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Review

Apelinergic system in endothelial cells and its role in angiogenesis in myocardial ischemia

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ABSTRACT

Apelin is a peptide known to have a vital role in cardiovascular diseases. It has been proven to induce proliferation and tube formation in endothelial cells, stabilise contacts between endothelial cells, and mediate pericyte recruitment. Since apelin level is reduced early after myocardial infarction, a supportive therapy with apelin is being investigated for its beneficial effect on blood vessel formation. It is becoming apparent, however, that the final effect of apelin often depends on stimuli the cell receives and the cross-talk with other molecules inside the cell. Hence, understanding the apelin pathway potentially can help us to improve angiogenic therapy. This review summarises recent knowledge regarding molecules involved in apelin signalling while focusing on their roles in angiogenesis within the ischemic environment after myocardial infarction.

Contents

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1. Introduction

Angiogenesis is impaired in many diseases, such as myocardial infarction (MI), atherosclerosis and diabetes. Understanding the pathways of angiogenesis helps us to find new therapeutics or improve already established methods for restoring blood flow. Since the first angiogenic factors were purified in the 1980s, many new factors connected to angiogenic pathways have been discovered. To name just a few, these include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and angiopoietin-1 (Ang-1) [1–3].

In relation to acute MI, pro-angiogenic therapy has emerged as having promising potential in cardiac repair [4–6]. Nevertheless, recovery of cardiac function in acute myocardial infarction (AMI) patients is not always satisfactory [7]. It appears that delivery of a single growth factor or cell type is not sufficient for long-term cardiac repair. New strategies are therefore being investigated, including co-delivery of endothelial progenitor cells and vascular smooth muscle cells (SMCs) [8], preconditioning of transplanted cells [9], and application of genetically modified stem cells [10,11].

A combined gene therapy using several growth factors appears to be more efficient than is delivery of a single growth factor [12,13]. This approach is based on the multi-step process of angiogenesis regulated *in vivo* by several angiogenic factors [14]. Importantly, overexpression of a single pro-angiogenic factor may induce pathologic blood vessel formation and even lead to formation of vascular tumours [15]. Accordingly, emphasis is given to regulating stabilisation and maturation processes together with preserving a balance of pro-angiogenic and anti-angiogenic factors. New modifications and regulators of angiogenic therapy are being tested to further improve therapy [16,17]. One of the angiogenesis regulators intensively studied in recent years is apelin, which has been found to play an important role in blood vessel formation [17–21]. This review discusses how apelin and other molecules involved in the apelin pathway influence angiogenesis while focusing on those molecules' roles in ischemia after AMI.

2. Apelin isoforms and distribution

Apelin is a peptide first identified in its 36-amino acid isoform in 1998 in bovine stomach extracts [22]. It binds to a G-protein-coupled angiotensin II protein J (APJ) receptor [22,23]. Several active isoforms of apelin have been identified to date. The isoforms are produced by cleavage of 77-amino acid pre-proapelin [24], bind to APJ receptor, and cause similar cellular effects [25]. However, shorter peptides consisting of 13 to 17 amino acids have higher activity than does apelin-36 [22,26]. In human heart, apelin-13 is the predominant isoform, while in plasma apelin-13 and apelin-17 can be found [27–29].

Since its first identification, the apelin/APJ pathway has been described to be active in various cells, tissues and organs (including heart, brain, lung, kidney, endothelium, plasma, and adipose tissue) [22,26,30]. In the vascular system, apelin and APJ receptor are expressed by endothelial and smooth muscle cells [17,31,32].

3. Apelin in endothelial cells of sprouting vessels

Vessel sprouting during angiogenesis is co-ordinated by signals from nearby tissue and the vessels themselves [33,34]. However, the responses of individual endothelial cells to the signals differ according to their positions in the vessels. Tip cells are specialised endothelial cells (ECs), localised at the leading edge of the vessels, which secrete cytokines, extend filopodia, and lead the migration in response to VEGF gradient [34]. The lumen of new vessels, by contrast, is formed of stalk cells which also secrete various factors [34]. Both tip and stalk cells express apelin, whereas APJ receptor is located on stalk cells only [35]. Surprisingly, APJ is little expressed in unstimulated ECs, but it is abundantly expressed under hypoxia and after stimulation with VEGF [36–38]. Although under normal conditions APJ receptor can be found mostly in the cell membrane, the receptor moves into the cytoplasm after stimulation with apelin [38].

4. Angiogenesis in ischemic tissue and apelin signalling

Hypoxia and the need for restoration of insufficient blood supply trigger angiogenesis in ischemic tissue. After VEGF stimulation, endothelial cells differentiate, proliferate, release platelet-derived growth factor (PDGF-BB), and attract mural cells (pericytes or smooth muscle cells) to newly formed blood vessels [39]. In the following phase, the pre-existing vessels are stabilised by mural cells releasing angiopoietin-1 (Ang-1), thereby activating tyrosine-protein kinase receptor Tie-2 on ECs and consequently stabilising intracellular junctions [40,41].

Apelin plays an essential role in blood vessel formation and ischemia repair [42,43]. It has been described as vital for all the steps of angiogenesis, upregulating both migration and tube formation [17] and having beneficial effect on stabilisation of contacts between endothelial cells generating non-leaky blood vessels [18]. Similarly, APJ receptor is necessary also for functional angiogenesis. In knockout mice that were APJ −/−, it has been observed that the blood vessels did not form appropriately and vascular smooth muscle cell layers were decreased even though the level of CD31 endothelial cells was preserved [44].

Hypoxia induces expression of APJ receptor on endothelial cells. This process leads to activation of apelin pathway [45]. In the next step, apelin produced by tip cells binds to APJ receptor on stalk cells and stimulates downstream signalling pathways in ECs [35].

To date, a rather large number of signalling molecules have been reported to be implicated in the apelin pathway during angiogenesis, both affecting apelin expression and acting downstream of apelin/APJ signalling. We discuss here the signalling networks and role of the molecules in ischemic tissue.

5. Apelin pathway and cross-talking signalling molecules

5.1. Hypoxia-inducible factor

The key step at the beginning of the apelin pathway is hypoxia. Hypoxia is regulated by hypoxia-inducible factor (HIF-1), which promotes the expression of genes containing hypoxia-responsive elements (HREs) [46]. In 2006, HREs were found in the apelin promoter, albeit without confirmed functionality [47]. One year later, it was demonstrated that apelin can be upregulated in response to hypoxia in cardiac tissue [48]. Finally, in 2008 it was shown that apelin expression in endothelial and vascular smooth muscle cells is mediated by the binding of HIF-1 to an HRE located within the first intron of apelin