Biochemical Interactions Between Angiotensin II and Apelin in Isolated Vascular Smooth Muscle Cells

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Angiotensin II, angiotensin receptors, apelins and recently de-orphanized APJ receptors are important control factors of cardiovascular system. The present studies aimed the effects of apelin-13 and [Pyr1]-apelin-13 on apoptosis of cultured rat vascular smooth muscle cells, induced by enhanced concentrations of angiotensin II, in the presence of losartan, a specific inhibitor of AT_1 angiotensin receptors type. The obtained data demonstrated that apelin-13 was less efficient in preventing the apoptosis of aortic smooth muscle cells in culture, induced by angiotensin II (in the presence of losartan), as compared to [Pyr1]-apelin-13. It could be concluded that the apoptotic effects induced by angiotensin II in rat aortic could be related to the involvement of AT_2 angiotensin receptor type, other types of receptors or pathways unrelated to angiotensin receptors. Furthermore, the apoptotic effects induced by angiotensin II are counteracted by apelins in a structure-related manner.

Keywords: angiotensin II, apelin-13, [Pyr1]-apelin-13, rat aortic smooth muscle cells, apoptosis

It was clearly demonstrated the involvement of toll-like receptor 4 in inflammatory cardiovascular diseases. On the other side, the roles played by toll-like receptor 4 (TLR4) in the pathogenesis of hypertension and the consecutive induced vascular damage (from the point of view of structure, mechanics and functionality) was not investigated. When infused in hypertensive rats, angiotensin II increased the aortic levels of mRNA for toll-like receptor 4. When the specific antibody against toll-like receptor 4 was used as pretreatment for angiotensin II infusion, some parameters as tumor necrosis factor-αand interleukin-6 levels, responsivity of aorta induced by phenylephrine and acetylcholine, levels of mRNA for NOX-1, superoxide anion occurrence, catalase and NAD(P)H oxidase activities, as well as decreased levels of NO production were normalized. These results were further strengthened by the administration of an inhibitor of toll-like receptor 4, CLI-095, which further decreased the levels of phospho-JNK1/2 and expression of the subunit p65 of nuclear factor NF-êB, both induced by angiotensin II treatment. These studies stated that angiotensin II was able to upregulate the toll-like receptor 4 expression and functioning, the last one being involved in the inflammatory processes, dysfunction of endothelium, remodeling and rigidity of vasculature, all associated with oxidative stress in hypertension [1].

Actually it is well known the existence of various components of renin angiotensin system inside the cells of the cardiovascular system, including the nuclear and mitochondrial compartments. There exists large debate on possible contrary effects of extracellular and intracellular angiotensin II on the adjustment of potassium currents and membrane potential at the level of smooth muscle cells of mesenteric arteries, with emphasis on regulating the tone and contractility of resistance vasculature. Furthermore, there are questions related to the involvement of epigenetic factors in the production and effects of angiotensin II and renin in resistance vessels [2].

There is very well established dogma in vascular smooth muscle contraction as the involvement of inositol 1,4,5-trisphosphate receptors in the consecutive induced cytosolic ionic calcium (Ca^{2+}) release as a consequence of vascular smooth muscle cells activation by contractile molecules. The unknown facet is represented by the involvement of inositol 1,4,5-trisphosphate receptors driven Ca^{2+} release from endoplasmic reticulum in the regulation of blood pressure *in vivo*. That's why it was induced a mouse model with an inositol 1,4,5-trisphosphate receptor triple-knockout specific for smooth muscle, achieved through a tamoxifen-inducible system. Following tamoxifen induction, the contractile properties of aorta and the blood pressure were assessed in close relation to the role of inositol 1,4,5-trisphosphate receptors release of ionic calcium. The specific deletion of inositol 1,4,5-trisphosphate receptors decreased the contractility of aorta when vasoconstrictors as phenylephrine, U46619, serotonin, or endothelin 1 were used. Moreover, the increased systolic blood pressure induced by angiotensin II in wild animals was reduced by the triple knockout procedure for inositol 1,4,5-trisphosphate receptors in genetic modified mice. All these results demonstrate the central role for inositol 1,4,5-trisphosphate receptors in the increased cytosolic Ca^{2+} concentrations, induced by vascular smooth muscle agonists as angiotensin II, as well as their involvement in normal contractility of these cell type and in hypertension pathogenesis [3].

The blood-brain barrier is critical for the maintaining of the metabolic and physical properties of the central nervous system. One of the pathological conditions altering deeply the blood-brain barrier is hypertension. This alteration might be the effect of high concentrations of angiotensin II down-regulating the proteins of endothelium tight junctions, the same actions being induced by circulating leukocytes and released cytokines having pro-inflammatory properties.

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The underlying mechanisms are not yet fully understood. The mechanisms through which heart failure and hypertension induce blood-brain barrier alteration could establish the basis for future effective treatments of associated autonomic imbalance [4].

It was previously showed that increased concentrations of endogenous angiotensin II are involved in the hypertrophic mechanisms of vascular smooth muscle cells. These hypertrophic mechanisms could be mediated by the transactivation of some growth factor receptors by angiotensin II in spontaneously hypertensive animals as rats. Resveratrol, a constituent of polyphenolic type of red wine, was demonstrated to reduce the vascular smooth muscle hypertrophic effects of angiotensin II, whether the fine mechanisms are yet not known. When administered as pretreatment of angiotensin II on vascular smooth muscle cells, resveratrol decreased the induced protein synthesis, as well as the enhanced levels of oxygen free radicals, functioning of NAD(P)H oxidase, and phosphorylation of epidermal growth factor receptor, platelet derived growth factor receptor, extracellular signal-regulated kinases 1/2 (MAP kinases), RAC-alpha serine/threonine-protein kinases 1/2. The clear conclusion of the above studies is that resveratrol reduced the observed hypertrophy of vascular smooth muscle cells induced by angiotensin II through the reduction of oxidative stress, and blocking of transactivation of c-Src and growth factor receptors, as well as the mitogen-activated protein kinases and/or RAC-alpha serine/threonine-protein kinases signaling [5-8].

Apelins are peptide hormones associated to the recently de-orphanized G protein-coupled receptors known as angiotensin II receptor-like 1 (APJ). There exist variate forms of apelins in the circulation, but the main endogenous ligands are apelin-13, apelin-36 and [Pyr-1]-apelin-13. The system comprising the apelins and their receptors are mainly expressed in the central nervous system (e,g. hypothalamus), adipose tissues, smooth muscle cells, fibroblast cells, as well as endothelial cells. Aortic and left internal mammary artery structures are enriched in apelins ELISA and immunohistochemical correlations. The apelins main biological functioning could be associated to food intake, important releases of vasopressin and histamine, secretions of gastric acid, bicarbonate and insulin, enhanced diuresis, proliferation of cells, angiogenesis, maintaining the balance of glucose and fluid, as well as the control of motility of gastrointestinal system [9, 10] and homeostasis of cardiovascular system [11].

The apelins and apelins specific receptors systems are now considered to be critical controllers of cardiovascular system homeostasis. Their alterations are really involved in diseases affecting cardiovascular system, beside angiotensin II. However, there are many unclarified issues of the involvement of e.g. [Pyr1]-apelin-13 in heart disease mediated or induced by enhanced levels of angiotensin II. These involvements could be unveiled using knockout mice models (e.g. apelin-deficient $APLN^{-/y}$ and apolipoprotein E). The cardiac dysfunction was magnified by angiotensin II infusion in 1-year aged $APLN^{-/y}$ mice, manifested through fibrosis and hypertrophy of myocardium, enhanced oxidative stress and decreased angiotensin-converting enzyme 2 levels. In the knockout mice model of apolipoprotein E the similar effects (including apoptosis) induced by prolonged infusion of angiotensin II were reduced by [Pyr1]-apelin-13 administration. Angiotensin II also increased apoptosis of neonatal rat cardiofibroblasts in culture, in close correlation to enhanced oxidative stress (superoxide anion generation) and decreased ACE 2, effects effectively prevented by co-treatment with Pyr1]-apelin-13. Thus, Pyr1]-apelin-13 might represent an important player in the future treatment of heart disease, being able to reduce the aging pathophysiological consequences of angiotensin II [12].

The present study aimed the effects of apelin-13 and [Pyr1]-apelin-13 on apoptosis of cultured rat vascular smooth muscle cells, induced by enhanced concentrations of angiotensin II, in the presence of losartan, a specific inhibitor of AT_1 angiotensin receptors. Such data are lacking from the principal flow of actual scientific literature.

Experimental part

Vascular smooth muscle cells in cultures, obtained from 5 rats, applying a previous described and adapted technique [13], were used for actual experiments, in accordance with anterior developed protocols [14-16]. Apoptosis of rat aortic smooth muscle cells was detected and evidenced as previously described for other types of cells using flow cytometry as technique [17-24].

The apoptosis of rat aortic smooth muscle cells in culture was assessed using angiotensin II (1 μM in culture medium) in the presence of losartan (1 μM in culture medium), a specific antagonist of AT_1 receptors, after 5 passages.

Apelin-13 and [Pyr1]-apelin-13 were administered as treatment (100 ng/mL) for 24 h, the same time as of angiotensin II, the inductor of apoptosis in our experiments. The absolute apoptosis control was represented by valinomycin (1 μM).

All experimental studies were performed in the light of the uniformly accepted ethical principles stated by the Helsinki Declaration [25-31].

Results and discussions

The obtained flow cytometry results showed a significant apoptotic effect of angiotensin II (63.54±6.43%, 1 μM in culture medium, fig. 1) in the presence of losartan, as compared to non-treated rat aortic smooth muscle cells (10.52±2.84%, fig 2).

When assessed, the apoptotic aortic vascular smooth muscle cells were found in a proportion of 52.12±5.97% of the cells in the case of [Pyr1]-apelin-13 treatment (70 ng/mL, fig. 3), 58.71±4.99% in the case of apelin-13 treatment (70 ng/mL, fig. 4), as well as 98.01±0.45% for valinomycin (1μM, absolute apoptosis control, fig. 5).

Our obtained data demonstrated that apelin-13 was less efficient in preventing the apoptosis of rat aortic smooth

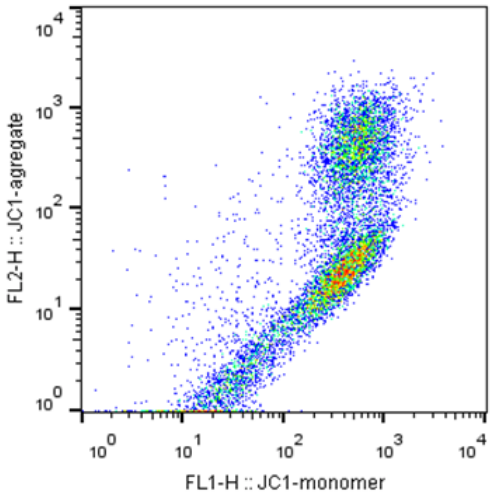

Fig. 1. When administered for 24 h, angiotensin II in the presence of losartan significantly induced aortic smooth muscle cells apoptosis. The experiments were performed in triplicate

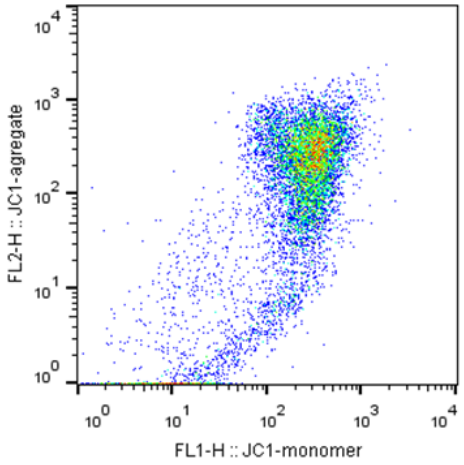

Fig. 2. A very small and non-significant fraction of aortic smooth muscle cells are apoptotic in normal conditions, without treatment

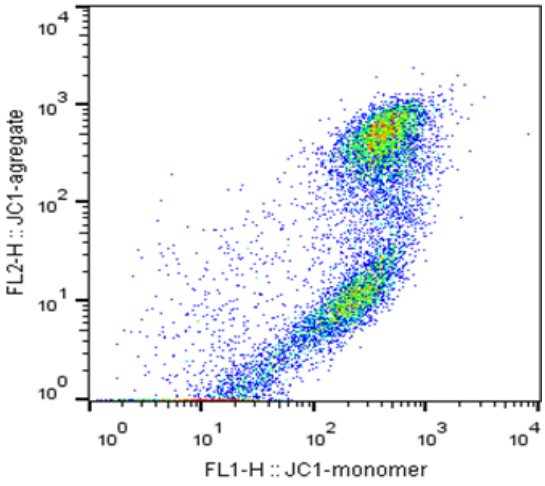

Fig. 3. When administered as treatment for 24 h, [Pyr1]-apelin-13 significantly reduced the apoptotic effects of angiotensin II on rat aortic smooth muscle cells in culture. The experiments were performed in triplicate

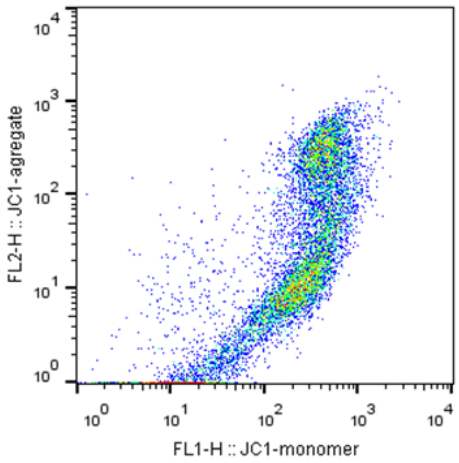

Fig. 4. The effects of treatment with apelin-13 were less evident when compared to those of [Pyr1]-apelin-13 in the same inductive apoptotic conditions (angiotensin II) for 24 h. The experiments were also performed in triplicate

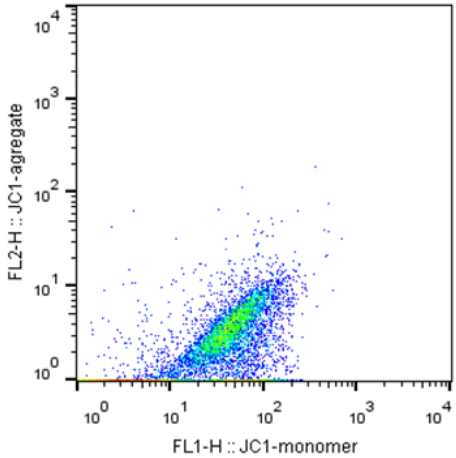

Fig. 5. The absolute control of aortic smooth muscle apoptosis, induced by valinomycin treatment for 24 h. The experiments were also performed in triplicate

muscle cells in culture, induced by angiotensin II (in the presence of losartan), as compared to [Pyr1]-apelin-13.

Apelin is considered as an adipokine fundamentally acting on de-orphanized APJ receptor, thus being its endogenous ligand. Apelin, including all its physiological variants and APJ receptors as a whole, is highly expressed in cardiovascular system, contributing to its homeostasis. Thus, apelin variants could be deeply involved in the pathogenesis of cardiovascular diseases. The variants of apelin are having important positive inotropic effects and induce vasodilatation which is dependent of endothelium and involves nitric oxide production [32].

On the other hand, it was demonstrated that apelin-13 was able to induce the proliferation of vascular smooth muscle cells, an intriguing effect. The cellular signaling transduction pathway mediating this apelin-13 effect is involving the Jagged-1/Notch3 and further expression of cyclin D1. When signal-regulated protein kinase 1/2 was inhibited, the above proliferative effects of apelin-13 were also blocked [33].

Several types of adipocytes, merely visceral, subcutaneous, and perivascular ones are secreting the adipokines functioning as circulating hormones (e.g., apelin, leptin, adiponectin) and intensively regulating especially the arterial tone. In addition, there was described also the involvement of perivascular adipocytes, which are also able to release relaxant or contractile vascular factors). The involvement of adipokines in the complex regulation of vascular tone is altered in obesity and related disorders, associating important hypoxia [34].

The mRNA levels of adiponectin were finely correlated with the degree of obesity, patients ages, levels of plasmatic triglycerides, levels of total cholesterol, body-mass index, gender, and eventual hypothyroidism. There was no evident correlation with glycemic values for the patients included in the study. The conclusion of the study was that level of expression of adiponectin might be used as a molecular marker to manage the patients with obesity [35].

There are actual data demonstrating the regulation of apelin in hypoxic conditions, effects mediated by hypoxia inducible factor-1αand bone morphogenetic protein receptor-2. In patients with pulmonary arterial hypertension, the plasmatic levels of apelin and its expression in pulmonary endothelial cells are highly reduced. Apelin variants are known playing important roles in angiogenesis, apoptosis of endothelial cells and vascular smooth muscle cells and could act opposite to vascular endothelial growth factor and angiotensin II effects. Moreover, their cardioprotective actions are evident and induce positive inotropic effects. Pulmonary hypertension could be prevented to develop by apelin variants in animal models [36].

Angiotensin II is a fundamental mediator of vascular dysfunction elicited by an enhanced oxidative stress. The vast majority of angiotensin II effects on endothelial cells are associated with increased production of O^{2-}

(superoxide anion). On the other hand, in isolated murine vessels it was demonstrated the enhanced production of other type of reactive oxygen radicals, merely H_2O_2, involving the impairment of further production of nitric oxide [37].

Hydrogen peroxide (H_2O_2) is also the result of monoamine oxidase functioning, a mitochondrial membrane-closely associated enzyme. Monoamine oxidase-induced production of such type of reactive oxygen species could mediate the endothelial dysfunction induced by angiotensin II exposure. The obtained results obtained for dog carotid artery demonstrated that monoamine oxidase represents a major source of reactive oxygen species, activated in response to angiotensin II stimulation, and blocked in the presence of one of its specific inhibitor named moclobemide. When L-NAME was also administered, the effects of inhibitor were abolished, suggesting the production of nitric oxide to be involved in the improvement of vascular functions related to moclobemide actions [38].

Bisphenol A, a plastic bottles component and one of the inner coatings of beverage cans, is considered to be a risk factor for cardiometabolic diseases. Several epidemiologic studies reported the correlation between bisphenol A and hypertension, beside others involving cardiovascular disease, hyperglycemia, weight gain and diabetes. For all of the above disorders there was established a positive correlation with urinary bisphenol A [39].

The exposure to bisphenol A is almost ubiquitous. Beside hypertension, bisphenol A was associating decreased heart rate variability. The question if the consumption of beverages from cans, and consecutively a higher rate of exposure, will affect the blood pressure and/or the heart rate variability is an important one for human health. The tests involving aged volunteers demonstrated that the urinary bisphenol A was increased by >1600% after the consumption of beverages from cans as compared to glass bottled ones. Meanwhile, the adjusted blood pressure values were increased by ≈4.5 mm Hg when 2 consecutive beverages were consumed from cans as compared to the same 2 consumed from glass bottles, with a statistically significant difference. The clear conclusion is that the consumption of beverages from cans will induce an acute increase of blood pressure [40].

The importance of the above results is found in the risk induced by plastics components to alter the blood pressure and to induce hypertension, possibly further modifying the involvement of renin-angiotensin system, since the plastics and their components are spread as waste all around the environmental medium [41-51].

Conclusions

Our obtained data demonstrated that apelin-13 was less efficient in preventing the apoptosis of rat aortic smooth muscle cells in culture, induced by angiotensin II (in the presence of losartan), as compared to [Pyr1]-apelin-13. Since losartan, a specific inhibitor of AT_1 angiotensin receptors, was present in the culture medium all over the time, the apoptotic effects induced by angiotensin II in rat aortic could be related to the involvement of AT_2 angiotensin receptor type, other types of receptors or pathways unrelated to angiotensin receptors. These data need further exploration to unveil the involved mechanisms.

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