The Emerging Role of Dairy Proteins and Bioactive Peptides in Nutrition and Health

Hypotensive Peptides from Milk Proteins[1,2]

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ABSTRACT   Hypertension is the major controllable risk factor associated with cardiovascular disease (CVD) events such as myocardial infarction, stroke, heart failure, and end-stage diabetes. A 5 mm Hg decrease in blood pressure has been equated with ~16% decrease in CVD. In the U.S. alone current annual antihypertensive drug costs are approximately $15 billion. The renin-angiotensin-aldosterone system is a target for blood pressure control. Cleavage of angiotensinogen by renin produces angiotensin I which is subsequently hydrolyzed by angiotensin-I-converting enzyme (ACE) to angiotensin II (a potent vasoconstrictor). Various side effects are associated with the use of ACE inhibitory drugs in the control of blood pressure including hypotension, increased potassium levels, reduced renal function, cough, angioedema, skin rashes, and fetal abnormalities. Milk proteins, both caseins and whey proteins, are a rich source of ACE inhibitory peptides. Several studies in spontaneously hypertensive rats show that these casokinins and lactokinins can significantly reduce blood pressure. Furthermore, a limited number of human studies have associated milk protein-derived peptides with statistically significant hypotensive effects (i.e., lower systolic and diastolic pressures). The advent of effective milk protein based functional food ingredients/nutraceuticals for the prevention/control of blood pressure therefore has the potential to significantly reduce global healthcare cost.   J. Nutr. 134: 980S–988S, 2004.

KEY WORDS: • ACE inhibitors • casokinins • lactokinins • hypertension • functional foods

Increasing consumer knowledge of the link between diet and health has raised the awareness and demand for functional food ingredients and nutraceuticals. This is leading to a mind-set of self-medication often driven by the desire to avoid undesirable side effects associated with consumption of organically synthesized drugs and to also avoid the increasing cost of drug therapy. It is well recognized that apart from their basic nutritional role many food proteins contain encrypted within their primary structures peptide sequences capable of modulation specific physiological functions.

The application of specific foods or food components in the prevention and/or treatment of disease are of particular relevance in the management of cardiovascular disease (CVD).[4]

High blood pressure (BP), or hypertension, is a controllable risk factor in the development of a range of cardiovascular conditions. Therefore, any food component that on ingestion has the ability to reduce BP is a potential candidate component in the prevention/treatment of CVD. This review outlines the current situation on how milk protein-derived peptide sequences may be exploited as natural hypotensive agents.

Metabolic pathways associated with control of blood pressure in humans

The seventh Joint National Committee report by the National Heart, Lung, and Blood Institute recommended changes to the pre-existing guidelines used to classify adult BP (1). Due to the fact that the risk of heart disease and stroke increases at BPs above systolic BP (SBP)/diastolic BP (DBP) values of 115/75 mm Hg, health experts have now decreased the previously accepted BP range in order to encourage more proactive and earlier treatment of high BP. The new guidelines divide BP into 4 categories as follows: (i) normal, SBP < 120 mm Hg, DBP < 80 mm Hg; (ii) prehypertension, SBP 120–139 mm Hg, DBP 80–90 mm Hg; (iii) stage 1 hypertension, SBP 140–159 mm Hg, DBP 90–99 mm Hg; and (iv) stage 2 hypertension, SBP ≥ 160 mm Hg, DBP ≥ 100 mm Hg. As a
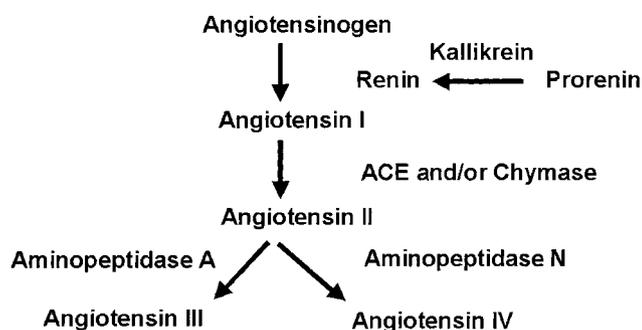
FIGURE 1 The renin-angiotensin system.

result of these changes many individuals whose BP was previously considered normal or borderline will now fall into the "prehypertension" category. By carefully controlling intracellular levels of specific metabolites, normal BP can be maintained. Modifications to the levels of these metabolites, due very often to environmental factors, may lead to the development of hypertension and in some instances CVDs. There are 2 general classes of hypertension, namely, primary/essential hypertension and secondary hypertension (2). Primary/essential hypertension, of which there is no known cause, accounts for 95% of all hypertensive complaints (3). Secondary hypertension may result from several disease states including kidney disease (4) and Cushing's syndrome (5), and treatment with certain medications such as estrogen-containing contraceptives (6). Secondary hypertension has been reported to complicate some 7–10% of all pregnancies (7).

Hypertension is a controllable risk factor in the development of a number of CVDs including stroke, left ventricular hypertrophy, smooth muscle cell hypertrophy and coronary infarct (8,9). Hypertension is reported to affect ~25% of the population (10). In the U.S. alone, hypertension and its associated complications led to ~35 million medical consultations in 2002 (11). The annual drug costs associated with the treatment of hypertension and related diseases is estimated to be in the region of $15 billion per annum in the U.S. (12). The risk of developing CVD is directly related to BP level. For each 5 mm Hg reduction in diastolic pressure the risk of CVD is reduced by about 16% (13,14). Two main strategies are currently recommended for the regulation of BP, lifestyle changes and drug treatments. Studies such as dietary approaches to stop hypertension (DASH) have shown that a balanced diet rich in fruits, vegetables, and low fat dairy products is an effective means of lowering BP (15,16). Additionally, modest increases in physical activity above sedentary levels have also been shown to effect clinically significant decreases in BP (17). Such dietary and lifestyle modifications have been endorsed by the World Health Organization (18). Alternatively, a range of targeted drug treatments may be employed including calcium channel blockers, angiotensin II receptor blockers, vasodilators, diuretics, and angiotensin-I-converting enzyme (ACE, EC 3.4.15.1) inhibitors (19).

BP is controlled by a number of different interacting biochemical pathways. Classically, BP control has been associated with the renin-angiotensin system (RAS). However, RAS is not an exclusive regulator of BP as the kinin-nitric oxide system (KNOS), the neutral endopeptidase system, and the endothelin-converting enzyme system have been shown to generate additional vaso-regulatory peptides independent of ACE (20–23). Together these systems generate a variety of regulatory peptides that collectively modulate BP, fluid, and electrolyte balance via membrane bound receptors located on

different tissues throughout the body. Additionally, BP can also be affected by an increase or decrease in fluid volume inside or outside the blood vessels or by an obstruction within the vessels.

RAS

The RAS is 1 of the major regulators of BP, electrolyte balance, renal, neuronal, and endocrine functions associated with cardiovascular control in the body. RASs specific to the brain (24,25), placenta (26), bone marrow (27), and pancreas (28) have been identified. As can be seen in **Figure 1**, RAS begins with the inactive precursor angiotensinogen (ATN). ATN is a glycopeptide with a molecular weight of ~60 kDa (29). ATN is distributed in numerous tissues in addition to plasma and cerebrospinal fluid. ATN is the only known precursor of angiotensin I as well as the only known substrate for renin (EC 3.4.23.15).

Renin is an acid proteinase containing ~350 amino acids. It is generated from the inactive precursor prorenin, by the action of kallikrein (EC 3.4.21.34) (30). The main source of renin is the juxtaglomerular cells of the kidney; however, renin has also been isolated from the submaxillary gland and from amniotic fluid. Several factors influence the release of renin, including renal perfusion pressure, salt depletion, and stimulation of β_2-receptors by aldosterone (31). Renin is responsible for liberation of angiotensin I from ATN (29). Inhibition of renin activity may be achieved as a result of angiotensin (Ang) II production and numerous pharmacological agents. The concentration of ATN in plasma is generally never high enough to saturate renin; therefore changes in the concentration of ATN may influence the rate of Ang II production (29).

Angiotensin I, the decapeptide released from the N-terminal portion ATN by the action of renin, has been located in plasma and most of the organs of the body including the brain, heart, lungs, kidneys, and reproductive systems. ACE removes the C-terminal dipeptide HL from angiotensin I resulting in the formation of angiotensin II, a potent vasoconstrictor. ACE also removes the C-terminal dipeptide from bradykinin (a potent vasodilator) resulting in the formation of inactive peptide fragments (**Fig. 2**). The levels of both angiotensin II and bradykinin are mainly dictated by ACE allowing for the regulation of peripheral BP.

Liberation of Ang II from Ang I results in a number of responses within the body, the particular response being dependent on the specific Ang II receptor activated on the target organ. The main receptors of Ang II are AT_1, AT_2, and AT_3, which are located in numerous tissues throughout the body (32). The major effects of Ang II are the control of BP, fluid
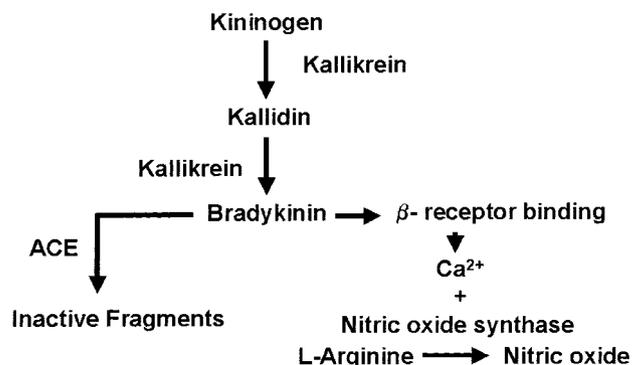

FIGURE 2 The kinin nitric oxide system.

TABLE 1

Vasoactive peptides and their receptors

Name	Peptide sequence[1]	Action[2,3]	Receptor	Reference
Angiotensin I	DRVYIHPFHL	—	—	—
Angiotensin II	DRVYIHPF	Vc	AT_1, AT_2	34
Angiotensin III	RVYIHPF	Vc	AT_1, AT_2	35
Angiotensin IV	VYIHPF	Vc and Memory	AT_1, AT_4	36
Angiotensin (1–7)	DRVYIHP	Vd	AT_1	37
Angiotensin (1–9)	DRVYIHPFH	—	—	—
Kallidin	KRPPGFSPFR	Vd	B_2	38
Bradykinin	RPPGFSPFR	Vd	B_2	38
[des-Arg]9-bradykinin	RPPGFSPF	Vd	B_1	38
[des-Arg]10-kallidin	KRPPGFSPF	Vd	B_1	38
Endothelin-1	CSCSSLMDKECVYFCHLDIIW	Vc	ETa, ETb	39

[1] Amino acids represented by one letter code.
[2] Vc, vasoconstriction.
[3] Vd, vasodilation.

volume and neurotransmitter interactions, and control of the activity of gonadotrophic hormone releasing hormones and pituitary hormones (20,24,33).

Ang II is a substrate for the angiotensinase group of enzymes resulting in the generation of other biologically active peptides (Fig. 1 and **Table 1**). The action of aminopeptidase A (EC 3.4.11.7) and aminopeptidase N (EC 3.4.11.2) on Ang II results in the formation of angiotensin III and IV, respectively (32,40). Chymase (EC 3.4.21.39), which has been isolated from mast and endothelium cells of the human heart, also hydrolyzes Ang I to Ang II (22). Chymase-dependent Ang II formation appears to be most active in the left ventricle of the human heart. In the other chambers of the heart ACE dependent Ang II formation dominates (41).

The kinin-nitric-oxide system

In the KNOS system, kallidin, a potent vasodilator, is formed from kininogen by the action of kallikrein (38). Further hydrolysis of kallidin by kallikrein results in the formation of other vasodilatory peptides including bradykinin (BK), [des-Arg]9 (minus carboxyl terminus arginine at position n)-BK and [des-Arg]10-kallidin (Fig. 2, Table 1). These molecules mediate a vasodilatory response by binding to β-receptors resulting in a series of reactions leading to increased intracellular Ca^{2+} levels (38). These increased Ca^{2+} levels stimulate nitric oxide synthase (EC 1.14.13.39) to convert L-arginine to nitric oxide (21). This vasodilatory pathway can be inhibited by the action of ACE, which degrades BK [Fig. 2 (42)]. The vasoregulatory action of nitric oxide has been outlined elsewhere (43).

Neutral endopeptidase and endothelin-converting enzyme systems

Neutral endopeptidase (NEP, EC 3.4.24.11), also called enkephalinase or neprilysin, is a transmembrane zinc metaloendopeptidase. NEP is found at the surface of several tissues including endothelial cells of the kidney, lungs, vascular wall, brain, heart, intestine, and adrenal glands (23,44). NEP hydrolyzes BK to inactive fragments. NEP may also hydrolyze Ang I and II to generate the vasodilatory hexapeptide, Ang (1–7) (**Fig. 3**, Table 1).

In response to stimulation by Ang II and/or damage to the endothelium, endothelin I (End I), a potent vasoconstrictor, is formed from big-endothelin by the action of endothelin-converting enzyme [ECE, EC 3.4.24.71, Fig. 3 (45)]. End I is present in coronary, renal, mesenteric, and cerebral arteries. End-I mediates vasoconstriction via 2 receptors (ETa and ETb), each of which is present on a variety of tissues [(46), Table 1]. End I can also regulate sodium re-absorption in the nephron (47). The release of nitric oxide from the surface of the vascular endothelium can inhibit the release of End I (43,48).

Natriuretic peptides

Natriuretic peptides are a group of peptides present in the body that can exhibit vasodilatory and tissue protective properties. For example, atrial natriuretic peptide has been reported to inhibit production of End I (Table 1). Natriuretic peptides can cause vasodilatation, natriuresis, diuresis, decreased aldosterone release, decreased cell growth, and inhibition of the sympathetic nervous system. These responses suggest natriuretic peptides may be ACE or renin inhibitory peptides produced naturally within the body (23).

It is clear therefore from the above (Figs. 1, 2, and 3) that ACE plays a central role in the control of BP by regulating the level of key vasoactive peptides (Table 1).

Characteristics of ACE

ACE is a chloride dependent metallocarboxydipeptidase (30). Three forms of ACE have been identified, i.e., somatic
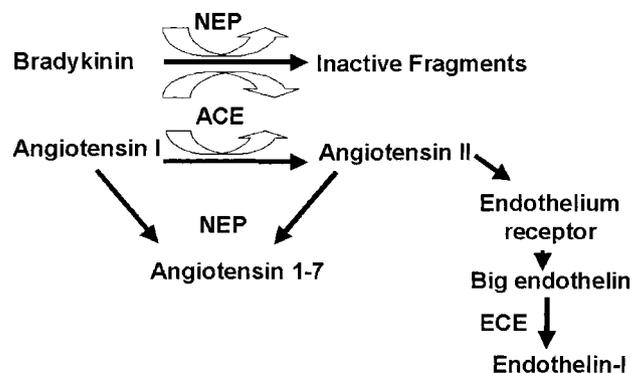

FIGURE 3 The neutral endopeptidase and endothelin converting enzyme systems.

ACE, germinal or testicular ACE, and an ACE 2 homologue (32). ACE is located on the surface of vascular endothelial cells in organs such as the brain, heart, lungs, liver, intestine, pancreas, spleen, skeletal muscle, adrenal gland, and placenta (29). Somatic ACE is a transmembrane peptidase, which binds to the external surface of the plasma membrane of cells via a hydrophobic anchor domain (20,49). A secretease activity can release membrane bound ACE into the plasma. Somatic ACE contains 2 active sites whereas germinal or testicular ACE only contains 1 active site (20). Recently, human genome studies have isolated a third form of ACE known as ACE homologue or ACE 2. This third form of ACE contains a single active site and has the ability to hydrolyze Ang I and II but does not hydrolyze BK (32,50). Peptides with a free C-terminal carboxylate anion serve as substrates for ACE (Table 1). The C-terminal must be anchored to a positively charged basic group and the scissile peptide bond must be in juxtaposition to the Zn^{2+} ion in the active site (29).

In addition to its role as a vasoregulatory activity, ACE has been reported to act as a digestive peptidase in the intestinal tract (51). ACE has also been shown to act as an endopeptidase on C-terminal amidated peptides such as substance P, a potent neuropeptide transmitter, and luliberin, a leuteinizing-releasing hormone. ACE can hydrolyze other substrates such as enkephalins, neurotensin, and the β-chain of insulin. Due to the ability of ACE to inactivate both bradykinin and substance P it has a particular role in inflammation. ACE may also be involved in immunity as well as reproduction due to its presence in the reproductive organs (20).

There are a number of methods used to quantify ACE activity. These include using hippuryl-L-histidyl-L-leucine, which can be quantified spectrophotometrically (52) or by reversed-phase high performance chromatography (53). Additionally, ACE activity may be quantified using 2-furanacryloyl-L-phenylalanyl-L-glycyl-L-glycine (54,55). Fluorometric analysis of ACE activity is also possible using the fluorophore-labeled tripeptide dansyltriglycine (56). The potency of an ACE inhibitor is usually expressed as an IC_{50} (concentration of material mediating 50% inhibition of ACE activity) value, which is equivalent to the concentration of inhibitor mediating 50% inhibition of activity.

Inhibitors of ACE

First reports of exogenous inhibitors of ACE displaying an anti-hypertensive effect in vivo were from snake venom (57,58). Although these peptides were potent inhibitors of ACE they had limited pharmacological application due to their lack of oral activity. Subsequently, peptidomimetic ACE inhibitors such as Captopril having potent anti-hypertensive activity were generated (59,60). Several adverse side effects such as hypotension, increased potassium levels, reduced renal function, cough, angioedema, skin rashes, and fetal abnormalities have been associated with synthetic ACE inhibitory drugs (61–64). Natural inhibitors of ACE have been identified within the primary sequences of a range of food proteins (65).

Milk protein derived inhibitors of ACE

Milk proteins contain ACE inhibitory peptides encrypted within their primary structures. These peptides can be released by enzymatic hydrolysis either during gastrointestinal digestion or during food processing. The sequences of the individual milk proteins displaying ACE inhibitory activity in vitro have been reviewed elsewhere (66,67). **Table 2** summarizes some properties of the more potent ACE inhibitory peptides from

TABLE 2

Potent casokinin and lactokinin sequences from individual bovine milk proteins

Protein	Peptide fragment	Primary Sequence[1]	ACE[2] IC_{50}[3]	Reference
			$\mu mol/L$	
Casokinins				
α_{s1}-casein	f(25–27)	VAP	2.0	68
α_{s2}-casein	f(174–179)	FALPQY	4.3	69
β-casein	f(74–76)	IPP	5.0	70
κ-casein	f(185–190)	VTSTAV	52.0	71
Lactokinin				
α-lactalbumin	f(104–108)	WLAHK	77.0	72
β-lactoglobulin	f(142–148)	ALPMHIR	42.6	73
BSA[4]	f(208–216)	ALKAWSVAR	3.0	74

[1] One letter amino acid code.
[2] Angiotensin-I-converting enzyme.
[3] Concentration of peptide mediating 50% inhibition of ACE activity.
[4] Bovine serum albumin.

the individual caseins (casokinins) and whey proteins (lactokinins). It is seen that, of the individual caseins, f(25–27) from α_{s1}-casein is a potent in vitro inhibitor of ACE having an IC_{50} of 2.0 $\mu mol/L$ and that f(208–216) of bovine serum albumin has an IC_{50} of 3.0 $\mu mol/L$. While the structure activity relationship for food-derived ACE inhibitors has not been established, it appears that binding to ACE is strongly influenced by the C-terminal tripeptide sequence. Many substrates and competitive inhibitors of ACE contain hydrophobic amino acids in this region. A number of potent food protein derived ACE inhibitors contain proline at the C-terminus. Furthermore, several ACE inhibitors contain lysine or arginine as the C-terminal residue. It has been postulated that the positive charge associated with the side-chain groups of these amino acids contributes to ACE inhibitory potency (58,65,75,76). In addition, the structure adopted by a specific peptide inhibitor of ACE, particularly the longer chain inhibitors, may also contribute to potency (66,76).

Generation and characterization of ACE inhibitory peptides

It is well established that in vitro incubation of milk proteins with gastrointestinal proteinase preparations enriched in pepsin, trypsin, and chymotrypsin activities results in the release of ACE inhibitory peptides. Therefore it is likely that ACE inhibitory peptides are generated during gastrointestinal transport. Bacterial and plant proteinases can also be used to release ACE inhibitory peptides (71,77). Therefore hydrolysates of whole milk protein, caseinates, whey proteins, and fractions enriched in individual milk proteins are potentially good sources of ACE inhibitory peptides.

The proteinases in various bacterial strains, many of which may be used in the manufacture of fermented dairy products, are capable of releasing ACE-inhibitory peptides from milk proteins [for review see (78,79)]. Proteinase from lactic acid bacteria such as *Lactobacillus lactis* and *Lactobacillus helveticus* CP790 (80,81) produce potent ACE inhibitory peptides in vitro. Pihlanto-Leppälä et al. (82) reported that the in vitro release of ACE inhibitory peptides from casein or whey by commercial yoghurt starters required further incubation with pepsin and trypsin activity. *Lactobacillus helveticus* strains were capable of releasing ACE inhibitory peptides into fermented

TABLE 3

Bovine casein-derived peptides displaying hypotensive effects in spontaneously hypertensive rats

Peptide	Sequence[1]	IC$_{50}$[2]	Maximum decrease in SBP[3]	Reference
		$\mu mol/L$	mm Hg	
α_{s1}-casein				
f(1–9)	RPKHPIKHQ	13	−9.3	88
f(23–34)	FFVAPFPEVFGK	77	−34.0	93
f(104–109)	YKVPQL	22	−13.0	80
f(146–147)	YP	720	−32.1	81
f(194–199)	TTMPLW	16	−14.0	93
α_{s2}-casein				
f(189–192)	AMPKPW	580	−5.0	80
f(190–197)	MKPWIQPK	300	−3.0	80
f(198–202)	TKVIP	400	−9.0	80
β-casein				
f(59–61)	VYP	288	−21.0	77
f(59–64)	VYPFPG	221	−22.0	77
f(60–68)	YPFPGPIPN	15	−7.0	88
f(74–76)	IPP	5	−28.3	70
f(80–90)	TPVVVPPFLQP	749	−8.0	77
f(84–86)	VPP	9	−32.1	70
f(140–143)	LQSW	500	−2.0	80
f(169–174)	KVLPVP	5	−32.2	80
f(169–175)	KVLPVPQ	1000	−31.5	80
f(177–183)	AVPYPQR	15	−10.0	93

[1] One letter amino acid code.
[2] Concentration of peptide mediating 50% inhibition of ACE activity.
[3] Systolic blood pressure (mean value).

milk drinks or yoghurt-type products (81,83–85). The potent casokinins IPP and VPP were found in skim milk fermented with *Lactobacillus helveticus* CP790 and *Saccharomyces cerevisiae* (81). Yoghurt-type products fermented with *Lactobacillus delbreukii* spp *bulgaricus* and *Lactobacillus lactis* spp *cremoris* were also shown to contain ACE inhibitory peptides (79). A number of studies have shown that ACE inhibitory peptides can be produced during cheese making (86–89). In general, it appears that ACE inhibitory peptide release increases during cheese ripening. However, extended ripening times result in the degradation of ACE inhibitory peptides. A low fat cheese containing ACE inhibitory peptides has been reported (90).

The general strategy for identification of potent in vitro ACE inhibitory peptides has been to isolate these peptides from in vitro digests of milk proteins or from fermented dairy products. Synthetic peptide studies are then usually carried out as confirmatory tests for primary structure and for the determination of IC$_{50}$ values (66).

Hypotensive effects

The ability to inhibit ACE in vitro is indicative of the potential of a given lactokinin or casokinin to act as a hypotensive agent in vivo. However, in order to mediate a hypotensive effect in vivo, the peptide(s) must reach the target organ. The oral ingestion of milk protein hydrolysates or fermented dairy proteins containing ACE inhibitory peptides therefore presents many challenges to the stability of the peptides therein. These peptides need to survive degradation by gastrointestinal proteinases and peptidases, they need to pass from the intestine to the serum where they may be susceptible to brush border and intracellular peptidase activi-

ties, and they need to be resistant to degradation by serum peptidases (91). Many studies in vitro have been performed to determine the stability of different ACE inhibitory peptides to survive gastrointestinal passage and to determine if ACE inhibitory peptides can be transported through intestinal cells. Peptides with potent in vitro ACE inhibitory activity such as α_{s1}-casein f(23–27) (68), and α_{s1}-casein f(104–109) (80), were subsequently shown to have no hypotensive effects in vivo; this was presumably due to degradation to inactive fragments during oral ingestion. During in vitro studies with the lactokinin, β-lactoglobulin f(142–148), Vermeirssen et al. (92) demonstrated that this peptide could be transported intact through a Caco-2 Bbe cell monolayer. However, the concentrations transported were reported to be too low to exert an ACE inhibitory effect in vitro. While valuable information can be obtained from in vitro model systems with respect to the proteolytic/peptideolytic stability and susceptibility to intracellular passage, however, it is only through in vivo studies that the hypotensive effects of a given peptide or peptide preparation can be reliably assessed (91).

Rat studies

Numerous rat studies have been performed to determine the hypotensive effects of milk protein derived ACE inhibitors. The maximal decrease in SBP achieved in spontaneously hypertensive rats (SHRs) using various casokinins and lactokinins are summarized in **Tables 3** and **4**, respectively. SBP decreases ranging from 2–34 mm Hg have been reported. Peptide studies using SHRs have been carried out with fragments derived from all the major casein and whey proteins. However, to date more SHRs studies have been performed with casokinins than lactokinins. There appears to be no direct relationship between the extent of SBP decrease and the IC$_{50}$ values for the different peptides tested to date. For example, β-casein f(169–175) having an IC$_{50}$ of 1000 μmol/L gave a maximal decrease in SBP of 31.5 mm Hg (Table 3). Of the casokinin related SHR studies reported, the highest reduction in SBP (34 mm Hg) was observed for α_{s1}-casein f(23–34) (Table 3). The casein-derived tripeptides IPP and VPP were reported to reduce SBP in SHR by 28.3 and 32.1 mm Hg,

TABLE 4

Bovine whey protein-derived peptides displaying hypotensive effects in hypertensive rats

Peptide	Sequence[1]	IC$_{50}$[2]	Maximum decrease in SBP[3]	Reference
		$\mu mol/L$	mm Hg	
α-la[4]				
f(50–53)	YGLF	733	−23	94, 95
β-lg[5]				
f(78–80)	IPA	141	−31	77
BSA[6]				
f(221–222)	FP	315	−27	77
β_2-m[7]				
f(18–20)	GKP	352	−26	77

[1] One letter amino acid code.
[2] Concentration of peptide mediating 50% inhibition of ACE activity.
[3] Systolic blood pressure (mean value).
[4] α-lactalbumin.
[5] β-lactoglobulin.
[6] Bovine serum albumin.
[7] β_2-microglobulin.

respectively. Highest lactokinin induced reduction in SBP (31 mm Hg) was obtained with β-lactoglobulin f(78–80) (Table 4). There appears to be no relationship between casokinin chain length and the observed hypotensive response in SHR. With regard to lactokinins, potent hypotensive responses have been observed for peptide fragments with 4 or less amino acid residues (Table 4). The differences in the range of SBP responses observed in Tables 3 and 4 may not only relate to compositional differences in the test material investigated but also to the study design where the dosage, duration, means of administration, and choice of control differed.

SHR studies with the Calpis sour milk drink, which has been shown to contain the potent casein-derived ACE inhibitory tripeptides IPP and VPP, yielded an SBP decrease of 17.7 mm Hg following consumption of 5 mL/kg body weight over an 8 h period (70). Using the tail-cuff method to measure SBP in SHR, the antihypertensive activity of IPP and VPP was dose-dependent up to 5 mg/kg body weight. Furthermore, neither the Calpis sour milk (25 mL/kg) nor a mixture of IPP and VPP changed the SBP of a normotensive strain of Wistar-Kyoto rats. Interestingly, BP in SHR returned to previous levels 24 h after discontinuation of consumption of Calpis sour milk. Consumption of a sour milk beverage fermented with *Lactobacillus helveticus* LBK-16 H (also shown to contain IPP and VPP) was reported to reduce SBP by 21 mm Hg during a 14 wk SHR feeding trial (84). In this long term study the attenuation of hypertension development in young SHRs was evident after 6 wk administration of fermented milk, whereas normal skim milk had no effect. The authors suggested that several mechanisms may have been responsible for the observed hypotensive effect of the fermented milk products in SHR (84). These include the inhibition of ACE by IPP and VPP as demonstrated by in vitro experiments (70) and the elevation of plasma renin levels in SHR. The latter case is indicative of a lack of negative feedback by angiotensin II due to inhibition of ACE. The authors stated that calcium in the fermented milk sample fed to SHR might also have contributed to retarding the development of high BP (84).

Human trials

A limited number of human studies have been performed on the hypotensive effects of different milk protein hydrolysates and fermented dairy products shown in vitro to contain ACE inhibitory peptides. In the majority of in vivo studies reported the anti-hypertensive effects were attributed to casokinins. **Table 5** summarizes the results obtained from human studies. Sekiya et al. (96) were the first to demonstrate that consumption of 20 g/d of a tryptic hydrolysate of casein could bring about a reduction in both DBP and SBP in hypertensive human volunteers (Table 5). More recently it was reported that a tryptic hydrolysate of casein containing a potent ACE inhibitory twelve residue (C12) α_{s1}-casein peptide f(23–34) could also reduce BP in hypertensive humans (100). In this study, the C12 containing hydrolysate was orally administered (160–200 mg/kg) on a daily basis to an unspecified number of human volunteers over a 4-wk period resulting in a BP reduction of between 4 and 6 mm Hg (Table 5). The antihypertensive effect was evident after wk 2 of the study. The double blind placebo controlled study of Hata et al. (97) was the first to demonstrate that a fermented sour milk drink could significantly reduce DBP and SBP following oral ingestion of 95 mL Calpis per day by mildly hypertensive human volunteers. In this study 30 elderly male and female patients, the majority of whom were taking antihypertensive medication, were divided into 2 groups and administered with either the Calpis soured milk or acidified milk as a placebo. In the test group significant SBP reductions of −9.4 and −14.1 mm Hg were recorded at 4 and 8 wk after initiation of the trial, respectively. DBP was reduced by 6.9 mm Hg at the end of the 8-wk trial. The authors reported that no significant changes in BP were observed on ingestion of the acidified milk placebo. In this trial the test and placebo samples had similar mineral levels. In addition, ingestion of the test material or the placebo had no effect on heart rate, body weight or blood serum variables, i.e., HDL or triacylglycerol concentrations. In 2 independent studies on the ingestion of fermented milk (150 mL/d) containing similar quantities of IPP and VPP, a larger hypotensive response was reported after 8 as opposed to 21 wk ingestion of the fermented milk [(98, 99), Table 5].

Evidence is also beginning to emerge suggesting that consumption of whey protein hydrolysates may result in significant reductions in BP [(101), Table 5]. In a recent study, a whey protein hydrolysate (20 g/d) and a whey protein isolate control (20 g/d) were orally ingested by 30 male and female, unmedicated, nonsmoking, hypercholesterolemic, borderline hypertensives over a 6 wk period (102). The study indicated that significant reductions in SBP and DBP occurred 1 wk after ingestion of the hydrolysate and that these BP reductions persisted for the remaining 5 wk of the study. It was also indicated that white cell counts were significantly increased and LDL levels were decreased in participants who ingested the whey protein hydrolysate sample.

An important observation from the in vivo trials is that consumption of specific hydrolysates or fermented dairy prod-

TABLE 5

Hypotensive effects of fermented milks and milk peptides in humans

Sample	Peptide sequence[1]	Dose	Duration	DBP[2]	SBP[3]	Reference
			wk	*mm Hg*		
Tryptic casein	—	20 g/day	4	−4.6	−6.6	96
Calpis	VPP/IPP	95 mL/day	8	−6.9	−14.1	97
Fermented milk	VPP/IPP	150 mL/day	8	−8.8	−14.9	98
Fermented milk	VPP/IPP	150 mL/day	21	−3.6	−6.7	99
C12	FFVAPFEVFGK	>0.2 g/kg	4	−6.5	−4.5	100
BioZate	whey peptides	20 g/day	6	−7.0	−11.0	101

[1] One letter amino acid code.
[2] Diastolic blood pressure.
[3] Systolic blood pressure.

TABLE 6

Commercial developments in hypotensive dairy protein derived products

Product type	Brand name	Active compound[1]	Manufacturer
Sour milk	Calpis	IPP, VPP	Calpis Co., Japan
Fermented milk	Evolus	IPP, VPP	Valio, Finland
Casein hydrolysate	Casein DP	FFVAPFEVFGK	Kanebo Ltd., Japan
Casein hydrolysate	C12 peptide	FFVAPFEVFGK	DMV International, The Netherlands
Whey protein hydrolysate	BioZate	Whey peptides	Davisco, U.S.

[1] One letter amino acid code.

ucts has no effect on BP in either normotensive rats or humans. Furthermore, dose-dependent effects are generally observed and there is a lag time between consumption of a given test sample and the appearance of hypotensive effects. In addition, on removal of the test compound a lag period takes place before a return to the high BP values existing prior to consumption of the test compounds. In the study of Hata et al. (97) the reduction in BPs observed at the end point of the administration period were still maintained 4 wk after oral ingestion of Calpis sour milk ceased. Finally, no adverse effects have been reported following oral consumption of the different test materials [(96–101), Table 5].

Current commercial developments

There are a number of products on the market or under development by international food/food ingredients companies aimed at exploiting the functional food ingredient potential of milk protein derived hypotensive peptides. These products are either in the form of fermented milk drinks or as milk protein hydrolysates. In most cases, some of the peptides thought to contribute to the hypotensive effect have been identified in these products (**Table 6**).

CONCLUSION AND FUTURE CHALLENGES

Considerable time and resources have been devoted to studying the potential hypotensive effects of milk protein derived peptides. To date, the major target for screening these peptides is their ability to inhibit ACE activity in vitro since ACE plays a central role in controlling BP. In vivo studies with SHRs and hypertensive human volunteers report significant blood pressure reducing effects of consuming specific milk protein hydrolysates and fermented dairy products.

More detailed studies are required for a better understanding of the blood pressure reducing mechanism(s) of food-derived peptides as the hypotensive effects may not be entirely due to inhibition of ACE activity. It was recently shown, for example, that α-lactorphin [α-lactalbumin f(50–53), Table 4] reduced BP in SHR and normotensive Wistar Kyoto rats in a dose-dependent manner following subcutaneous administration. However, the BP reducing effect was absent in the presence of naloxone, an opioid receptor antagonist, indicating that the hypotensive effect was mediated through the vasodilatory action of binding to opiate receptors (94). The BP reducing effects of complex systems such as fermented milk drinks and milk protein hydrolysates may only be in part due to ACE inhibition. These products contain a complex mixture of peptides that may also have opioid binding capabilities. Furthermore, the hypotensive effects of fermented milk drinks may also be in part due to the high levels of biologically available calcium present in these products (99). The hypotensive effects of high calcium, low fat dairy product diets have

been well documented (15). There is a need for detailed, peer-reviewed unequivocal evidence demonstrating the hypotensive effects of consuming specific milk protein based ingredients/products. This information is required by food processors and legislative authorities to provide consumers with functional foods having validated health claims.

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