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Review Article

Biochemistry Section

Apelin and its Receptor: An Overview

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ABSTRACT

Apelin protein is an endogenous ligand of Apelin Receptor (APJ). APJ is a member of G-protein coupled receptor family. Both apelin and its receptor express extensively in the human body. Apelin receptor activation occurs by its cognate peptide ligand, apelin and many physiological effects, including vasodilation, vasoconstriction, angiogenesis, fluid homeostasis, neuroendocrine response to stress and energy metabolism. Apelin derived from its precursor might yield a number of bioactive peptides. Apelin is synthesised as an immature single peptide (preproapelin) which consists of 77 amino acids. In the endoplasmic reticulum, preproapelin is cleaved by endopeptidases to a 55 amino acid proapelin and subsequently, to various biologically active apelin-36, apelin-17 and apelin-13 isoforms. Post-translation, the apelin containing the pyroglutamate group at N-terminus of the peptide is modified to pyroglutamate apelin 13. In adipocytes, proprotein convertase subtilisin/kexin 3 directly cleaves the proapelin to apelin 13 and does not produce any longer isoforms. In contrast, Angiotensin Converting Enzyme 2- (ACE-2) cleaves at proline-phenylalanine site at C-terminus and renders apelin 13 and apelin 36 inactive. To date, ACE-2 is the only known enzyme for apelin degradation. The C-terminal region is responsible for receptor binding and subsequent activation. Prior research suggests the role of apelin and its receptor in pathogenesis of various conditions including preeclampsia, hypertension, cardiovascular diseases, diabetes mellitus, obesity and cancer. Despite its established importance and link to therapeutic target, the precise role of this apelin/APJ remain obscure. In this attempt, we summarised the structure, chemistry, biosynthesis, expression and gene regulation, distribution, receptor binding mechanism, biological functions and therapeutic applications along with the associated recent advances.

Keywords: Angiogenesis, Angiotensin converting enzyme-2, Apelin receptor, G-protein coupled receptor, Vasodilator

HISTORY AND DISCOVERY

Apelin Receptor Structure and Properties

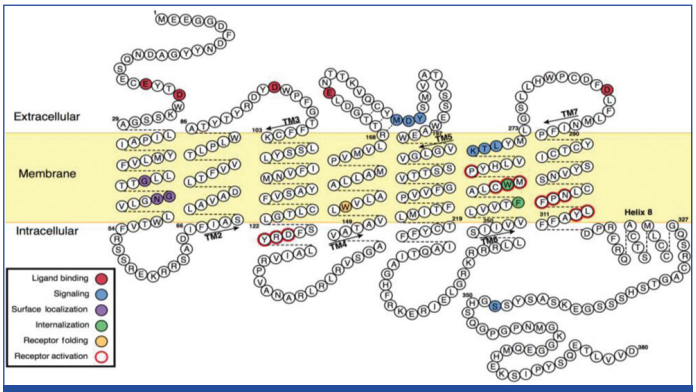
In 1993, O'Dowd BF et al., discovered a type of receptor protein and named it Apelin Receptor (APJ/AR) [1]. They characterised a 700 base pair sequence of the Polymerase Chain Reaction (PCR) fragment, which encoded a protein of 380 amino acids protein. The APJ gene (Gene symbol *APLNR*) is located on chromosome 11q12 and does not contain intron. APJ receptor resembles Angiotensin Type 1 receptor (AT1). APJ and AT1 have structural homology of 115 amino acids (30%) and 86 amino acids in the transmembrane regions (54%) [2].

APJ receptor contains seven hydrophobic transmembrane regions characteristic of G-Protein Coupled Receptor (GPCR) family [2]. GPCR is activated by neuropeptides, polypeptide hormones and non-peptides such as biogenic amines, nucleotides, lipids and ions [3]. Some novel GPCRs do not have their endogenous natural ligands, and are termed as "orphan receptors". The cAMP-dependent protein kinase phosphorylates the APJ receptor at its consensus sites for palmitoylation and glycosylation.

The glycosylation of N-terminal GPCR, has been implicated in the expression, correct folding of nascent protein, stability and ligand binding of receptor [4]. Studies on the APJ receptor structure showed that amino acids in the N-terminal (e.g., Asp23 and Glu20) and C-terminal portions of the APJ receptor are required for internalisation [5,6] [Table/Fig-1].

Regulation of APJ (*APLNR*) Gene

The regulation of APJ gene expression has not been extensively studied till date. At the transcriptional level, the highest, rat APJ gene promoter activity is found between -966 and -165 bp. Electrophoretic mobility shift, super-shift and competitive immunoassays have indicated that promoter is under complex regulation by specificity protein 1 (Sp1), oestrogen receptor, glucocorticoid receptor and CCAAT enhancer binding protein γ (C/EBP-γ or CEBPG) transcription



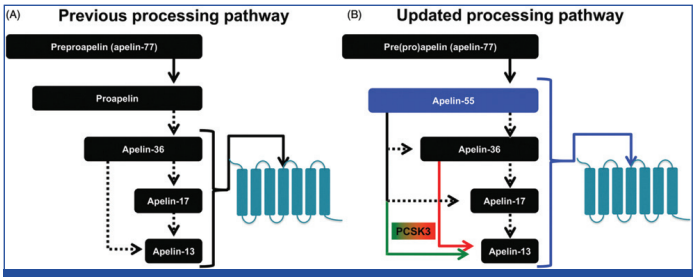
[Table/Fig-1]: Human apelin receptor (APJ/AR) structure, with seven delineated transmembrane (TM) helices [6].

factors, with Sp1 as a major regulator of rat APJ gene promoter activity [7]. Various studies on Single Nucleotide Polymorphisms (SNPs) of *APLNR* gene and reported an association between SNP (rs9943582 (G/A) in 5' flanking region of Sp1 binding site of *APLNR* gene with susceptibility to brain infarction [8,9]. Two *APLNR* gene polymorphisms, in Han Chinese population, rs7119375 (G/A) and rs10501367 (G/A), in Italian patients, rsG212A, suggests association of SNPs with hypertension [10]. In animal model, up-regulation of APJ gene, in response to acute and repeated stress is observed and these changes are likely to be dependent on glucocorticoids [11]. The endogenous ligand, apelin also regulates APJ expression within the Gastrointestinal (GI) tract and up-regulation of APJ expression was observed in animal adipose tissue by insulin [12].

APELIN STRUCTURE AND PROPERTIES

The term apelin denotes APJ endogenous ligand. APJ receptor remained as "orphan receptor" until 1998, when Tatemoto K et al., identified this ligand and termed it as apelin [2]. In humans, the *APLN* gene is located on chromosome X at Xq25-26.1 position; a 6 kb open reading frame is an apelin gene (*APLN*) with one intron.

In human chromosome X, apelin gene (*APLN*) located at Xq25-26.1 and contains ~6 kb intron within its Open Reading Frame (ORF) [2]. Apelin is synthesised as 77 amino acid preproprotein (preproapelin), an immature single peptide, with hydrophobic rich N-terminal region. The amino acid sequence of apelin is similar to that of angiotensin II [2]. Bovine, human, rat and mouse preproapelin precursors have 76-95% homology and appear to exist endogenously as a dimeric protein, due to disulphide bridges. Dimerization of pre(pro)apelin occurs by disulphide bridge formation. Previous studies, reported that the dimerization is a prerequisite for proper processing in bioactive peptides, such as stomatostatin-II, suggesting that dimerization is necessary for proper processing of apelin [6,13]. In the endoplasmic reticulum, prepropeptide is cleaved to a 55 amino acid proapelin by removal of an N-terminal signal peptide, presumed to be an inactive precursor, containing the receptor binding site [14]. The 55 amino acids proapelin peptide further generate several smaller bioactive peptides, which includes apelin-36 (apelin 42-77), apelin-17 (apelin 61-77) and apelin-13 (apelin 65-77) [15]. Additionally, pyroglutamate apelin 13 {(Pyr1)-apelin 13} is a post-translationally modified form of apelin and it contains pyroglutamate group at N-terminus of the peptide [16]. These peptides have distinct activities and shorter peptides are more potent activators for APJ [Table/Fig-2,3] [17].



[Table/Fig-2]: Apelin processing pathways. (a). Previously 'theorised' sequential processing pathway of "apelin-36 precursor" (b). Current 'Myriad' processing pathway based on recent findings of apelinergic system which identifies proapelin as apelin-55, an additional bioactive member [6].

[Table/Fig-3]: Primary structure of apelin peptides and its amino acid sequences (A-Pyr1-apelin 13, B-Apelin 13, C-Apelin 17, D Apelin-36) [6].

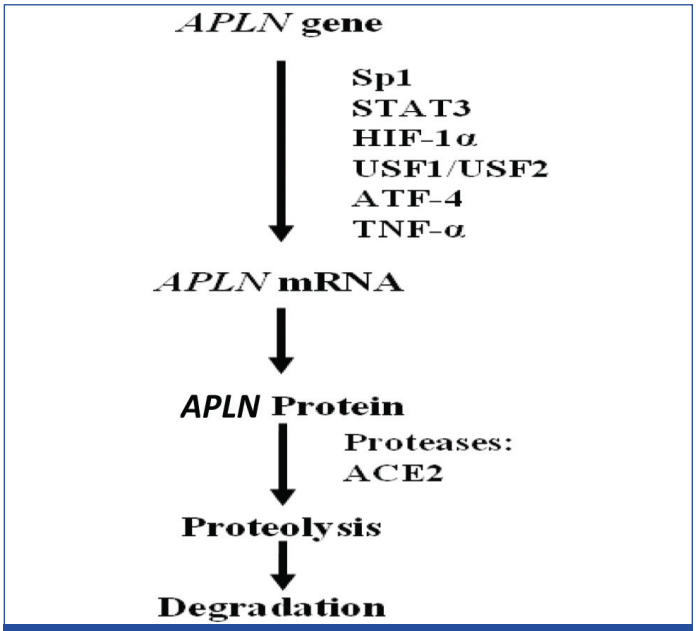
In adipocytes, proprotein convertase subtilisin/kexin 3 (PCSK3 or furin) directly cleaves proapelin to apelin 13, with no production of longer isoforms [14]. Apelin peptides, which lack cysteine residues, are probably present in monomeric form. Apelin protein biological activity depends on carboxy terminal of the peptide comprising 12 amino acids [16]. Carboxy-terminal of preproapelin is rich in basic amino acids, which are potential cleavage sites during post-translational processing [2]. Among all apelin peptides, apelin-13 and (Pyr1) apelin-13 are the shortest, most prevalent and biologically active forms.

The processing of apelin precursor (prepropeptide) is complex. Aminopeptidases, serine proteases and to some extent carboxypeptidases, are responsible for the processing of apelin peptides. Angiotensin converting enzyme-2 (ACE-2) degrade apelin-13 and apelin-36 into inactive peptides by cleaving Pro-Phe bond and removes phenylalanine on C-terminus with high catalytic efficiency [16]. It has been suggested that (Pyr1) apelin-13 is more resistant to enzymatic cleavage by ACE-2. To date, ACE-2 is the only known enzyme for degradation of apelin. In the circulation, apelin is cleared very rapidly within a short plasma half-life of no longer than 8 minutes [18].

REGULATION OF APELIN (*APLN*) GENE

The Apelin gene expression is regulated by various transcription factors. A single-nucleotide polymorphism (SNP) study reported Sp1 role in the regulation of apelin gene expression [8]. The expression of Apelin gene is enhanced by tumour necrosis factor-α (TNF-α), via phosphoinositide-3 kinase (PI3K), c-Jun N-terminal kinase (JNK) and MEK1/2 (mitogen activated protein kinase 1/2) in adipocytes [19]. Apelin core promoter sequences in rat and humans contain putative binding sites for Upstream Stimulatory Factor (USF) 1/2, and overexpression of USF up-regulates apelin transcription [20,21].

Hormone Response Elements (HREs) are present in promoter and intron sequence of apelin gene in various species that causes up-regulation of apelin expression [Table/Fig-4]. In hypoxic conditions, apelin expression is up-regulated in cardiac myocytes wherein Hypoxia Inducible Factor-1α (HIF-1α) binds to HRE (-813/-826) located within the first intron of human Apelin gene and increases apelin expression in vascular cells [22]. In white adipocytes, peroxisome-proliferator-activated receptor γ co-activator 1α also up-regulates the Apelin gene expression [23].



[Table/Fig-4]: Regulation of Apelin gene.

Sp1-Specificity protein 1; STAT3-Signal Transducer and Activator of Transcription 3; HIF-1α-Hypoxia Inducible Factor-1 α; USF1/USF2-Upstream Stimulatory Factor (USF) ½; ATF4-Activating Transcription Factor 4; TNF-α-Tumour Necrosis Factor α; ACE-II-Angiotensin Converting Enzyme-II [24].

SECRETION AND DISTRIBUTION OF APELIN AND APELIN RECEPTOR

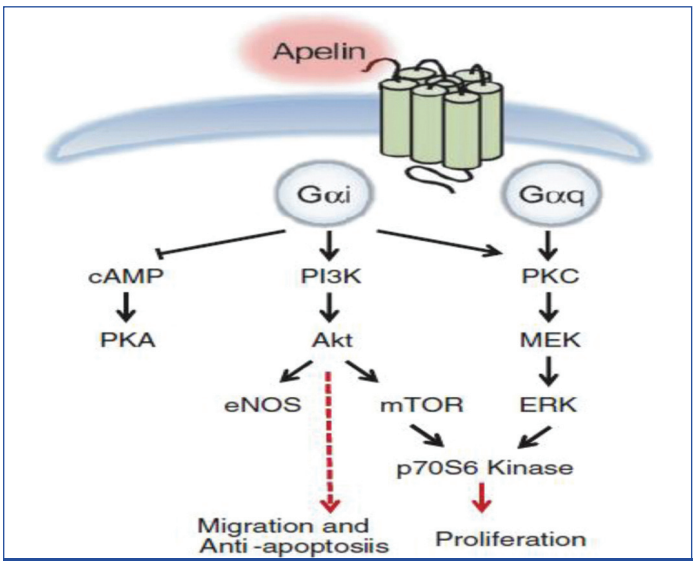
In mammals, APJ expression is widely distributed in embryo and various peripheral tissues in adults. Maximum expression of APJ are found in the heart, lung, mammary gland and placenta, and significant but lower levels of APJ mRNA are present in kidney, pituitary gland, ovary and skeletal muscle, [25]. APJ expression in these tissues is localised to vascular, endothelial and smooth muscle cells. The apelin expression is also observed in a range of peripheral tissues, including heart, liver, kidney, adipose tissues, and brain, with highest levels found in the lung, mammary gland and placenta [26].

RECEPTOR BINDING AND MECHANISM OF ACTION

Apelin is believed to be the only endogenous ligand for APJ [27]. All apelin peptides bind to APJ [14]. Binding of apelin peptides leads to conformational changes in the receptor, which causes activation of its associated G-protein that induces Guanosine Diphosphate (GDP) dissociation and Guanosine Triphosphate (GTP) binding. N-terminal domain of APJ plays a critical role in ligand binding [15]. APJ is suggested to couple with $G_{i/o}$ protein, this findings was supported by the inability of (Pyr1) apelin 13 and apelin 36 to generate calcium mobilisation or to release arachidonic acid metabolites into the cells which leads to the positive or negative regulation of various intracellular effectors. By using transfected cells, similar results were obtained with neurons differentiated from NT2 (NTERA-2) cells and to a lesser extent with astrocytes. Interestingly, the rate of receptor recycling to the surface is faster for apelin 13 than for apelin 36 [28].

CELLULAR EFFECTS AND INTRACELLULAR RESPONSES

Apelin induces the proliferation and migration of APJ expressing endothelial cells [29]. The major signaling pathways of apelin are mediated initially by Gαi (inhibitory)-protein coupled to APJ and Protein Kinase C (PKC) [Table/Fig-5]. Apelin causes concentration-dependent inhibition of cAMP production and increases phosphorylation of protein kinase B in endothelial cells of umbilical cord. The binding of apelin to APJ, phosphatidylinositol-3 kinase (PI3K) and ERK pathway leads to phosphorylation of P70S6K, that results in endothelial cell migration and proliferation [30,31]. Studies have reported that APJ forms a heterodimer with k-opioid receptors and leads to ERK phosphorylation, causing increased cell proliferation [32]. Furthermore, apelin inhibits pulmonary arterial endothelial cell apoptosis in mouse [33]. The apelin-36 and apelin-13 induce intracellular effectors activation and exhibit varied coupling and desensitization through Gαi-protein [34]. Substitution of the C-terminal phenylalanine residue of apelin13 by alanine results in the generation of an antagonist of APJ. The only available nonpeptidic antagonist of APJ is the compound ALX40-4C (N-alpha-acetyl-nona-D-arginine amide acetate), which was primarily shown to interact with second extracellular loop of the CXCR4 (C-X-C motif Chemokine Receptor 4). However, it should be noted that binding affinity of ALX40-4C for CXCR4 is much higher than APJ receptor and thus it is not a specific antagonist to APJ [28].



[Table/Fig-5]: Schematic representation of intracellular signal transduction pathway and cellular effects in apelin/APJ system [6].
cAMP-Cyclic adenosine monophosphate; PKA-Protein Kinase A; PI3K-Phosphatidylinositol 3-Kinase; Akt-Protein Kinase B; eNOS-Endothelial Nitric Oxide Synthase; mTOR-Mechanistic Target of Rapamycin; PKC-Protein Kinase C; ERK-Extracellular Regulated Kinase; MEK-Mitogen-activated Protein/Extracellular Signal-regulated Kinase

QUANTIFICATION OF APELIN

Human apelin is estimated in serum, plasma and other biological fluids by enzyme-linked immunosorbent assay (ELISA) and liquid chromatography/tandem mass spectrometry [35,36].

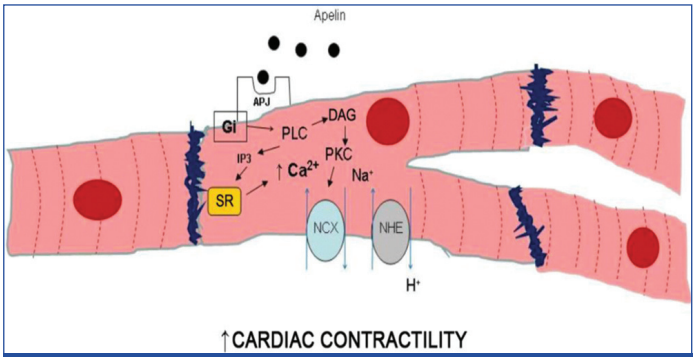
BIOLOGICAL FUNCTIONS OF APELIN

Apelin and APJ are distributed throughout the human body. Higher concentration of plasma apelin as compare to tissue concentration, suggests its paracine and autocrine nature.

APELIN AND CARDIOVASCULAR FUNCTIONS

The well-established apelin and its receptor APJ's signaling pathways are associated with various cardiac and vascular functions. Apelin expression is primarily linked to the endothelium and APJ expression to the myocardium, suggesting a paracrine mode of signaling [37]. Apelin acts as a potent cardiac contraction activator; the apelin-APJ activation controls systemic perfusion and effect both heart and the vascular system [38]. In vascular system, Nitric Oxide (NO), mediates the effect of apelin as a vasodilator. In anaesthetised intact rats, peripherally administered apelin reduced the Mean Arterial Pressure (MAP) [39]. The effect of apelin and the specific apelin peptide in conscious state of the animal remains unclear but numerous studies suggests that apelin plays a cardinal role in blood pressure regulation [40-42]. Peripheral administration of apelin causes vasodilation in human saphenous vein; in contrast it acts as a vasoconstrictor in vessels denuded of endothelium [42]. Central administration of (Pyr1) apelin-13 increases the MAP [43]. While intracerebroventricular (ICV) administrations of (Pyr1) apelin 13 has no effect on MAP or heart rate in anaesthetised rats, ICV administrations increases the MAP and heart rate in conscious rats [44].

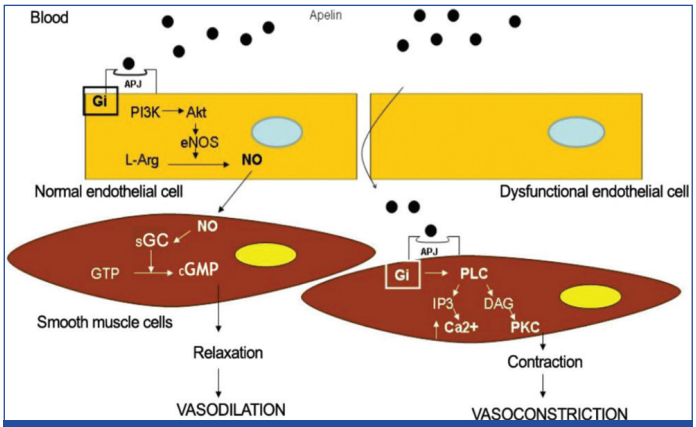
Apelin has been documented to have positive inotropic effects in normal rats and negative effect on failing heart rate of rats after myocardial infarction. Hence, it may be used as a positive inotropic agent in ischaemic heart failure patients. Apelin administration causes peripheral and coronary vasodilation and increases cardiac output. The inotropic effects of apelin may be caused by increased intracellular calcium levels mediated by Na^+/H^+ and Na^+/Ca^{2+} exchanger activation [Table/Fig-6] [45].



[Table/Fig-6]: Intracellular pathways responsible for positive inotropic effect of apelin/APJ interaction.
DAG-Diacylglycerol; Gi-Inhibitory G protein; IP3-Inositol Triphosphate; NCX-Na^+/Ca^{2+} Exchanger; NHE-Na^+/H^+ Exchanger; PKC-Protein Kinase C; PLC-Phospholipase C; SR-Sarcoplasmic Reticulum [45]

Studies have shown that apelin 13 administration causes reduction in the blood pressure in animal models and humans, which indicates that apelin can regulate blood pressure [13,43]. The ability of apelin's blood pressure lowering effects differed depending on the dose, anaesthesia, route of administration and experimental model. Apelin causes endothelium dependent vasodilation by stimulating the phosphorylation of endothelial Nitric Oxide Synthase (eNOS) at serine 1179 and releases NO. Nitric oxide, in turn, activates soluble guanylate cyclase in vascular smooth muscle cells, resulting in increased levels of cyclic Guanosine Monophosphate (cGMP) which mediates dilation of vascular smooth muscle cells [15]. Apelin triggers L-arginine transport and

increases the production of NO in isolated aorta of the rat [46]. Pyr1 apelin 13 also increases serum NO levels in infarcted treated rats [47]. Japp AG et al., reported that the use of apelin in humans stimulates peripheral vascular, coronary vasodilation and increases cardiac output [18]. However, administration of apelin in APJ knock-out mice indicated no change in blood pressure suggesting that apelin reduces blood pressure via its APJ receptor [48]. Apelin-induced endothelium-dependent vasodilation is reduced by co-administration of L-Nitro-Arginine-Methyl-Ester (L-NAME), suggesting an endothelium-derived NO dependent mechanism. L-NAME is an inhibitor of eNOS [Table/Fig-7] [16].



[Table/Fig-7]: Intracellular pathways responsible for the vasomotor effects in apelin/APJ interaction in the absence and in the presence of endothelial dysfunction.
DAG-Diacylglycerol; eNOS-Endothelial Nitric Oxide Synthase; Gi-Inhibitory G protein; sGC-Soluble Guanylate Cyclase; GTP-Guanosine Triphosphate; cGMP-Cyclic Guanosine Monophosphate; IP3-Inositol Triphosphate; L-Arg-L-Arginine; NCE-Na+/Ca2+ Exchanger; NHE-Na+/H+ Exchanger; NO-Nitric Oxide; PKC-Protein Kinase C; PLC-Phospholipase C [45]

ROLE OF APELIN IN VASOCONSTRICTION

Apelin increases the heart rate not directly through a chronotropic effect, but by a secondary baroreceptor reflex. An opposite effect was observed in conscious sheep; anesthetized and conscious rats, where apelin administration increased the heart rate and MAP [48]. Studies have shown that intravenous apelin-13 created a biphasic response in MAP and heart rate and this biphasic response consisted of a preliminary decrease, followed by a momentary increase and a decline back to the baseline level in the last 15 minutes. *In vitro* studies reported the apelin is involved in both vasodilation and vasoconstriction [49-51].

ROLE OF APELIN IN ANGIOGENESIS

Apelin, angiogenic factor in endothelial cells, stimulates vessel growth and endothelial cell proliferation. In frogs, apelin is required for normal development of heart and blood vessels [52]. Additionally, development of retinal vasculature is decreased in apelin knock-out mice. Apelin is important for the hypoxia-induced retinal angiogenesis and is also involved in non-neovascular remodeling of the retina. Apelin activates the cell transduction cascade ERKs, Akt and p70S6 kinase phosphorylation, which leads to the proliferation of endothelial cells and formation of new blood vessels. These findings indicate that apelin is required for angiogenesis. However, further research is required to establish human model.

ROLE OF APELIN IN FLUID HOMEOSTASIS

APJ mRNA expression detection by Immunohistochemistry (IHC) in brain, shows that apelin regulates the body fluid homeostasis. Studies showed that both apelin 13 and apelin17 induce a decrease in circulating plasma vasopressin levels and water intake after ICV administration. Increased water consumption was observed in rats, upon intra-peritoneally administration of apelin. These contrasting effects can be explained on the account of different routes of administration [29]. In APJ knockout mice, abnormal fluid homeostasis leads to decrease in drinking behaviour and inability to concentrate, indicating apelin's *in vivo* anti-diuretic effect [26].

ROLE OF APELIN IN NEURO-ENDOCRINE RESPONSE TO STRESS

The expression of apelinergic system has been observed in the Central Nervous System (CNS). Existing scientific evidences confirm that the *in vivo* effects of apelin and APJ on Hypothalamic-Pituitary-Adrenal neuro-endocrinal functions are mediated through Corticotrophin Releasing Factor (CRF) and vasopressin dependent mechanisms.

Central administration of (Pyr1) apelin 13 increases the expression of c-fos, an indicator of neuronal activity in the paraventricular nucleus [40]. Furthermore, apelin 13 administration stimulates the release of Corticotrophin Releasing Hormone (CRH) and vasopressin from hypothalamic extracts *in vitro*. In the paraventricular nucleus, APJ mRNA levels increase in response to acute and chronic stress and following adrenalectomy, implying negative regulation of APJ mRNA expression by glucocorticoids [53].

ROLE OF APELIN IN METABOLIC ACTIONS

Apelin and its ligand APJ are expressed in adipose tissue of mouse, human and rats. Apelin controls food intake and a positive correlation have been reported between plasma apelin and Body Mass Index (BMI). Apelinergic system is a therapeutic target for metabolic disorders. However, expression of plasma apelin is increased only in obese subjects and in mouse models of obesity associated with hyperinsulinaemia, indicating that obesity or high fat diet feeding may not be the main cause for the rise in the apelin expression thus implies a close relationship between apelin and insulin both *in vivo* and *in vitro* [54].

Insulin stimulates the production of apelin in adipocytes and apelin mRNA expression is down-regulated in the adipocytes of mice treated with the β-cell toxin streptozotocin, which leads to a fall in plasma insulin levels [55]. Intraperitoneal apelin administration in normal and obese mice reduced body adiposity without affecting food intake, decreased the insulin, leptin and triglyceride levels and increased adiponectin levels. Apelin is necessary for insulin sensitivity. Reduced insulin sensitivity, glucose intolerance and hyperinsulinaemia are observed in apelin knockout mice [56]. Peripheral administrated apelin have powerful glucose lowering effect and it increased glucose uptake in skeletal muscle, adipose tissue and improved insulin sensitivity in both apelin knockout and obese high-fat diet fed mice [57].

THERAPEUTIC APPLICATIONS OF APELIN

Studies suggest the therapeutic potential of apelin with respect to treatment of hypertension and Cardiovascular Diseases (CVD) in animal models, even though its short half-life limits the therapeutic uses of apelin. Based on these findings, the recent research is focused on the development of novel agonists, delivery methods, and/or improving the efficacy of agonists of APJ.

The role of apelin's blood pressure lowering effects, by virtue of its C-terminal phenylalanine, plays an important role, evident by its substitution for alanine results in apelin-specific antagonistic activity *in vivo* and reduced apelin binding affinity *in vitro* [58]. Apelin treatment in rats with isoprenalin-induced lesions promotes the restoration of cardiac function and is also cardioprotective against lipidic peroxidation [59]. Ventricular resynchronisation therapy promotes a long-term increase in plasma levels of apelin, which may be associated to cardiac function improvement [60]. Reduced levels of apelin are reported in coronary disease patients undergoing haemodialysis. It is speculated that apelin may be involved in the pathophysiology of CVD, secondary to chronic renal failure and that it may be utilised in the future as a treatment approach for uraemic cardiomyopathy [61].

Apelin is considered a possible marker for cardiac hypoxia (acute and chronic). Circulating levels of apelin do not seem to be of help for the clinical evaluation and prognosis of acute or chronic heart failure patients [62]. Apelin may be a useful marker to assess the

development of isolated atrial fibrillation as well as a diagnostic marker to distinguish dyspnoea due to pulmonary causes from that of cardiovascular causes.

Apelin gene therapy has also been proved to be cardioprotective by stimulating angiogenesis in diabetic mice or mice subjected to myocardial infarction [63]. As per the available information, the therapeutic potential of apelin in humans is scarce and draws much attention for researchers.

RECENT UPDATES

Van Mieghem T et al., analysed maternal serum concentration of apelin and cardiac output and total peripheral resistance in high risk pregnancies at 20 to 34 weeks of gestation. Placental apelin staining was done by IHC and placental apelin gene expression was assessed by quantitative polymerase chain reaction (qPCR). In 20 and 26 weeks of gestation, apelin levels were found to be positively correlated with total peripheral resistance (r=.57, p=0.01) and inversely correlated with stroke volume (r=-42, p=0.08). About 30% reduction in apelin serum levels was observed in pregnancies with Intrauterine Growth Restriction (IUGR) complications as compared to uncomplicated pregnancies or in women with preeclampsia (p=0.009). Apelin staining was strongly decreased in IUGR compared to controls. Preeclamptic placentas showed an intermediate staining. Serum and placental apelin were significantly reduced in IUGR condition and apelin levels mimicked cardiovascular changes during pregnancy [64].

Colcimen N et al., carried out a study on the role of Vascular Endothelial Growth Factor (VEGF), annexin A5 and apelin by IHC in placenta of preeclamptic and healthy controls. Between placenta samples of normotensive pregnant (controls), mild preeclampsia and severe preeclampsia cases. VEGF, annexin A5 and apelin were measured by immunohistochemical methods and Immunoreactivity (IRS) scores were calculated for each parameter. Results of the above study displayed increased levels of VEGF and apelin IRS in mild and severe preeclamptic group ($p<0.026$) in response to control group ($p<0.002$). However, annexin A5 IRS was significantly decreased in preeclampsia ($p<0.04$). Depending upon the intensity of the disease, it can be stated that elevated VEGF and apelin and low level of annexin A5 are responsible for alteration in fetoplacental circulation and structure in uteroplacental bed in preeclampsia [65].

Yamaleyeva LM et al., studied the expression pattern of apelin content in chorionic villi of normal pregnant and preeclamptic women at gestational age of 36-38 weeks. In preeclampsia, the preproapelin mRNA levels are reduced, which was associated with low total apelin content in preeclamptic women as compared with normal chorionic villi. Further, apelin peptide was characterised in pooled samples of each group by using HPLC-RIA, which revealed little or no apelin 36 or apelin 17. Pyroglutamate apelin 13 {(Pyr1)-apelin 13} was the predominant form in preeclamptic and normal villi. The activity of ACE-2 was higher in preeclamptic villi and decreased apelin levels were observed in placental chorionic villi of preeclamptic women [16].

Therefore, the conclusion is that angiotensin II involving regulation of apelin expression which is decreased in preeclamptic villi.

FUTURE PROSPECTIVE

Apelin may serve as a novel drug target linked to cardiovascular disease, hypertension and preeclampsia. The intensity of stress level alters the growth and development while contributing to physiological and metabolic changes. From the present knowledge, there is a possibility that apelin/APJ alters this balance. Acute stress triggers immunological reactions, alterations in blood pressure, GI symptoms, neurological and psychological responses while chronic stress causes behavioural and neuropsychiatric manifestations, cardiovascular and metabolic diseases. The apelinergic system has been implicated in most of these conditions. Hence, targeting apelin may bring new therapeutic options to overcome all cardiovascular related problems in the near future.

CONCLUSION

Apelin peptides and its receptor APJ are widely expressed *in vivo*, with interactions between apelin peptides and APJ. These participate in the regulation of key roles in cardiovascular system, angiogenesis, fluid homeostasis, neuro-endocrine response to stress, metabolic actions, therapeutic role in vasodilation and constriction. This article suggests the need for further augmentation of research evidences in human system with respect to various biological functions.

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