Apelin: a potent aquaretic neuropeptide with distict cardiovascular effects

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SUMMARY

Apelin is the endogenous ligand of the membrane G-protein-coupled receptor termed APJ. Apelin transcript and immunoreactivity are expressed in the central nervous system and in various peripheral tissues, including the heart, lung, and mammary gland. Although Apelin circulates in plasma at low picomolar concentrations, it probably operates mainly as a local paracrine hormone. There are various apelin peptides and their biological activity is inversely correlated with peptide length, with apelin-17 being more potent than apelin-36. Abundant expression of apelin and its receptor in the supraoptic and paraventricular nuclei of the hypothalamus, areas where vasopressin and oxytocin are synthesized, suggests its involvement in the regulation of water balance. The central apelinergic system is sensitive to changes in water balance. Hypothalamic apelin might inhibit ADH release under physiological conditions and water deprivation removes its inhibitory influence by suppressing apelin release. Apelin reduce blood pressure predominantly by dilating peripheral veins and reducing preload rather than by dilating arterioles and reducing peripheral resistance. This hypotensive effect of apelin is mediated by endothelium-derived NO. Apelin exerts chronic positive inotropic effect which distinguishes from that of other mediators in that it does not induce myocardial hypertrophy. Several lines of evidence also suggest that apelin may be involved in angiogenesis

RESUMEN

Apelina: Un potente neuropéptido acuarético con distintivos efectos cardiovasculares

La Apelina es el ligando endogenous de un receptor de membrana acplado a proteina G- llamado APJ. Los transcriptos y la immunoreactividad de la Apelina se expresan en el sisema nervioso central y en varios tejidos periféricos, incluyendo el corazón, pulmón, y glándula mamaria. A pesar de que la Apelin circula en el plasma a bajas concentraciones picomolares, es probable que opere principalmente como una hormona paracrine local. Existen varios péptidos de apelina y sus actividades biologicas se correlacionan en forma inversa con la longitud del peptido siendo la apelin-17 más potente que la apelin-36. Existe una abundante expresión de la apelin y de su receptor en los núcleos supraopticos y paraventriculares del hipotalamo, áreas donde la vasopresina y la oxitocina son sintesizadas, lo que sugiere su participación en la regulación del balance de agua. El sistema apelinérgico central es sensible a los cambios en el balance de agua. La apelina hipotalámica puede inhibir a la liberación de ADH bajo condiciones fisiológicas y la deprivación acuosa remueve su influencia inhibitoria al suprimir la liberación de apelina. La Apelina reduce la presión sanguínea predominantemente al dilatar las venas periféricas y reducir la precarga mas que dilatando las arteriolas y reduciendo la resistencia periférica./ Esta acción hypotensora es mediada el Oxido nítrico producido por el endotelio.

La Apelin ejerce también un efecto inotrópico positivo que se distingue de los otros mediadores en que no induce hipertrofia miocárdica. Varias líneas de evidencia también sugieren que la apelin puede estar comprometida en la angiogénesis.

INTRODUCTION

The history of Apelin discovery is curious because its receptor was first cloned as an orphan receptor and later the endogenous ligand was isolated and characterized. In 1993, O Dowd and coworkers cloned a gene

displaying considerable sequence similarity with the angiotensin receptor type 1 (AT-1) gene [1]. This novel gene encoded a putative plasma membrane G-protein-coupled receptor termed APJ which, despite high sequence homology to AT-1, did not bind angiotensin II. Afterwards in 1998, Tatemoto et al. observed that bovine stomach homogenates activated APJ transiently expressed in Chinese hamster ovary cells and they isolated a 36-amino-acid peptide which was named apelin (from APJ endogenous ligand) [2]. Apelin transcript and immunoreactivity are expressed in the central nervous system and in various peripheral tissues, including the heart, lung, and mammary gland [3-5]. Within the gastrointestinal tract, northern analysis showed a maximal apelin expression in the stomach with a lower expression level in the intestine. Apelin expression was not detected in the pancreas. Immunohistochemistry revealed abundant apelin-positive cells in the glandular epithelium of the stomach. Apelin-positive cells were identified in the rat stomach as mucous neck, parietal cells, and chief cells. Apelin is also identified in gastric epithelial cells that produce chromogranin A, a marker of enteroendocrine cells. This widespread expression of apelin in peripheral tissues is associated with its synthesis by endothelial cells [6] Although Apelin circulates in plasma at low picomolar concentrations [7, 8], it probably operates mainly as a local paracrine hormone.

BIOCHEMISTRY AND METABOLISM

In humans, the apelin gene is located on chromosome Xq25-26.1 and encodes a 77-amino-acid prepropetide [4]. Considerable sequence homology of preproapelin exists across different mammalian species, with 23 C-terminal residues being identical in rat, mouse, cattle, and human. Although initially isolated as a 36-residue peptide, shorter C-terminal fragments, apelin-13 and apelin-17, display even higher potency than apelin-36 [2]. Apelin-36 is the most abundant preproapelin-derived peptide, but significant amounts of apelin-13 containing N-terminal pyroglutamate residue are also detected in rat and human tissues [8,9]. In the mammary gland, apelin mRNA increases progressively during pregnancy, reaches a peak during parturition, and decreases progressively during lactation. Apelin is secreted in substantial amounts in colostrum and milk and is even detected in commercially available bovine milk [3]. In the bovine colostrum, both apelin-36 and apelin 13 are present [10]. Conversion of N-terminal glutamate to pyroglutamate is catalyzed by glutaminyl cyclase (QC, E.C. 2.3.2.5) and is a postranslational modification common in many biologically active peptides which prevents enzymatic breakdown and preserves their biological activity [11,12]. The main apelin peptides circulating in plasma are apelin-13 and, to a lesser extent, apelin-17. The biological activity of apelin peptides inversely correlates with peptide length, with apelin-17 being more potent than apelin-36 and less potent than apelin-13. Apelin-12 is even more active but, according to the current knowledge, it is not synthesized in vivo. The twelve C-terminal amino acids peptide is the shortest active sequence, since apelin-11 and shorter peptides are inactive [9].

The only currently known pathway of apelin metabolism is by angiotensin-converting enzyme-2 (ACE2), a recently discovered zinc-containing carboxypeptidase which cleaves a C-terminal phenylalanine of apelin-13 and apelin-36, thus converting them to biologically inactive peptides [13]. ACE2 also converts angiotensin I to inactive angiotensin 1–9 and angiotensin II (angiotensin 1–8) to angiotensin 1–7; the latter reaction may be a major role of ACE2 because angiotensin 1–7 has many activities opposite to those of angiotensin II, such as stimulation of natriuresis, vasodilation, and inhibition of vascular cell growth and proliferation. ACE2 is insensitive to classical ACE inhibitors. Although specific ACE2 inhibitors have been synthesized, it is unclear if blocking ACE2 activity would be beneficial, since despite enhancing favorable apelin signaling, this treatment might limit the availability of angiotensin 1–7. In contrast to the ubiquitous expression of ACE in different vascular beds, ACE2 is limited mainly to endothelium of arteries, arterioles and venules of the heart and kidney, renal tubular epithelium, and testis [14].

APELIN RECEPTOR

Apelin receptor (APJ) is widely expressed in the brain and in almost all peripheral tissues. In particular, APJ immunoreactivity was detected in endothelial cells lining intramyocardial, renal, pulmonary, and adrenal vessels as well as in endocardial endothelial cells. Lower levels of APJ staining are present in cardiomyocytes and in vascular smooth muscle cells [15]. In the brain, APJ mRNA is preferentially expressed in glial cells of the white matter [16]. APJ is also expressed in phytohemaglutinin- and interleukin-2-activated peripheral blood mononuclear cells and supports the efficient entry of primary T-helper tropic human immunodeficiency vi-

rus (HIV) as a coreceptor with CD4 [17]. Interestingly, apelin inhibits HIV infection of cells coexpressing CD4 and APJ. The rat homologue of APJ, B78/apj, was detected in lung, heart, skeletal muscle, kidney, brain, liver, ovary, and anterior pituitary [5].

APELIN AND WATER BALANCE

Abundant expression of apelin and its receptor in the supraoptic and paraventricular nuclei of the hypothalamus, areas where vasopressin and oxytocin are synthesized, suggests its involvement in the regulation of water balance. The central apelinergic system is sensitive to changes in water balance. Water deprivation for 24 or 48 hours markedly increases hypothalamic apelin content and decreases plasma apelinn level, suggesting suppressed apelin release from hypothalamic stores. The changes were the opposite to those of ADH, which increases in plasma and decreases in the hypothalamus [18]. This suggests that hypothalamic apelin might inhibit ADH release under physiological conditions and that water deprivation removes its inhibitory influence by suppressing apelin release [8]. Hyperosmolality induced by water deprivation or drinking a hypertonic NaCl solution increased APJ receptor expression in the supraoptic nucleus in the rat, which may be a response to decresed apelin secretion [19]. Itracerebroventricular (icv) administered apelin-13 significantly decreased plasma ADH level in normally hydrated conscious mice [21]. In a similar way ivc injection of apelin-17 reduced plasma ADH in mice water deprived for 2 days. Icv injected apelin-17 suppressed electrical activity of supraoptic ADH-producing neurons ans decreased plasma ADH levels in lactating mice [8].

Reduction in ADH release results in markedly increased diuresis after icv apelin administration without changing sodium or potassium excretion. Various studies have addressed the effect of apelin on water intake. In normally hydrated rats, acute intraperitoneal injection of apelin increases water intake within the subsequent 30 min [4].

Similarly, icv-administered apelin stimulated drinking behavior in the first hour post-injection [20]. In contrast, Reaux *et al.* [21] reported that centrally administered apelin reduced water intake by approximately 30% in Wistar-Kyoto rats that were water-deprived for 24 hours, but had no effect on animals with free access to water.

CARDIOVASCULAR EFFECTS OF APELIN

Apelin has distinct effects on blood pressure and vascular tone In the rat, intravenous injection of apelin at 10 nmol/kg induced a rapid (within 1 min) decrease in mean arterial pressure ranging from 5% for apelin-36 to 25% for apelin-12. The effect is transient and lasts 3–4 min [4,9]. Hypotension is accompanied by a slight increase in heart rate in conscious, but not in anaesthetized animals. Apelin induced tachycardia results from the baroreceptor reflex-mediated stimulation of the sympathetic nervous system [22]. In vivo studies revealed that apelin is a very potent venodilator, more efficacious than Ca2+-antagonists, hydralazine, isoprenaline, or nitroglycerin. Thus, apelin could reduce blood pressure predominantly by dilating peripheral veins and reducing preload rather than by dilating arterioles and reducing peripheral resistance[22].

The hypotensive effect of apelin is mediated by endothelium-derived NO, since the NO synthase inhibitor L-NAME abolished this effect both in rats [9] and in mice [23]. In addition, apelin increases plasma concentration of NO metabolites, nitrites+nitrates. In cultured mice endothelial cells, apelin stimulates the phosphorylation of endothelial NO synthase (eNOS) at Ser1176 by protein kinase B/Akt.

This pathway plays an important role in the regulation of eNOS activity; it is activated, for example, by several other vasodilators, such as insulin and leptin [39]. In contrast to these studies, apelin-13 potently contracted endothelium-denuded human saphenous vein with a potency similar to endothelin-1 [24]. Thus, apelin may exert both endothelium-dependent NO-mediated vasodilatation and endothelium-independent vasoconstriction by acting directly on vascular smooth muscle cells. Studies performed in intact animals strongly suggest that the former effect prevails under physiological conditions when endothelial function is normal. APJ receptor knockout mice have been generated [23]. These animals develop normally during the embryonic stage and demonstrate no histological abnormalities. Baseline blood pressure and heart rate of APJ-/- mice do not differ from wild-type animals. However, whereas pyroglutamylapelin-13 significantly decreases blood pressure in wildtype mice, it has no effect in APJ-deficient animals, indicating that this receptor mediates the hypotensive response to apelin. In addition, apelin fails to stimulate eNOS phosphporylation in APJ-deficient mice. APJ-null mice are also more sensitive to the hypertensive action of angiotensin II administered

at low doses. This suggests that apelin-APJ signaling plays an important role in counteracting angiotensin II-induced vasoconstriction. This is further supported by the observation that baseline blood pressure is higher in double-knockout mice lacking both APJ and angiotensin receptor type 1a (AT1a) than in AT1a-knockout mice with intact APJ [23]. Ishida *et al.* [23] observed that the hypotensive effect of apelin was less marked in spontaneously hypertensive rat (SHR) than in control normotensive Wistar-Kyoto (WKY) rats. In contrast, in another study [13] apelin-13 exerted a more marked hypotensive effect in SHR than in WKY.

Recently, a significant reduction in apelin and APJ gene expression in the heart and aorta was observed in SHR in comparison with WKY [25]. In contrast to peripheral administration, intracerebroventricular (icv) injection of apelin-13 elicited a dose-dependent increase in mean arterial pressure in conscious animals [26].

Myocardial contractility

Szokodi *et al.* [27] first demonstrated that apelin dose-dependently increases myocardial contractility in isolated perfused rat heart. The time-course of apelin s effect was similar to that of the potent inotropic mediators endothelin-1 and adrenomedullin, i.e. a significant increase in contractility was observed at 2 minutes after adding apelin to the perfusion medium and persisted for >20 min. In contrast, the b-adrenergic receptor agonist isoproterenol exerts a more immediate but transient effect. The increase in developed tension induced by apelin was 70% of the maximal response to isoproterenol, which places apelin among the most potent inotropic agents known so far. In contrast to its vascular effect, the apelin-induced increase in myocardial contractility is not mediated by NO [27]. The positive inotropic effect of apelin was attenuated by specifi c inhibitors of phospholipase C or protein kinase C. More detailed studies revealed that apelin increases the activity of sarcolemmal Na+/H+-exchanger, leading to: 1) intracellular alkalinization (which increases the sensitivity of contractile apparatus to Ca2+) and 2) increase in intracellular Ca2+ by activating Na+/Ca2+ exchanger working in a reverse mode (Na+ out, Ca2+ in).

Interestingly, apelin significantly improved systolic and diastolic function of rat myocardium in an experimental model of heart failure induced by coronary artery ligation [28]. Despite increasing myocardial contractility, apelin has only a weak effect on cardiac output, probably because it induces venodilation and reduces preload. In contrast to the decrease in arterial pressure and increase in heart rate after acute apelin injection, chronic peptide infusion has no effect on these variables, suggesting that the vasodilatory effect is curtailed over time. However, a positive inotropic effect persists if apelin is administered for two weeks [29]. Therefore, apelin increases cardiac output more markedly after chronic than after acute administration. The unique beneficial feature of apelin which distinguishes it from other mediators which exert chronic positive inotropic effect is that it does not induce myocardial hypertrophy [29].

Effect of myocardial overload, hypertrophy, and failure on the apelin-apj system

The effect of myocardial overload and failure on the apelin- APJ pathway is controversial. In isolated neonatal rat ventricular myocytes, cyclic mechanical stretch for 12 or 24 hours decreased apelin and APJ mRNA by more than 50% and 30%, respectively [27]. In contrast, in two in vivo models of chronic pressure overload induced by chronic arterial hypertension, spontaneously hypertensive rat and double transgenic rats harboring human angiotensinogen and renin genes, the left ventricular apelin mRNA level was significantly lower compared with the respective controls, whereas APJ expression was unchanged [27]. In humans with severe heart failure, mechanical offloading of the heart after implantation of the left ventricular assist device resulted in the upregulation of APJ gene expression and apelin level in the left ventricle, suggesting that myocardial overload per se leads to the down-regulation of the cardiac apelin system [30].

Plasma apelin concentration is increased in patients with early stages of heart failure (NYHA class I and II), whereas in those with severe disease (NYHA class III and IV) it decreases to a level similar to that in healthy individuals.

Foldes *et al.* [31] have shown increased apelin mRNA content in the left ventricular myocardium of patients with idiopathic dilated cardiomyopathy or ischemic heart disease (NYHA class II and III). In this study, APJ mRNA in the left ventricle of patients with heart failure was lower than in healthy subjects. Taken together, these data suggest that myocardial apelin synthesis is up-regulated in early stages of heart failure, possibly in an attempt to improve myocardial contractility, whereas decreased APJ expression may be a "down-regula-

tion" phenomenon induced by an excess of ligand [31]. Interestingly, although apelin is expressed in myocytes of embryonic heart, in adult animals it is not synthesized by cardiomyocytes, but only by endothelial cells of coronary vessels. However, in decompensated heart failure, myocardial apelin synthesis is reactivated [27,29]. In contrast to ventricular myocytes, apelin gene expression does not increase in the atria of failing hearts [31]. This resembles the regulation of atrial and brain natriuretic peptides (ANP and BNP), which are selectively up-regulated in the ventricular, but not in atrial myocardium of the failing heart. Thus, apelin may be a novel component of a "fetal program" derepressed in the failing myocardium.

Angiogenesis

Several lines of evidence suggest that apelin may be involved in angiogenesis. First, apelin is abundantly expressed in the endothelium of embryonic vessels. Second, APJ expression is upregulated during formation of new vessels and down-regulated after vessel stabilization [32]. Finally, apelin-13 potently stimulates the proliferation of cultured human umbilical vein endothelial cells in a manner involving phosphatidylinositol 3-kinase (PI3K), PKB/Akt, protein kinase C, and extracellular signal-regulated kinase (ERK) [33].

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