Health relevance of antihypertensive peptides in foods

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Abstract

Food-derived bioactive peptides are promising components for the prevention and treatment of cardiovascular diseases including hypertension. Recently, there has been an increase in knowledge about the variety of complex and interrelated mechanisms for blood pressure regulation as well as the potential targets for peptides to exert their antihypertensive effects. Empiric and bioinformatics studies have provided large amounts of data regarding characteristics, structure-activity relationships and bioavailability of antihypertensive peptides through the use of *in vitro* assays, cell cultures, and animal studies. However, the scarce number of robust clinical trials to prove their efficacy in humans is the main reason of the limited use of antihypertensive peptides as functional foods for promoting health. Further research is needed to overcome the challenges for the application of food-derived antihypertensive peptides as functional ingredients with health benefits in the human body.

Introduction

Hypertension is considered as one of the most important risk factors in the development of cardiovascular diseases, which are currently the main cause of death in the first world. Hypertension consists of a long-term elevation of blood pressure (BP) over 140/90 mm Hg (systolic/diastolic) that frequently can be improved with healthy lifestyles such as medical nutrition care including reduction in sodium intake and calcium supplementation, as well as regular physical activity and reduction of alcohol consumption and stress conditions [1]. Different pharmaceutical drugs are available for the treatment of hypertension, but their high costs and negative side-effects has led to an increasing interest in the research on natural food-derived bioactive peptides for the management of BP [2].

Bioactive peptides are short sequences of amino acids that exert different biological properties with a positive health impact on the human body, being antihypertensive peptides the most studied to date. The use of empirical and bioinformatics approaches have allowed the discovery of numerous peptides with antihypertensive effects derived from milk, egg, meat, fish, and their by-products, but their use as nutraceutical and functional food ingredients for BP regulation is still very limited due to their insufficient proven efficacy in clinical trials [3-5].

The present paper gives a general overview about the multiple and complex mechanisms of action of antihypertensive peptides as well as the current status of bioavailability studies and future research needs for their use as functional components to promote human health.

Antihypertensive mechanisms of action

The control of BP can be carried out through a variety of interrelated metabolic pathways, being the inhibition of angiotensin converting enzyme (ACE) by the renin-angiotensin system (RAS) the most studied mechanism to date. However, there are other control systems that target nitric oxide system (NOS), endothelin system function, and receptor blockers, among others [6**]. A simplified scheme of the main systems for BP regulation is shown in Figure 1.

Renin-angiotensin system (RAS)

The RAS is one of the most important pathways for BP control and electrolyte balance in the human body. In this system, angiotensinogen is cleaved by renin generating angiotensin I. This inactive peptide is hydrolysed by ACE into angiotensin II, which is a potent vasoconstrictor that mediates its action through binding with AT_1 and AT_2 receptors (-R). AT_1-R induces vasoconstriction by increasing the Ca^{2+} level in vascular smooth muscle cells (VSMC), releases aldosterone that increases salt and water retention leading to renin inhibition, and increases inflammation and oxidative stress. Binding to AT_2-R produces vasodilation through the NOS, protecting against hypertension [7]. Additionally, ACE can hydrolyse the vasodilator bradykinin into inactive fragments, inhibiting indirectly the production of nitric oxide (NO) that occurs during BP regulation when bradykinin binds to β-R [8]. Angiotensin I can also be cleaved by ACE 2 to generate the inactive angiotensin 1-9, which can be further converted to angiotensin 1-7 by the action of ACE. A more efficient pathway of ACE 2 is the hydrolysis of angiotensin II into angiotensin 1-7, whose binding to the MAS-R inhibits angiotensin II-induced vasoconstriction [9].

Most strategies in BP control involve the inhibition of ACE by food-derived peptides, which reduces both angiotensin II generation and bradykinin inactivation leading to a BP decrease [5,10]. However, other antihypertensive mechanisms in RAS would imply

98 AT_1-R blockers, ACE 2 up-regulation, β-R activation, and renin inhibitors that may be
99 even more specific than ACE inhibitors [7,11].
100 *Nitric oxide system (NOS)*
101 This pathway is assumed to be, together with RAS, one of the main mechanisms in BP
102 regulation. In the NOS, the oxidation of L-arginine to L-citrulline by the action of
103 endothelial nitric oxide synthase (eNOS) generates NO which leads to vasodilatory
104 effects and BP reduction [12]. So, the use of peptides that increase NO production
105 through eNOS up-regulation would be a possible pathway for hypertension treatment.
106 *Endothelin system*
107 Endothelin converting enzyme (ECE) cleaves big endothelin-1 (bET-1) to generate
108 endothelin-1 (ET-1), which mediates vasoconstriction in VSMC due to its binding to
109 ET_A-R and ET_B-R. However, ET_B-R also induces dilatation of endothelial cells through
110 eNOS activation [13]. The decrease in the release of ET-1 by endothelial cells through
111 the use of ECE inhibitors is an alternative antihypertensive mechanism to ACE
112 inhibition. ET-1 levels can also be reduced indirectly by ACE inhibition due to the
113 accumulation of bradykinin and consequently increases the generation of NO, which
114 antagonises the release of ET-1. Additionally, peptides can block calcium channels,
115 reducing the influx of Ca^{2+} produced by the activation of AT_1-R and $ET_{A,B}$ -R and thus
116 increasing vasodilation [14].
117 *Other systems*
118 Antihypertensive activity of food-derived peptides can be exerted through other less
119 studied mechanisms related to sympathetic nervous system (SNS), vascular
120 inflammation and oxidative stress. Angiotensin II induces the formation of reactive
121 oxygen species (ROS) that scavenge NO and increase SNS activity, leading to renin
122 production and RAS activation. Moreover, ROS stimulate the production of cytokines

123 that generate over-expressed inflammatory responses. Therefore, peptides showing anti-
124 inflammatory and antioxidant activities may also exert lowering BP effects due to the
125 increased bioavailability of NO through the control of cytokine levels and the
126 scavenging of free radicals, respectively [15,16]. Additionally, binding of peptides to
127 opioid receptors can exert lowering effects on BP due to the release of the vasodilator
128 NO or the decreased SNS activity. The presence of opioid receptors in the intestinal
129 tract would be an advantage as peptides would not need to be absorbed and reach the
130 blood stream to exert their antihypertensive action [17].
131
132 **Structure-activity modelling**
133 The development of bioinformatic tools and predictive models has allowed the
134 discovery of bioactive peptides and study their chemical structures as well as enzyme-
135 peptide interactions linked with specific bioactivities. Quantitative structure-activity
136 relationship modelling (QSAR) and molecular docking simulation are widely used as
137 cost- and time-effective tools prior to *in vitro* and *in vivo* assays, allowing a better
138 understanding of the characteristics and mechanisms of antihypertensive peptides
139 [18,19]. Such *in silico* models have shown that the C-terminal sequence has a major
140 effect on antihypertensive mechanisms such as ACE and renin inhibition, as well as the
141 formation of hydrogen bonds between peptides and the active sites of enzymes is
142 mainly responsible for the inhibition of these enzymes [20-22]. Abdelhedi et al. [23]
143 noted that binding of peptides to ACE can also be performed through hydrophobic, van
144 der Waals and electrostatic interactions with the residues coordinated with the zinc ion
145 of the enzyme. On the other hand, Liu et al. [24] suggested that the structural stability of
146 the ACE-peptide complex may be due to covalent cation–pi interactions. Molecular
147 docking would appear to give better results for competitive ACE inhibitors because

structure-activity correlation studies are based on a competitive-type binding mechanism in which ligands occupy the active site of the enzyme. However, non-competitive inhibitors of ACE do not fit the model based on these studies and the relationship between the inhibition mechanism and the structure of these peptides is not yet clear [25]. ~~However~~Therefore, more research is needed in order to increase the knowledge about targets and mechanisms of action of bioactive peptides, which would improve the development of meaningful models for the discovery of promising antihypertensive peptides.

Bioavailability of antihypertensive peptides

Bioactive peptides need to reach their targets in a significant quantity and an active form to exert their health effects. Low bioavailability of peptides is mainly attributed to their instability during gastrointestinal (GI) digestion, selective transport, and degradation by blood plasma peptidases, which are important factors that may seriously limit their *in vivo* antihypertensive effects. Moreover, food processing conditions and matrix-peptide interactions can modify the structural characteristics of antihypertensive peptides or generate new compounds, affecting their bioavailability and bioactivity [~~25~~26*]. Several methods or models including static and dynamic *in vitro* assays, cell and *ex vivo* cultures, and animal studies are used to assess the bioaccesibility and bioavailability of antihypertensive peptides prior to further clinical studies to prove its hypotensive effect in humans.

In vitro studies

Digestion models are simple and useful tools to evaluate the effects of food processing and predict the stability of bioactive peptides in the GI tract [~~26~~27,~~27~~28]. These models do not reproduce all the dynamic aspects of the GI process, but show very good

correlations with *in vivo* outcomes even in the case of complex foods that can be subjected to structural changes due to the digestive environment [~~28~~29*]. Furthermore, it should be noted that gut endogenous proteases constitute a little explored source of bioactive peptides such as ACE inhibitory, renin inhibitory and antioxidant peptides that need to be considered, together with dietary protein-derived peptides, for their impact on various regulatory systems in the GI and human body [~~29~~30].

Cell cultures are also frequently used to study the stability and mechanism of transport of antihypertensive peptides through the intestinal epithelium [~~30~~31-~~32~~33]. As an example, Gallego et al. [~~30~~31] evidenced that the transport through Caco-2 cells can result in the degradation of ACE inhibitory peptides into smaller fragments, most of them also showed high *in vitro* activity (see Table 1). Peptides are easily hydrolysed by brush border peptidases generating di- and tri-peptides that are likely to be transported *via* intestinal peptide transporter T1 (PepT1). In this sense, Wang and Li [~~33~~34] reported that peptides transported by this active route showed higher bioavailability than peptides transported by paracellular route but also the amino acid sequence of short peptides could also affect their bioavailability. The use of cell cultures has improved the knowledge about the multiple functional modalities of antihypertensive peptides including ACE inhibition, control in the release of NO and endothelin, and reduction in oxidative stress and inflammation [~~34~~35,~~35~~36].

Current challenges involve the quantification of low abundant bioactive peptides for an adequate characterisation of their bioavailability, which is possible through the use of modern mass spectrometry techniques [~~36~~37]. Recently, Yang et al. [~~37~~38] evaluated the transport rate of ACE and renin dual inhibitory peptides LY, RALP and TF for potential *in vivo* antihypertensive activity. Grootaert et al. [~~38~~39**] went one step further as their study quantified egg antihypertensive peptides in an *in vitro* model that

combined luminal digestion with intestinal transport, also evaluating the food matrix influence.

Ex vivo and in vivo animal studies

Spontaneously antihypertensive rats (SHR) are one of the most common animal models used to study human hypertension. Numerous studies have been focused on studying the effects of oral administration of ACE inhibitory peptides on systolic blood pressure (SBP) of SHR. Some recent examples are shown in Table 2, evidencing that some peptides with low IC_{50} values exerted a notable and short-term antihypertensive effect in SHR after administration of low doses of peptide. It is worth noting the study by Sánchez-Rivera et al. [~~46~~47*] that identified and quantified the fragments generated from the peptide HLPLP by the action of rat plasma peptidases, which were still exerting an antihypertensive effect (reduction of 21.1 mm Hg for an intake of only 7 mg/Kg body weight of rat).

Ex vivo and *in vivo* studies are also useful to examine the molecular mechanisms of action [6**] and routes of intestinal transport of antihypertensive peptides. According to Jahandideh et al. [~~49~~50], egg hydrolysates exerted BP reduction through multiple mechanisms of vascular relaxation and RAS modulation such as reduced ACE and AT_1-R expression, as well as enhanced AT_2-R expression and NO bioavailability. On the other hand, Gleeson et al. [~~50~~51] evaluated the PepT1 and paracellular transport of peptides IPP and LKP through a combination of *in vitro*, *ex vivo* and *in vivo* models.

Human studies

Clinical trials are the most accurate way to evaluate the efficacy and health effects of food-derived antihypertensive peptides, but the complexity, high cost and time consuming make them scarce. Most of human studies performed to date involve dairy peptides, whereas the bioactivity of animal and vegetal products has been scarcely

confirmed in clinical trials. Several milk-derived ACE inhibitory peptides have been detected in the circulation of humans [~~51~~52,~~52~~53] and have shown significant BP-lowering effects [6**,~~51~~52,~~53~~54*]. Sanchón et al. [~~54~~55] monitored the degradation of milk proteins and peptide release in digests from human jejunum, evidencing a good correlation with a standardised *in vitro* model and characterising those peptides more resistant to GI digestion. In meat products, Montoro-García et al. [~~55~~56] suggested that the consumption of dry-cured ham, a product rich in bioactive peptides, did not impair BP in humans even though its high salt intake probably due to the participation of ACE inhibition and other antihypertensive mechanisms. Marine sources may have a positive health impact on oxidative stress and hypertension due to their relatively high content in amino acid taurine, which is known for its antioxidant activity and BP reducing effects [~~56~~57]. Salmon and sardine peptides have also shown antihypertensive effects in humans, although other clinical trials on hypertension and inflammation parameters have been inconclusive [~~57~~58]. *In vivo* conditions such as genetic factors, health status, diet, and other sources of inter-individual variability across different populations can lead to controversial outcomes when compared with animal or other human intervention studies. So, it is needed to consider these aspects to draw adequate conclusions and support health claims for food-derived antihypertensive peptides.

Conclusions

Despite the advances in knowledge about the potential of food-derived antihypertensive peptides to exert health benefits, this is still a subject of ongoing research. Further studies are needed for an indepth knowledge about the molecular mechanisms and pharmacokinetics of antihypertensive peptides, structural features that can affect their stability and bioavailability, and the development of robust clinical trials to conclusively

248 determine their efficacy in humans. Additionally, the use of antihypertensive peptides as
249 ingredients of functional foods requires the use of efficient strategies for industrial scale
250 production and oral delivery, as well the assurance of the safety and quality of the
251 product for consumer acceptance. The use of empirical and bioinformatics studies can
252 effectively help to overcome the current challenges existing for the use of food-derived
253 antihypertensive peptides in functional foods for promoting health.
254
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Figure captions

Figure 1. Simplified scheme of blood pressure regulation showing the relationship between the main mechanisms (renin-angiotensin, nitric oxide, and endothelin systems). Enzymes are shown in red whereas potential targets of peptides to mediate antihypertensive effects are in yellow squares. Adapted from Majumder and Wu [6**].

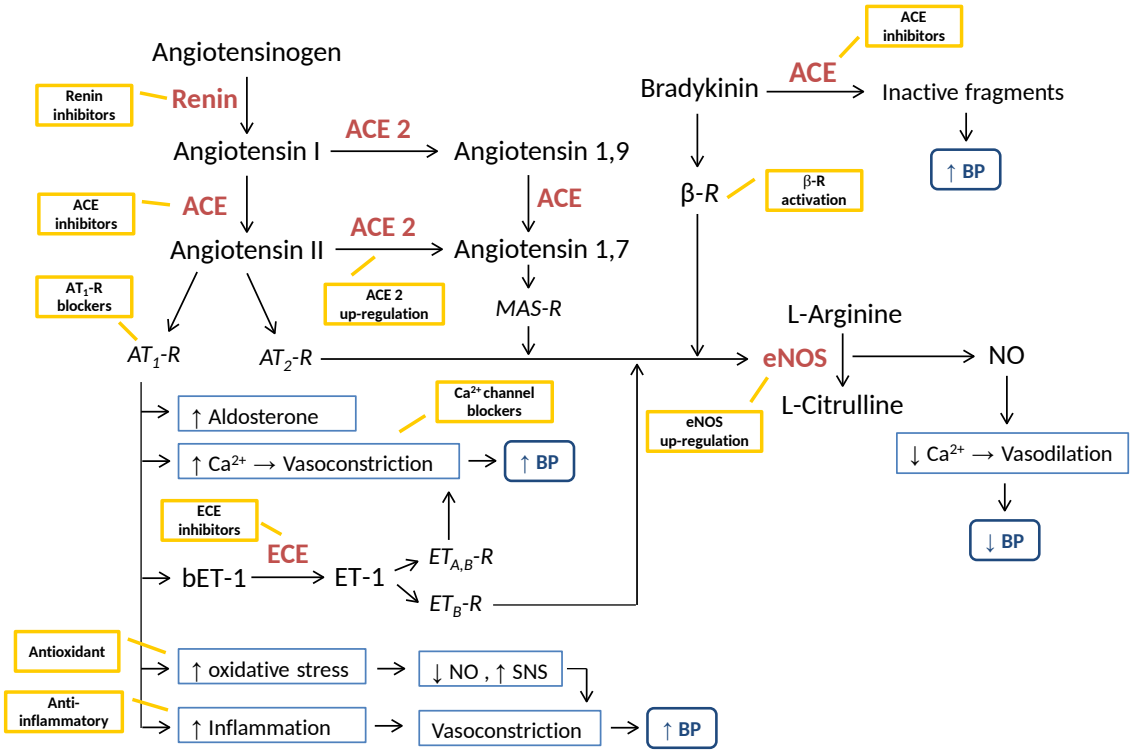
Angiotensinogen
Renin inhibitors
Renin
Angiotensin I
ACE 2
Angiotensin 1,9
ACE inhibitors
ACE
ACE
Angiotensin II
ACE 2
Angiotensin 1,7
ACE 2 up-regulation
MAS-R
AT1-R blockers
AT1-R
AT2-R
Bradykinin
ACE
ACE inhibitors
Inactive fragments
↑ BP
β-R
β-R activation
L-Arginine
eNOS
NO
L-Citrulline
eNOS up-regulation
↓ Ca2+ → Vasodilation
↓ BP
↑ Aldosterone
Ca2+ channel blockers
↑ Ca2+ → Vasoconstriction
↑ BP
ECE inhibitors
ECE
bET-1
ET-1
ETA,B-R
ETB-R
Antioxidant
↑ oxidative stress
↓ NO , ↑ SNS
Anti-inflammatory
↑ Inflammation
Vasoconstriction
↑ BP

Figure 1.

Table 1. Transport through Caco-2 cell monolayers of three ACE inhhibitory peptides derived from dry-cured ham.

Precursor peptide	Peptide fragments[a]	IC_{50} (μM)	Monoisotopic mass (Da)[b]	Apical – times (min)[c]				Basal – times (min)[d]		
				0	15	30	60	15	30	60
AAATP		100	429,22	x	x					
	AATP	300,74	358,19		x	x	x			
	AAAT	513,65	332,17						x	x
	ATP	406,56	287,15		x			x	x	x
	AAA	111,47	231,12						x	
AAPLAP		14,38	538,31	x	x	x	x			
	PLAP	76,5	396,24		x	x	x			
	APLA	> 1000	370,44					x	x	
	AAPL	> 1000	370,22					x	x	
	PL	337,32	228,15					x	x	x
	LA	310	202,13							x
KPVAAP		12,37	581,35	x	x	x	x	x	x	x
	VAAP	16,75	356,21			x		x	x	
	KPV	> 1000	342,23			x		x	x	
	KP	22	243,16		x		x			
	VA	607,96	188,12		x	x	x	x	x	x
	AP	230	186,10		x	x	x			x

[a] Fragments derived from the degradation of the precursor peptide detected by using MALDI-ToF/ToF MS.

[b] Monoisotopic molecular mass in Daltons of the matched peptide.

[c] Peptides detected in the apical compartment at different transport times.

[d] Peptides detected in the basal compartment at different transport times.

Adapted from Gallego et al. [31] with permission from Elsevier.

Table 2. Examples of ACE inhibitory peptides with proved antihypertensive effects in spontaneously hypertensive rats.

Peptide sequence	Source	IC_{50} (μM)	Dose (mg/kg bw)[a]	SBP (mm Hg)[b]	Time (h)[c]	Reference
GAAGGAF	Adlay seed glutelin	14,19	30	− 49.7	6	[40]
VLIVP	Bay scallop mantle	19,7	1,5	− 21.67	3	[41]
YQKFPQYLQY	Bovine casein	11,07	9	− 40	4	[42]
QIGLF	Egg white	75	50	− 13	10	[43]
MEVFVP	Flounder fish	79	40	− 44.25	6	[44]
VSQLTR	Flounder fish	105	40	− 34.25	6	[44]
YLVR	Hazelnut	15,42	10	− 39.97	8	[24]
FQPS	*Kacang* goat	27	2,39	− 10.6	8	[45]
IPIK	Krill	57,4	20	− 17	6	[46]
HLPLP	Milk casein	21	7	− 21.1	2	[47*]
LPLP	Milk casein	720	7	− 16.2	4	[47*]
WALKGYK	Mushroom	0,4	25	− 18	2	[48]
GNGSGYVSR	Sipuncula	29	5	− 31	2	[49]
YASGR	Sipuncula	184	5	− 25	2	[49]

[a]Oral administration of the peptide expressed as mg /kg body weight of rat.

[b]Maximum decrease in systolic blood pressure measured in mm Hg.

[c]Time in hours after peptide administration to exert the maximum decrease in systolic blood pressure.