Artykuł przeglądowy *Review*

Role of adropin in cardiovascular physiology

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Summary

Adropin is a new regulatory peptide participating in the maintenance of energy homeostasis, metabolic adaptation, and modulation to insulin sensitivity. Low serum levels of adropin are associated with obesity and insulin resistance, which are related to cardiovascular incidents. Thus, it can be hypothesized that adropin may also exert direct effects on blood vessels. Obtained results indicate that, apart from its metabolic effects, adropin exerts angiogenic effects. It stimulates the proliferation, migration, and capillary-tube formation of endothelial cells, and improves endothelial barrier functions. In endothelial cells, adropin markedly elevates nitric oxide release and eNOS bioactivity through VEGFR-PI3K-Akt and VEGFR-PI3K-ERK1/2 pathways. Since endothelial dysfunction plays a role in different cardiovascular disorders, e.g. atherosclerosis, acute myocardial infarction, and cardiac syndrome X, adropin deficiency might be involved in the pathogenesis of these diseases. Therefore adropin may serve as a biomarker and therapeutic candidate for prevention of the above-mentioned conditions. However, further studies are required to shed more light on this problem, as plasma adropin levels were significantly increased depending on the severity of heart failure.

Keywords: adropin, nitric oxide, eNOS, endothelium

Adropin (ADR) was identified as a new regulatory peptide by Kumar et al. in 2008 (13). The term adropin is a combination of the first letters of two Latin words: *aduro*, which means "to set fire to," and *pinquis*, meaning "fats" or "oils". Adropin contains 76 amino acids, with the first 33 residues comprising the signal peptide, and has a molecular weight of 4499.9 Da. The amino acid sequences of adropin in humans, rats, and mice are identical. Adropin is coded by the Energy Homeostasis Associated Gene (*Enho*), which is expressed in the liver and brain of mice (13). High *Enho* expression in the brain was detected in the areas of the thalamus, hypothalamus, midbrain, cerebellum, and medulla oblongata, which are involved in the control of different behaviors and regulate peripheral metabolism through the autonomic nervous system. The pioneering study of Kumar et al. (13) indicated that hepatic *Enho* expression is regulated by fasting and the macronutrient composition of diet. Chronic exposure to diet-induced obesity (high-fat diet) and genetically-induced obesity reduce *Enho* expression. However, high *Enho* expression was observed during a short-term intake of high-fat diet (13).

Recent research demonstrated that ADR is expressed in the brain (vascular area, pia matter, neuroglial cells, and neurons), cerebellum (Purkinje cells, neuroglial cells, vascular areas, granular layer), kidneys (glomerulus, peritubular capillary endothelial cells, peritubular interstitial cells), heart (epicardium, myocardium, endocardium), liver (sinusoidal cells), pancreas (serous ancini), vascular tissues (endothelial cells of umbilical vein and coronary arteries), and muscles (3, 17). Aydin et al. (3) list adropin amounts in descending order in the following tissues: pancreas, liver, kidney, heart, brain, and cerebellum. However, the current literature provides no information on adropin levels in vascular tissues and muscles. Adropin immunoreactivity increases in a greater number of tissues in diabetic animals (3). Nonetheless, there was no change in ADR immunoreactivity in the pia matter and neurons of the brain, or in the endocardium of diabetic animals (3). ADR, as secreted protein, could be released into circulation. It is also present in the milk, cheese whey, and plasma of dairy cows (2).

Kumar et al. (13, 14) demonstrated that adropin is a peptide hormone which participates in the maintenance of energy homeostasis, metabolic adaptation to macronutrients, and modulation to insulin sensitivity. A role of ADR in metabolic homeostasis is supported

by the observation that a transgenic overexpression or systemic administration of adropin attenuates hepatosteatosis, insulin resistance, and glucose intolerance (13). Therefore, adropin may protect against hepatosteatosis and hyperinsulinemia associated with obesity (13, 14). Serum levels of ADR were suppressed by fasting and diet-induced obesity in experimental animals (14). ADR levels were high in mice fed a high-fat, low-carbohydrate diet, whereas lower levels were observed in mice fed a low-fat high-carbohydrate diet (14). Lower levels of ADR were also observed in diabetic humans (5, 7, 23). On the other hand, the serum ADR concentration in rats with streptozotocin-induced diabetes was significantly higher than in control rats (3).

A decreased serum level of ADR participates in metabolic disorders associated with obesity and insulin resistance, which are related to cardiovascular events. Since insulin sensitivity and vascular function are closely related, it may be hypothesized that adropin may also exert a direct effect on blood vessels. The first evidence suggesting a vascular effect of adropin was provided by Lovren et al. (17). These authors reported that adropin is expressed in the endothelial cells of the umbilical vein and coronary arteries. In an *in vitro* study, a greater proliferation, migration, and capillary-like tube formation were observed in adropin-treated endothelial cells of the umbilical vein. Adropin attenuated the permeability of these cells, which suggests its role in improving endothelial barrier function. Moreover, the protective effect of ADR is also associated with the abrogation of TNF-alfa induced apoptosis. In endothelial cells, ADR markedly elevates nitric oxide (NO) release, endothelial nitric oxide synthase (eNOS) protein levels, and mRNA expression. Adropin activates serine/threonine protein kinase (Akt), which leads to a post-transcriptional activation of eNOS via phosphorylation of Ser^{1177}. The ability of adropin to enhance Akt phosphorylation may reveal NO-dependent and NO-independent signaling pathways that serve to regulate endothelial survival and function, and, as a consequence, improve vascular function. Additionally, the reduction of adropin-induced Akt phosphorylation by a specific inhibitor of the phosphoinoside-3 kinase (PI3K) indicates that the PI3K-Akt signaling pathway is activated by adropin, and suggests that adropin-induced eNOS activation is at least in part PI3K dependent. A specific antagonist of the mitogen-activated protein kinase 1 (MAPK1) attenuated adropin-stimulated Ser^{1177} eNOS phosphorylation, which may imply that adropin also exerts its effects via the extracellular signal-regulated kinases 1/2 (ERK1/2) signaling pathway (17).

The regulation of endothelial survival and function, and of angiogenesis via the activation of PI3K-Akt and ERK1/2 pathways is also associated with vascular endothelial growth factor receptor 2 (VEGFR2) expressed in endothelial cells. Data from Lovren et al. (17) indicate that adropin potently upregulates VEGFR2 in endothelial cells and that the gene silencing of VEGFR2 significantly impairs the effects of adropin on Akt, ERK1/2, and eNOS phosphorylation. Collectively, these data indicate that adropin may modulate eNOS bioactivity partially through the upstream activation of VEGFR2 with a resultant activation of the PI3K-Akt and ERK1/2 pathways (17).

In an *in vivo* study, ADR exerted a positive angiogenic effect. Adropin-treated animals demonstrated improved perfusion after ischemia, and this effect corresponds with higher capillary density in ischemic muscles (17). Additionally, muscles isolated from adropin-treated animals are characterized by significantly higher VEGFR2 protein levels and greater eNOS, Akt, and ERK1/2 phosphorylation (17).

Data collected by Lovren et al. (17) suggest that adropin is a novel regulator of endothelial function via upregulating endothelial eNOS. Endothelial cells play a crucial role in maintaining vascular tone by releasing NO. NO synthetized from L-arginine by eNOS is a potent endogenous vasodilatator which is released in response to shear stress (9). Thus, it plays an important role in flow-mediated vasodilatation. NO also inhibits the activation of plaque, adhesion of leukocytes to endothelium, and smooth muscle cells proliferation (4, 12). As eNOS is responsible for the production of NO in blood vessels (9), ADR deficiency may be associated with a reduction of NO bioavailability in the endothelium. Low bioavailability of NO is a consequence of endothelial dysfunction that precedes the development and progression of atherosclerosis (1, 22). What is more, the reduced plasma adropin concentration correlates with insulin resistance and obesity, which are related to the progression of coronary atherosclerosis and an increased incidence of cardiovascular events (5, 7, 13, 18). Since both low plasma adropin and diabetes are linked to endothelial function, Wu et al. (23) hypothesized that decreased serum adropin levels may be involved in the development of atherosclerosis independently of or synergistically with type 2 diabetes. Their data indicated that the blood adropin level was significantly lower in type 2 diabetic individuals than in non-diabetic individuals and was inversely and independently related to the severity of coronary atherosclerosis, suggesting that serum adropin serves as a novel predictor of coronary atherosclerosis (23).

It is a well-known fact that endothelial dysfunction plays a role in acute coronary syndromes (11, 20). Concluding from these findings, Yu et al. (24) hypothesized that adropin deficiency might be involved in the pathogenesis of acute myocardial infarction (AMI). They found significantly decreased serum adropin levels in AMI patients compared with patients with stable angina pectoris and controls. Moreover, patients with stable angina pectoris displayed a significantly lower serum ADR level in comparison to controls. Statistical analysis proved that lower serum ADR levels were independently associated with the presence of AMI in patients with coronary artery disease. These results

indicate that adropin appears to be a potential serum biomarker for early diagnosis of AMI in coronary artery disease patients. In addition, the lack of correlation between serum adropin levels and cardiac troponin T (cTnT) or creatine kinase (CK) in AMI patients, may suggest that adropin is not a marker for cardiac necrosis or injury.

Endothelial dysfunction is regarded as one of the main reasons for cardiac syndrome X (CSX). This syndrome manifests itself by increased resistance in coronary blood flow, which is attributed to endothelial dysfunction in microcirculation (8). In a study by Celik et al. (6) adropin was found to be an independent risk factor for CSX. The circulating levels of adropin and serum nitrite/nitrate levels were significantly lower in patients with CSX than in healthy subjects.

Endothelial dysfunction has also been reported as a factor in failing heart function, since it leads to the higher peripheral resistance observed in heart failure (19). Thus, myocardial microcirculation disorders and an increased afterload contribute to the development of cardiac damage and decompensation. In turn, left ventricular attenuation may negatively influence endothelial function by reducing shear stress and vascular NO bioavailability (10). According to Lian et al. (15), adropin is a functional link between endothelium cells and heart failure, and it might decelerate left ventricular dysfunction in heart failure. In a study by Lian et al. (15), plasma ADR levels were significantly increased depending on the severity of heart failure, and showed a positive correlation with the plasma level of brain natriuretic peptide (BNP) and a negative correlation with left ventricular ejection fraction (LVEF). Both BNP and LVEF indicate the severity of heart failure (21). Since natriuretic peptides are involved in fat metabolism as stimulants of lipolysis (16), Lian et al. hypothesize that lipolysis stimulated by BNP is associated with adropin synthesis in heart failure (15). The data on serum ADR levels obtained by Lian et al. (15) are contrary to the results of other authors (6, 23, 24), who observed suppressed levels of adropin in subjects with cardiovascular incidents. An elevated serum adropin level was also observed in diabetic rats (3). Moreover, this increase was associated with a marked increase in adropin immunoreactivity in the myocardium and epicardium of the heart (3). In view of these contradictory data, further studies are necessary to elucidate the precise role of adropin in heart failure and coronary artery diseases.

According to current data, apart from its metabolic effects, adropin has non-metabolic properties, such as the regulation of endothelial function. This peptide has a potential endothelial protective role via the upregulation of endothelial NO synthase bioactivity and improvement of endothelial barrier functions. Therefore, adropin may serve as a biomarker and therapeutic candidate for prevention of diseases related to endothelial dysfunction. However, further studies are required to shed more light on this problem.

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