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Bioactive peptides on endothelial function

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Abstract

Cardiovascular diseases (CVD) such as myocardial infarction and stroke are a major cause of morbidity and mortality worldwide. Impairment of the normal vasorelaxant functions of the vascular endothelium, termed endothelial dysfunction; appear to underlie the pathogenesis of CVD. Endothelial dysfunction is often secondary to abnormal increases in oxidative stress, inflammation and overactivity of the renin–angiotensin system (RAS), which makes these pathways attractive targets for therapeutic interventions. Given the side-effects associated with synthetic pharmaceutical agents, there is growing interest in using natural products such as bioactive peptides for treating chronic diseases like CVD. In this review, we discuss the potential for bioactive peptides with antioxidant, anti-inflammatory and RAS modulating properties for treating endothelial dysfunction and preventing CVD.

Keywords: Bioactive peptide; Endothelial dysfunction; Oxidative stress; Inflammation; RAS

1. Introduction

Cardiovascular diseases such as myocardial infarction and stroke are leading causes of morbidity and mortality [1]. Taken together, these two conditions caused 249.7 deaths/100,000 persons in 2013 and contributed to 28.2% of all deaths worldwide [2]. The key underlying pathology in cardiovascular disorders is atherosclerosis, the inflammatory thickening of the blood vessel wall [3]. Additional vascular factors predisposing to atherosclerosis include hypertension [4], the persistent elevation of blood pressure above 140/90 mmHg [5]. Both atherosclerosis and hypertension originate from impaired functioning of the endothelium, the monolayer of cells that line the lumen of blood vessels [6,7]. Thus, the endothelium is a key factor for maintaining vascular health and prevention of cardiovascular diseases.

While a number of pharmacological agents are widely used for prevention, treatment and long-term management of vascular diseases, these drugs are not without the risk of significant side-effects [8,9]. Not surprisingly, there has been an increased interest in developing alternative therapies from natural sources, which are commonly perceived to be safer than synthetic drugs. Naturally occurring proteins and their constituent peptides are an attractive source for novel natural compounds with various biological activities [10]. Bioactive peptides are defined as relatively short peptides (typically containing 2–20 amino acids) derived from their parent proteins (by enzymes, heat, chemical treatments or microbial fermentation) that demonstrate additional biological activities over and above their expected nutritional value [11,12]. Recent years have seen much interest in using bioactive peptides (and peptide-rich protein hydrolysates) from food sources as safe and natural alternatives for promoting and enhancing health [13–15]. This review will provide an overview of the potential roles of bioactive peptides in maintaining endothelial functions and/or preventing endothelial dysfunction through modulation of different physiological pathways.

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2. Endothelial functions and dysfunction

Over the past few decades, the roles of the endothelium in vascular physiology have become better understood. While

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originally it was believed to be simply an inert layer of cells separating blood from the structural tissues, it is now well known for its critical roles in regulating vascular tone. Research from the last 20 years has defined many crucial roles for the endothelium in the regulation of vascular tone, modulation of inflammation, promotion or inhibition of vascular growth and of platelet aggregation and coagulation, and in the development of atherosclerosis [16].

The vascular endothelium is considered to be the largest endocrine organ in the body. Endothelial cells secrete various vasoactive agents, such as the vasodilatory nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF), as well as the vasoconstrictory endothelin I, angiotensin II (Ang II) and thromboxane [17,18]. Endothelial dysfunction is a broad term that implies diminished production or availability of NO and/or an imbalance in the relative contribution of endothelium-derived relaxing and contracting factors [19]. Endothelial dysfunction has been shown in the elderly, in patients with hypertension, diabetes and hypercholesterolemia as well as in those subjected to air pollution or smoking [16,20,21]. Such dysfunction is involved in the pathophysiology of metabolic syndrome and cardiovascular diseases such as atherosclerosis, hypertension and heart failure [22,23]. Reduced bioavailability of endothelium-derived NO is the key for endothelial dysfunction. NO relaxes blood vessels (vasodilation), prevents thrombus formation, suppresses smooth muscle proliferation, and inhibits the leukocyte attachment to the activated endothelium [16,24]. Loss of endothelial NO bioavailability, a key manifestation of endothelial dysfunction, is increasingly accepted as a common trait of essentially all cardiovascular risk factors, showing profound prognostic implications in prediction of adverse cardiovascular events and long-term outcomes [25]. A number of pathological mechanisms appear to mediate endothelial dysfunction in general, including oxidative stress, dysregulated inflammation and overactivity of the renin–angiotensin system (RAS) all of which are potential targets for modulation by bioactive peptides. The key endothelial functions and their altered dysfunctional counterparts are summarized in Fig. 1.

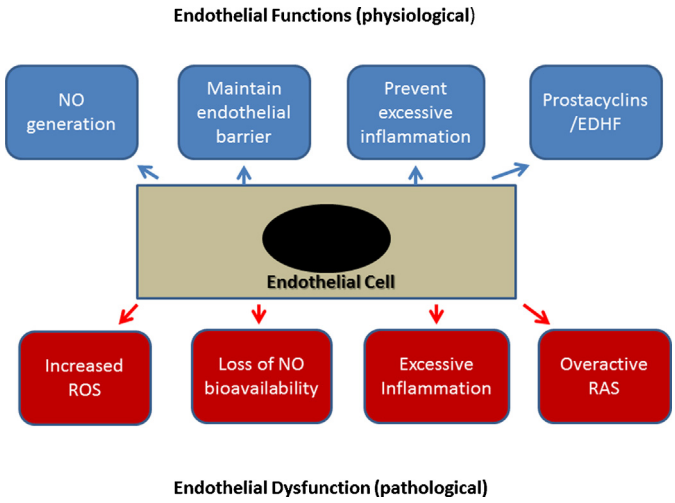

Fig. 1. Endothelial functions under normal (physiological) and abnormal (pathological) conditions.

3. Bioactive peptides on NO bioavailability and endothelial function

3.1. Oxidative stress, NO and endothelial dysfunction

Oxidative stress, *i.e.* the excessive and/or dysregulated generation of reactive oxygen species (ROS) such as superoxide and hydrogen peroxide, is a major contributor to disease pathologies. In the vascular endothelium, an excess of superoxide leads to its reaction with NO to generate peroxynitrite ($ONOO^-$), a highly reactive and toxic molecule that causes nitration at the tyrosine residues of various proteins. Not only does peroxynitrite formation reduce NO bioavailability (and hence contributes to endothelial dysfunction), tyrosine nitration can also adversely affect physiological functions of many cellular proteins, predisposing the endothelial cells toward inflammation and cell death [26,27]. In addition, high ROS levels also induce uncoupling of the NO generating enzyme endothelial nitric oxide synthase (eNOS), further jeopardizing the vascular functions [28]. Given the critical roles played by NO and its alterations observed under oxidative stress, regulation of ROS levels is a key area for enhancing or maintaining normal endothelial functions.

3.2. Antioxidant bioactive peptides

Recently, antioxidant peptides derived from hydrolysis of food proteins such as those from milk, egg, meat, wheat and soy have been reported [29–34]. Most peptides were characterized based on either radical-scavenging activity or metal-chelating activity; however, all these chemical assays are performed under cell-free *in vitro* conditions, making it impossible to extrapolate the results to *in vivo* situations [35]. Hence it is of great importance to evaluate the bioactivity of antioxidant peptides under physiological conditions to establish their protective roles in diseases. To date, only a limited number of studies have evaluated the role of antioxidant peptides under physiologically relevant conditions.

For example, glutathione (GSSH), was found to reverse the impaired relaxation of aortas from spontaneously hypertensive rats (SHR) to acetylcholine; an effect which was of comparable magnitude to other antioxidants such as aminotriazole and ascorbic acid, when given intraperitoneally at the same dose [36]. The effect of GSSH was also observed in human subjects, particular with vascular dysfunction [37,38]. Data from our laboratory have shown antioxidant properties of egg derived peptides Ile-Arg-Trp and Ile-Gln-Trp which may contribute toward their anti-hypertensive and vasorelaxant effects observed in animal studies as described later [39,40]. Milk derived peptides with antioxidant activity can improve vascular function and reduce blood pressure although additional mechanisms such as modulation of vasoactive factors are likely to contribute toward the observed beneficial effects [29].

3.3. NO generation by bioactive peptides

Apart from improving NO bioavailability through antioxidant effects, certain peptides can also directly induce endothelial NO formation and hence, contribute to improved endothelial functioning. For example, the whey derived peptide NOP-47, a direct stimulator of endothelial NO, has been shown to improve endothelium-dependent vasorelaxation and increase plasma nitrate/nitrite levels (a measure of physiological NO formation *in vivo*) in a randomized clinical trial in healthy human subjects [41]. Similarly, flaxseed protein hydrolysate, containing arginine-rich peptides, can reduce blood pressure in SHRs, presumably through the enhanced NO generating effects of arginine [42]. The relaxing effect of peptide (Arg-Ala-Asp-His-Pro-Phe), an ovokinin (2–7), derived from chymotryptic digest of egg white protein ovalbumin, showed endothelium-dependent vasorelaxation in an isolated SHR mesenteric artery *via de novo* NO generation [43]. Carnosine is a dietary endogenous dipeptide (beta-Ala-*L*-His) present mainly in skeletal muscle tissue [44]. Apart from directly stimulating NO formation in cultured endothelial cells [45], carnosine also showed dose-dependent relaxation effects on isolated aortic rings [46]; this relaxation appeared to be endothelium independent and is likely due to the generation of cGMP although additional effects of endothelium-generated second messengers could not be ruled out. Interestingly, vasoconstriction was the response to carnosine in rabbit saphenous vein rings, suggesting its opposite vasoactive roles in different vascular beds [47].

3.4. Opioid peptides and vasorelaxation

Bioactive peptides with opioid activity are another class of vascular regulators. Two opioid peptides, α-lactorphin (Tyr-Gly-Leu-Phe) and β-lactorphin (Tyr-Leu-Leu-Phe), derived from milk protein α-lactalbumin and β-lactoglobulin, respectively, bind to opioid receptors and show weak opioid activity *in vivo* [48]. Indeed, α-lactorphin reduces the blood pressure in SHRs showing direct vasoactive effects [49]. Both peptides demonstrated endothelium-dependent vasorelaxation *ex vivo*, involving NO since the relaxation was abolished by the NO synthase (NOS) inhibitor NG-nitro-L-arginine methyl ester (L-NAME) rather than endothelium-derived hyperpolarizing factor (EDHF) or vasorelaxant prostanoids [50]. Surprisingly, Casoxin D (Tyr-Val-Pro-Phe-Pro-Pro-Phe), an anti-opioid peptide identified from pepsin-chymotrypsin digest of human casein, also promoted vascular relaxation mediated by bradykinin B1 receptors which could be antagonized by a cyclooxygenase inhibitor [51]. In contrast, casomokinin L (Tyr-Pro-Phe-Pro-Pro-Leu) relaxed canine mesenteric artery rings in endothelium-dependent and NO-mediated manner, suggesting further complexity in the roles of opioid receptors and vasorelaxation [52].

4. Bioactive peptides on inflammation

4.1. Inflammation and endothelial dysfunction

Inflammation plays an essential role for the vascular endothelium. In response to sub-lethal injury, endothelial cells respond with increased permeability and upregulated leukocyte adhesion molecules which enable recruitment of leukocytes on the endothelium followed by their migration across the vascular barrier [53]. While inflammation is essential for the body's defenses against microbes and other injurious stimuli, excessive and persistent inflammatory changes can lead to vascular pathologies like atherosclerosis and its complications. Inflammation by itself can activate the endothelial cells; the associated increase in permeability could alter its vasodilatory properties, while the concomitant increase in ROS formation would further attenuate the vascular relaxation [54]. In addition, inflammatory recruitment of leukocytes to the sub-endothelial space is a major contributor to atherosclerotic plaque formation and subsequent cardiovascular disease [55].

4.2. Anti-inflammatory bioactive peptides

Bioactive peptides with known anti-inflammatory effects are potential candidates for improving endothelial dysfunction. In contrast to antioxidant peptides, nearly all anti-inflammatory ones have been validated in physiologically relevant systems like cultured cells and animal models, which improve their chances of *in vivo* biological actions. In spite of this, few of the anti-inflammatory peptides identified to date have been specifically tested for improvements in vascular/endothelial functions.

Research in our laboratory had identified an egg protein ovotransferrin derived tripeptide, Ile-Arg-Trp, as a potential ACE (angiotensin converting enzyme) inhibitory molecule [40]. Further testing of this peptide determined its additional anti-inflammatory and antioxidant effects on cultured endothelial cells [56]. These functions were validated in an *in vivo* study involving SHRs where this peptide reduced blood pressure, improved endothelium-dependent vasorelaxation (as shown in *ex vivo* study) and attenuated markers of inflammation, showing correlation between cell-based and whole animal studies [57]. Another ovotransferrin derived peptide, Ile-Gln-Trp also showed similar anti-inflammatory effects on endothelial cells and comparable vasculo-protective roles in SHRs [39,58]. It is likely that a combination of factors such as anti-inflammatory properties, ACE inhibition and improved NO bioavailability all contributed to the final outcomes in both cases.

Peptide-rich milk protein hydrolysates have shown anti-inflammatory effects in cultured endothelial cells and appear to inhibit leukocyte-endothelial interactions [59], which may explain some of their beneficial vascular/endothelial roles in addition to better known ACE inhibitory effects. A gastric enzyme hydrolysate of Spirulina (a type of sea weed) yielded 2 peptides (Leu-Asp-Ala-Val-Asn-Arg and Met-Met-Leu-Asp-Phe) with similar anti-inflammatory effects on endothelial cells [60], while other bioactive peptides such as Ser-Ser-Ser, Glu-Glu-Glu and Val-Pro-Leu have all been shown to attenuate leukocyte-endothelial interactions [61]. Given the crucial role for inflammatory leukocyte recruitment in atherosclerosis, such peptides may help maintain normal endothelial functions in an inflamed environment.

Table 1
Endothelial functions of bioactive peptides.

Endothelial functions	Peptide	Source protein	Experimental system	References
Antioxidant	Glutathione		SHR/Male WKY rat, human subjects	[36]
Antioxidant, ACE-inhibitor, anti-inflammatory	Ile-Arg-Trp	Ovotransferrin	Endothelial cells, SHR	[56,57]
Antioxidant, ACE-inhibitor, anti-inflammatory	Ile-Gln-Trp	Ovotransferrin	Endothelial cells, SHR	[39,58]
NO generation	NOP-47	Whey protein	Human subjects	[41]
NO generation/opioid agonist	Aα-lactorphin (Tyr-Gly-Leu-Phe)	Aα-lactabumin	Isolated mesenteric arterial ring of SHR	[50]
NO generation/opioid agonist	Aβ-lactorphin (Tyr-Leu-Leu-Phe)	Aβ-lactoglobulin	Isolated mesenteric arterial ring of SHR	[50]
NO generation	Arg-Ala-Asp-His-Pro-Phe	Ovalbumin	Isolated mesenteric arterial ring of SHR	[43]
NO generation	Arginine-rich cationic peptides	Flaxseed protein	SHR	[42]
NO generation	Carnosine	Skeletal muscle protein	Endothelial cells (f-2)	[45]
NO generation	Casomokinin L (Tyr-Pro-Phe-Pro-Pro-Leu)	Human casein	Canine isolated mesenteric arterial ring	[52]
Anti-inflammatory	Leu-Asp-Ala-Val-Asn-Arg	Spirulina protein	Endothelial cells	[60]
Anti-inflammatory	Met-Met-Leu-Asp-Phe	Spirulina protein	Endothelial cells	[60]
ACE inhibition	Val-Tyr	Sardine	SHR, mice	[70,71]
ACE inhibition	Val-Pro-Pro	Casein	Endothelial cells, SHR, human subjects	[74,76]
ACE inhibition, ACE2 upregulation	Ile-Pro-Pro	Casein	Endothelial cells, SHR, human subjects	[74–76,80]
ACE inhibition	Peptide-rich hydrolysate	Thai rice bran protein	2K1-C rats	[77]
AT1R blocker	Leu-Ile-Trp-Lys-Leu	Lactoferrin	Isolated rabbit carotid arteries, receptor binding assays	[81]
AT1R blocker	Arg-Pro-Tyr-Leu	Lactoferrin, egg protein	Isolated rabbit carotid arteries, receptor binding assays	[81]
Renin downregulation, AT1R blocker	Arg-Val-Pro-Ser-Leu	Egg white protein	SHR	[82]

5. Bioactive peptides as RAS modulators

5.1. Renin–angiotensin system (RAS) and endothelial dysfunction

RAS is a significant regulator of vascular functions, with Ang II being one of the most potent vasoconstrictors known [62]. While much of these effects occur at the VSMC level, excessive RAS actions can also be deleterious to the endothelium. For example, Ang II can lead to inflammatory changes, matrix metalloproteinase-2 (MMP-2) release and ROS generation in endothelium, with their adverse consequences on endothelial vasorelaxant properties [63–65]. In addition, endothelial cells are a source of ACE, the critical enzyme involved in generation of Ang II from its inactive precursor Ang I. On the other hand, ACE2 (angiotensin converting enzyme 2), the negative regulator of RAS, is associated with anti-inflammatory effects and improved vascular functions, suggesting a more complex role for RAS as an endothelial regulator [66,67].

5.2. RAS regulation by bioactive peptides

Initial studies of bioactive peptides as ACE inhibitors were mostly done by biochemical assays in cell-free *in vitro* systems. However, many such studies have now been followed up with cellular and animal studies validating the original observations. For example, previous work on egg white protein ovotransferrin hydrolysates in our laboratory identified a number of tripeptides with ACE antagonist properties, which were later confirmed to have significant effects on blood pressure reduction and attenuation of plasma Ang II levels in SHRs [57,58,68]. Another ACE inhibitor peptide Val-Tyr, derived from sardines, also reduced blood pressure in SHRs and transgenic hypertensive mice [69,70]. This peptide specifically inhibited Ang I-evoked contraction, but not those by Ang II since pre-incubation with this peptides did not affect the contraction evoked by directly adding Ang II to the bath; the inhibition of Ang I contraction was probably due to the inhibition of ACE within the vascular wall [71]. Two tripeptides derived from the milk protein casein, namely Ile-Pro-Pro and Val-Pro-Pro, have ACE inhibitory functions and have demonstrated improvements in both *ex vivo* vasorelaxation as well as *in vivo* blood pressure control [72–74]. It appears that additional vasculo-protective mechanisms such as enhancement of endothelial NO generation may contribute to these observed effects [75]. A study in human subjects also showed marked improvements in the augmentation index, a marker of endothelial dysfunction, upon long-term administration of a fermented milk drink rich in these peptides [76].

Recently, the enzymatic hydrolysate of rice bran protein, containing a number of different bioactive peptides, has been shown to reduce blood pressure and improve endothelium-dependent vasorelaxation in 2K-1C rats, likely through ACE inhibitory action of this preparation [77]. In a similar vein, casein hydrolysates with *in vitro* ACE inhibitory properties also demonstrated attenuation of *ex vivo* vasoconstriction as well as reduction of blood pressure *in vivo*, further validating the potential of ACE inhibitor peptides and peptide-rich protein hydrolysates to improve vascular functions [78].

Apart from ACE inhibition, other mechanisms targeting different components of RAS are under investigation. A recent study from our laboratory has shown the ability of egg derived peptide Ile-Arg-Trp to upregulate ACE2 expression in the vasculature of SHRs [79]; together with the well-characterized ACE-inhibitory and anti-inflammatory actions, this could be another pathway to exert its beneficial effects. Similar effects of Ile-Pro-Pro on ACE2 were also determined by Ehlers *et al*. [80]. As the vasoconstrictory effects of Ang II are largely mediated through its receptor AT1R (Ang II receptor type 1), it is also a target for modulation by bioactive peptides. For example, several peptides such as Arg-Pro-Tyr-Leu (from milk or egg) and the milk-derived Leu-Ile-Trp-Lys-Leu can act by inhibiting Ang II binding to AT1R, which shows *in vivo* reduction of blood pressure in SHRs [81]. A multi-functional egg peptide, Arg-Val-Pro-Ser-Leu, exerts its beneficial vasorelaxant effects through reductions in levels of ACE, renin, Ang II and kidney AT1R in SHRs, further demonstrating the potential of bioactive peptides in regulating RAS for vascular functions [82].

The bioactive peptides with endothelial functionalities are summarized in Table 1.

6. Conclusions

In conclusion, bioactive peptides are an attractive option for treating and managing endothelial dysfunction and its complications, based on potential modulation of oxidative stress, inflammation and RAS overactivity. However, several challenges remain to be overcome before widespread acceptance and commercial usage of such peptides can become the norm. Specific issues include the lack of knowledge regarding peptide receptors and pharmacokinetics, the incomplete understanding of potential allergenic/immunogenic peptide sequences as well as limited information regarding dosage regimens, safety data and side effects. Future studies should involve further basic biomedical research to elucidate the molecular mechanisms of action that underlie the promising results observed with these peptides on improving endothelial/vascular health. Clinical trials, especially those performed with strict randomization, would also help to move more bioactive peptides from the bench to the bed side, with expected benefits for both the people at risk of CVD and the health care system as a whole.

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