaviour and suppression of intestinal motility, the σ-receptor for emotional behaviour, and the κ-receptor for sedation and food intake. The opioid peptides derived from a variety of precursor proteins are called 'atypical' opioid peptides, since they carry various amino acid sequences at their N-terminal regions; only the N-terminal tyrosine is conserved. The N-terminal sequence of 'atypical' opioid peptides is Tyr-X-Phe or Tyr-X_1-X_2-Phe. The tyrosine residue at the N-terminal and the presence of another aromatic amino acid at the third or fourth position form an important structural motif that fits into the binding site of the opioid receptors [18,19]. β-Casomorphins were the first identified opioid peptides derived from food protein. Considerably more information has been collected about their structure and liberation during the past decade than about other opioid peptides [8,18,20].

Whey proteins contain opioid-like sequences in their primary structure, namely α-la (both bovine and human) f(50–53) and β-lg (bovine) f(102–105). These peptides have been termed α- and β-lactorphins [8]. The treatments needed to liberate lactorphins as well as their structure and activity are presented in Table 1. Proteolysis of α-la with pepsin produced α-lactorphin; whilst digestion of β-lg with pepsin and then with trypsin, or with trypsin and chymotrypsin, yielded β-lactorphin. α-Lactorphin exerts weak but consistent opioid activity in the guinea pig ileum and in connection with receptor-binding; whereas β-lactorphin—despite its similar receptor-binding affinity—exerts an apparent non-opioid stimulatory effect on guinea pig ileum. These peptides show very low affinity for opioid receptors and are μ-type receptor ligands. Both α- and β-lactorphin were found to displace ^{3}H-naloxone from its binding sites at micromolar concentrations. The reference opioid peptide, morphine, displaced ^{3}H-naloxone in the nanomolar range ($IC_{50} = 23 \pm 13$ nM) [12,21]. Furthermore, it was shown that digestion of β-lg with chymotrypsin produced His-Ile-Arg-Leu (β-lactotensin, β-lg f(146–149)). The pharmacological activity of β-lactotensin was similar to that of β-lactorphin, as they induced stimulation that was antagonised neither by naloxone nor by atropine [14].

Bovine milk contains approximately 0.9 g/l of α-la and 3.0 g/l of β-lg, whilst human milk contains 1.6 g/l of α-la but no endogenous β-lg [22]. If the yield of lactorphins is 100%, 1 l of bovine milk contains 32 mg (64 μmol)

Table 1. Opioid peptides and peptides acting on smooth muscle derived from bovine whey protein[a]

Protein	Enzyme(s)	Identified peptide[b]	Amount in hydrolysate (mg/g)	Pharmacological properties	
				IC_{50}[c]	GPI[d]
α-Lactalbumin	Pepsin	α-La f(50–53) YGLF α-Lactorphin	5.0	67±13 μM	Inhibition[e]
β-Lactoglobulin	Pepsin then trypsin	β-Lg f(102–105) YLLF β-Lactorphin	1.7	38±7 μM	Stimulation
	Chymotrypsin	β-Lg f(146–149) HIRL Morphine	4.0	N.D.[f] 23±12 nM	Stimulation Inhibition

[a] Results adapted from studies [12,14,21].
[b] One letter amino acid codes used.
[c] IC_{50} Concentration producing half-maximal displacement of ^{3}H-naloxone.
[d] GPI Effect on the contractions of guinea pig ileum *in vitro*.
[e] Observed at concentration of 10^{-4} M.
[f] N.D., not determined.

of α-lactorphin and 90 mg (162 μmol) of β-lactorphin, and human milk 56 mg (112 μmol) of α-lactorphin. These concentrations are sufficient to produce the observed *in vitro* effects. However, it is unlikely that this concentration of peptides would be released during *in vivo* digestion of milk. The concentrations of lactorphins and β-lactotensin observed in the hydrolysates, for example, were only 5–14% of the optimum concentrations. Furthermore, there is no current evidence for the liberation of lactorphins or β-lactotensin during gastrointestinal digestion; whereas liberation of the casomorphins has been shown [23].

Peptides with angiotensin I-converting enzyme inhibition activity

Angiotensin I-converting enzyme (ACE, peptidyl dipeptide hydrolase, EC 3.4.15.1) has been classically associated with the renin-angiotensin system, which regulates peripheral blood pressure. ACE raises blood pressure by converting angiotensin I released from angitonsinogen by renin into the potent vasoconstrictor angiotensin II. ACE also degrades vasodilative bradykinin and stimulates the release of aldosterone in the adrenal cortex. Consequently, ACE-inhibitors may exert an inhibitory effect [24] (Fig. 2). ACE is an exopeptidase, which cleaves dipeptides from the C-terminal of various peptide substrates. ACE is an unusual zinc-metallopeptidase, as it is activated by chloride and lacks a narrow *in vitro* substrate specificity [25].

Inhibitors of ACE were first obtained from snake venom [26]. Afterwards, various ACE-inhibitors have been discovered from enzymatic hydrolysates and related synthetic peptides of food proteins. These food proteins include bovine and human casein, zein, gelatin, yeast and corn (for a review, see [27,28]). Many casein-derived ACE-inhibitory peptides are described, and it has also been demonstrated that the major whey proteins contain peptides which inhibit ACE. The primary sequences of the α-la and β-lg-derived ACE-inhibitory peptides are summarized in Table 2.

The results suggest that the peptides originating from α-la f(99–110) may contribute considerably to the ACE-inhibitory activity of α-la hydrolysates, since ACE-inhibitory activity was found in α-la f(99–108), f(104–108) and f(105–110). Mullally *et al.* [11] reported ACE-inhibitory activity in α-la f(50–53) (IC_{50} = 733.3 μM) and the dipeptides (Tyr-Gly, IC_{50} = 1522.6 μM and Leu-Phe, IC_{50} = 349.1 μM). In our studies, the tripeptide Tyr-Gly-Leu (α-la f50–52) demonstrated ACE-inhibitory activity at about the same range. The ACE-inhibitory activity of β-lg hydrolysates is a result of various peptides liberated from various regions of the β-lg chain. Trypsin, for example, released several peptides with moderate activity, namely β-lg f(22–25), (32–40) and (81–83). The tryptic β-lg peptide f(142–148) reported by Mullally *et al.* [30], showed higher ACE-inhibitory activity with an IC_{50} value of 42.6 μM compared to the IC_{50} values found, within the 77–1682 μM range, in our studies. The most active ACE-inhibitory whey peptide in our studies was α-la f(104–108) with an IC_{50} value of 77 μM.

Production of bioactive peptides

There are a number of methods by which peptides with biological activity can be produced. They most common rely on food processing using heat, alkali or acid conditions which hydrolyse proteins; the enzymatic hydrolysis of food proteins; and the microbial activity of fermented foods. Biologically active peptides are released by limited hydrolyses of well-known proteins. So far, the most common way to produce bioactive peptides has been through enzymatic digestion utilising different scales and techniques. Pancreatic enzymes—preferably trypsin—have been used for the chemical characterization and identification of many known bioactive peptides. For example, ACE-inhibitory peptides are most commonly produced by trypsin [6].

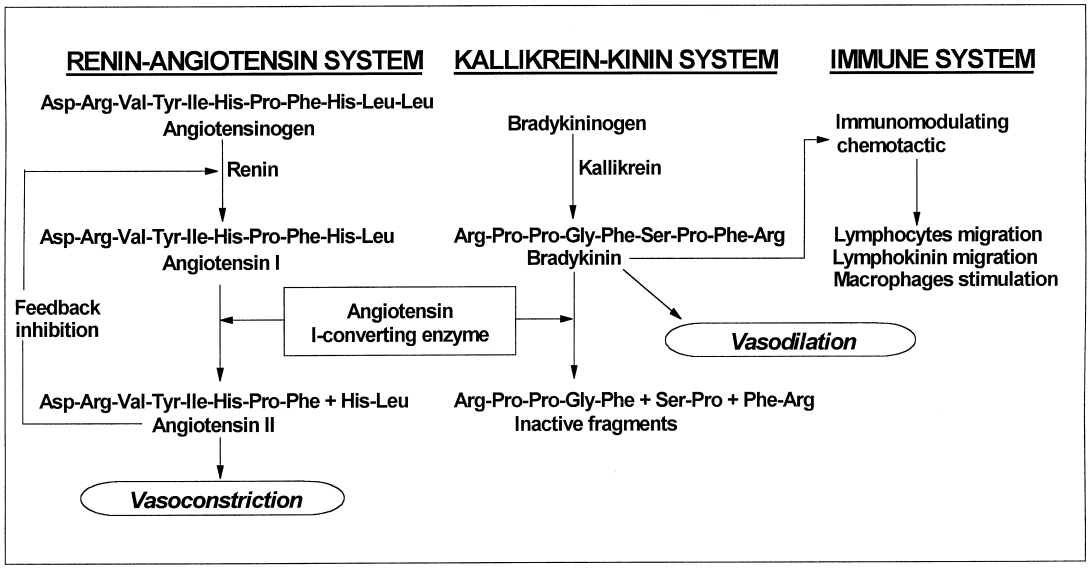

Fig. 2. The role of angiotensin I-converting enzyme in the renin-angiotensin, kallikrein-kinin and immune systems.

Table 2. Summary of the identified ACE-inhibitory peptides from whey, α-lactalbumin and β-lactoglobulin hydrolysates

Treatment	Identified peptide fragment	Sequence[a]	IC_{50} (μM)[b]	Reduction in BP (mg/kg)	Reference
α-La with pepsin then trypsin and chymotrypsin	α-la f(50–52)	YGL	409		[16]
α-La with pepsin	α-la f(50–53)	YGLF[c]	733	23 (0.1)	[11,29]
α-La with trypsin	α-la f(99–108)	VGINYWLAHK	327		[16]
	α-la f(104–108)	WLAHK	77		[16]
β-Lg with trypsin	β-lg f(22–25)	LAMA	556		[16]
	β-lg f(32–40)	LDAQSAPLR	635		[16]
	β-lg f(81–83)	VFK	1029		[16]
	β-lg f(142–148)	ALPMHIR	43		[30]
β-Lg with pepsin, then trypsin and chymotrypsin	β-lg f(94–100)	VLDTDYK	946		[16]
	β-lg f(106–111)	CMENSA	788		[16]
	β-lg f(142–146)	ALPMH	521		[16]
	β-lg f(102–105)	YLLF[c]	172		[11]
Whey with fermentation then pepsin and trypsin	α-la f(105–110)	LAHKAL	621		[15]
	β-lg f(9–14)	GLDIQK	580		[15]
	β-lg f(15–20)	VAGTWY	1682		[15]
Whey with proteinase K	β-lg f(78–80)	IPA	141	31 (8)	[31]

[a] One letter amino acid codes used.
[b] Concentration of an ACE-inhibitor needed to inhibit 50% of the ACE activity.
[c] Synthetic peptides used.

However, other enzymes and various enzyme combinations of proteinases—including alcalase, chymotrypsin pancreatin and pepsin as well as enzymes from bacterial and fungal sources—have been utilized to generate bioactive peptides. Microbial enzymes have also been successfully incorporated to produce ACE-inhibitory peptides [32,33].

After hydrolysis, the peptides in hydrolysates have been fractionated and enriched by means of various methods. Ultrafiltration membranes have been successfully used to enrich specific peptide fractions. An ultrafiltration membrane reactor has been applied for the continuous extraction of permeates enriched with bioactive fragments, in order to produce antithrombotic peptide [34]. We have reported that the <1 kDa fractions from α-la hydrolyzed with pepsin and β-lg hydrolyzed either with pepsin and trypsin or with pepsin, trypsin and chymotrypsin have no opioid properties, even though they contain whey protein-derived opioid peptides (α- and β-lactorphins) [13]. Later, we found that the ACE-inhibitory activity in the <1 kDa fraction was, in many cases, higher than in the other fractions

tested [16]. These results, together with those of Mullally *et al*. [35], indicate that it may be possible to exploit ultrafiltration in order to enrich ACE-inhibitory peptides derived from whey protein. Furthermore, Bordenave *et al*. [36] showed that α-lactorphin was generated with continuous hydrolysis of goat whey in an ultrafiltration reactor. The concentration of α-lactorphin in hydrolysate was higher than in our study. Membranes containing negatively charged materials have been used to desalt whey hydrolysates [37] as well as to enrich cationic peptides with antibacterial properties from cheese whey [38]. Accordingly, they assumed that this technique provides new possibilities for enriching peptides with a low molecular mass and that it is easily up-scaled to gram or even kilogram quantities.

Liberation of bioactive peptides during food processing

Bioactive peptides may also be liberated during the manufacture of milk products. Hydrolysed milk proteins used for hypoallergenic infant formulae, clinical applications and as food ingredients, for example, consist exclusively of peptides [39]. Proteases in food itself—such as plasmin in milk—can hydrolyse proteins during food processing and storage. Bacterial starter cultures contain several proteolytic enzymes which are responsible for the breakdown of protein into peptides and amino acids. During fermentation, various long oligopeptides are liberated by degradation of caseins, which could be precursors of peptides with biological activity when cleaved by other enzymes. In fermented milk products, intracellular peptidases of lactic acid bacteria will most likely contribute to further degradation after lysis [40,41].

Various studies have reported on casomorphins, ACE-inhibitory peptides and phosphopeptides found in fermented milk products. Cheese contains phosphopeptides as natural constituents [42,43] and secondary proteolysis during cheese ripening leads to the formation of other bioactive peptides, such as ACE-inhibitory [44–47]. Precursors of β-casomorphins have been identified in Parmesan cheese [48]. Moreover, ACE-inhibitory activity in milk has been found to increase during fermentation with Calpis sour milk starter containing *Lactobacillus helveticus* and *Saccharomyces cerevisiae*. Two ACE-inhibitory peptides, Val-Pro-Pro and Ile-Pro-Pro, have in fact been isolated and identified from Calpis sour milk [49]. Our studies showed that commercial lactic acid starters were not able to produce ACE-inhibitory peptides from whey or casein proteins; rather, further proteolysis was needed to produce this activity [15]. On the other hand, we have earlier identified an ACE-inhibitory peptide from a Finnish fermented milk product [50]. In addition, fermented milks produced by *Lb. delbrueckii* subsp. *bulgaricus* and *Lactococcus lactis* subsp. *cremoris* strains contains several casein-derived ACE-inhibitory peptides [51]. At present there are various industrial products available which contain bioactive peptides. These include Glutamine peptide, which is claimed to assist in maintaining the immune system and to regulate protein turnover and glycogen replenishment; Peptide FM, claimed to inhibit dietary fat deposition and to alter fat metabolism; and caseinophosphopeptides, claimed to enhance Ca^{2+} and Fe^{2+} absorption and prevent dental caries [52]. In Japan, there is a "Food for special health use" containing the casein dodecapeptide (α_{s1}-casein f [23–34]), which is shown to be a useful ingredient in the prevention of hypertension and diseases of the circulation system [53].

Physiological importance

To exert physiological effects *in vivo*, bioactive peptides must be released during intestinal digestion and must then reach their target sites at the luminal side of the intestinal tract or after absorption in the peripheral organs. Two types of experiments have been performed to obtain information as to whether bioactive peptides may be released from milk proteins after the ingestion of cows' milk in order to eventually elicit the special effects. Firstly, in studies in which bovine milk proteins were incubated under conditions imitating gastrointestinal digestion, these have demonstrated the release of, e.g. β-casomorphins, α-casein exorphins and casoxins [4,7,54,55]. Secondly, the contents of the small intestine have been examined both in animal and human studies after ingestion of milk proteins. Evidence for the liberation of β-casomorphins, caseinophosphopeptides and immunostimulatory peptides from casein into the intestinal lumen of mammals after the ingestion of milk or a diet containing casein have already, in several studies, been obtained [23,56–59]. An antimicrobial peptide, lactoferricin B, was detected in the gastric content of rats fed bovine lactoferrin. This finding indicates that active peptides of lactoferrin can be generated by gastric pepsin digestion *in vivo* [60].

The final digestion of dietary protein in the small intestine takes place via the enzymatic hydrolysis of oligopeptides through the agency of the brush-border peptidases. A variety of peptidases have been described as being localized to the intestinal brush-border membrane. These peptidases cleave free amino acids or dipeptides from the N- or C-terminal or the interior bond of oligopeptides [61–63]. Peptides which are not degraded by intestinal proteolysis can, in principle, be absorbed intact and enter the blood circulation. Di- and tripeptides—such as immunopeptides and several ACE-inhibitors—may pass across the intestine in quantitatively significant amounts in order to reach peripheral target sites. A carrier-mediated, peptide-sequence-independent transport mechanism of di- and tripeptides has been demonstrated [64]. After absorption at the intestinal tract, serum peptidases can further hydrolyze peptide bonds. Resistance to peptidase degradation may, in fact, be a prerequisite for a physiological effect following

oral ingestion and/or the intravenous infusion of biologically active peptides/hydrolysates. The absorption and degradation of natural β-casomorphins and its analogues have been intensively studied, whereas little is known about the fate of other bioactive peptides, especially those derived from whey proteins. For example, indirect evidence suggests the presence of β-casomorphins in the intestinal contents of humans after milk ingestion, whereas no intact transepithelial passage occurs since they are rapidly degraded by the intestinal enzymes [65–69]. On the other hand, Masuda *et al.* [70] found that two antihypertensive tripeptides (Ile-Pro-Pro and Val-Pro-Pro) were absorbed directly without being decomposed by digestive enzymes, reached abdominal aorta, inhibited the ACE and finally showed antihypertensive effect in spontaneously hypertensive rats.

Opioid peptides can be considered as compounds exerting possible effects on the nervous system. The existence of μ, κ and δ opioid receptors in the central nervous system is well-documented [71]. Furthermore, opiates are reported to influence gastrointestinal function in two ways: first, they affect the smooth muscles—which reduces transit time—and second, they affect the intestinal transport of electrolytes, which explains their antisecretory properties. These two kinds of effects may be related to the fact that opiates act on different subtypes of intestinal opioid receptors. At least five receptor subtypes have been suggested to exist in peripheral tissues: μ, δ, ϵ, σ, and κ [72]. As opioid receptor ligands, these peptides can be expected to behave in the manner of other opioids, i.e. to act as agonists or antagonists, bind to receptors and elicit effects in all cells or tissues in which opioids are known to be active. Natural β-casomorphins show a preferential affinity for the μ-receptors [19]. In rats, β-casomorphins have been shown to cause analgesia [73–75], apnea [76], as well as changes in the sleep of neonatal rats [77]. Naloxone pre-treatment reverses these effects, suggesting that opioid μ-receptors are involved. Blass and Blom [78] demonstrated that the behaviour of infant rats is sensitive to elevations in central β-casomorphin concentration, and that effectiveness against pain is mediated through central opioid pathways. These effects have been shown by intracerebral, intraperitoneal or intraventicular injections. It has been shown that casomorphins inhibit intestinal motility in isolated segments of rat ileum [79]. In healthy human volunteers, morphiceptin has been shown to delay gastrointestinal transit time [80]. Furthermore, an *in vivo* study showed that luminal administration of morphiceptin exerted a significant antisecretory effect at micromolar concentrations in rats [81]. The enhancement of net water and electrolyte absorption by β-casomorphin in the intestine leads to antidiarrheal action [82,83]. Their action seems to depend on the transfer of intact peptides from the luminal to the blood side of the tissue, where the opioid receptors are located. This action is prevented by the hydrolysis of natural peptides. Tome *et al.* [84] proposed that the β-casomorphin analogue act through a neuromediated mechanism, since it is inhibited by a neurotoxin. This is consistent with earlier findings showing that opiate receptors are not present on the enterocyte membrane of rabbit intestine, but are mainly located in submucosal and myenteric plexuses [85]. According to Brandsch *et al.* [86] it is likely that β-casomorphins bind to the opioid receptors present in the intestinal luminal membrane, which may induce the transport alterations.

The inhibition of ACE, located in various tissues (e.g. plasma, lung, kidney, heart, skeletal muscle, pancreas, brain), may influence different regulatory systems [25]. ACE plays a pivotal role in two independent humoral systems which affect blood pressure, since it is responsible for the generation of a vasopressor agent, angiotensin II, and for the inactivation of a vasodepressor agent, bradykinin. Many specific ACE-inhibitors have been developed for use as potent, orally administered antihypertensive drugs [87]. A number of studies with hydrolysates and peptides have been conducted in order to investigate the antihypertensive effect of ACE-inhibitory peptides in spontaneously hypertensive rats (SHR). Karaki *et al.* [88] demonstrated that a single intraperitoneal or oral administration of a tryptic hydrolysate of casein and oral administration of ACE-inhibitory peptides (α_{s1}-casein f(23–34), f(194–199) or β-casein f(177–183)) (100 mg/kg) decreases blood pressure in SHR. Repeated oral administration of 3% (1.8–2.0 g/kg day) casein hydrolysate decreases blood pressure in SHR, but not in normotensive rats. Tryptic casein hydrolysate was observed to have an antihypertensive effect in mildly hypertensive human volunteers [89]. So far, only few studies have shown the corresponding effect of whey peptides. Abubakar *et al.* [31] observed that cheese whey digested with proteinase K had a depressive effect on systolic blood pressure (−55 mm Hg), and that the highest antihypertensive activity among the identified active peptides was found with the tripeptide Ile-Pro-Ala (β-lg f(78–80)). Nurminen *et al.* [30] found that the subcutaneous administration of α-lactorphin dose-dependently lowered systolic and diastolic blood pressure in SHR as well as in normotensive Wistar Kyoto rats. Furthermore, these studies showed that the blood pressure—lowering mechanism of α-lactorphin was not ACE-inhibition, but rather appears to be due to interaction with opioid receptors, since the response was antagonised by pre-treatment with naloxone. This provides a further evidence for the multi-functional role of bioactive peptides. Accordingly—aside from ACE—inhibition-other mechanisms may also be involved in the blood pressure-reductive action of various peptides. Several studies have shown the cardiovascular effect of endogenous opioids [90–92]. The mechanism of this phenomena is difficult to clarify because of the numerous

opioid peptides and receptor subtypes and is, therefore, at least partly unknown. Further studies on the mode of action as well as the effect subsequent to peroral administration of α-lactorphin are in progress.

Conclusion

The occurrence of many biologically active peptides in bovine milk is now well established, but numerous scientific and technological issues have to be resolved before these substances can be optimally exploited for human nutrition and health. The efficacy and safety of bioactive peptides should be investigated further by carrying out animal studies in order to verify the effects and clarify adverse effects. In addition, the influence of opioid peptides on, e.g. pain control, behaviour and food intake regulation require clarification. The precise nature of the active substances and their *in vivo* fate, site and mechanism are also worthy of investigation. Will the peptides retain their activity during gastrointestinal digestion and enter into the blood circulation, and can they penetrate the blood–brain barrier? This information is required in order to determine whether the activity observed is peripheral or is the central effect. Molecular studies are needed to assess the mechanisms by which these peptides exert their activities. The modification of technologies—or the developments of alternative processes—should also be examined. At present, most peptides are synthesized for use in biological studies including animal studies, but their production quantities are limited. The amount is sufficient for animal studies, but in the case of human studies larger amounts are needed. The high cost of these preparations will further restrict their use in human studies. Accordingly, there is a need to develop specific chromatographic and membrane techniques by means of which active peptide fractions can be produced and enriched. Since it is envisaged that bioactive peptides will be mainly ingested by consumers through foods, an important step shall be to study the technological properties of the active peptide fractions and to develop model foods which both contain these peptides and retain their activity for a certain period. It is recognized that peptides are more reactive than proteins due to their lower molecular weight, and the peptides that are present in the food matrix may react with other food components. The interaction of peptides with carbohydrates and lipids as well as the influence of the processing conditions (especially heating) on peptide activity and bioavailability should also be investigated.

Clearly, if some of the anticipated effects suggested by the positive results from animal studies can be substantiated in human studies, this will be a major factor of success in the marketing of dairy foods on the basis of their health-enhancing properties. In the future, milk-derived bioactive peptides may be important components in foods sustaining health and in the prevention of diseases and conditions such as cardiovascular diseases, obesity, osteoporosis and stress.

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