MINIREVIEWS

Expanding role for the apelin/APJ system in physiopathology

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Apelin is a bioactive peptide known as the ligand of the G protein-coupled receptor APJ. Diverse active apelin peptides exist under the form of 13, 17 or 36 amino acids, originated from a common 77-amino-acid precursor. Both apelin and APJ mRNA are widely expressed in several rodent and human tissues and have functional effects in both the central nervous system and peripheral tissues. Apelin has been shown to be involved in the regulation of cardiovascular functions, fluid homeostasis, vessel formation and cell proliferation. More recently, apelin has been described as an adipocyte-secreted factor (adipokine), up-regulated in obesity. By acting as circulating hormone or paracrine factor, adipokines are involved in physiological regulations (fat depot development, energy storage, metabolism or eating behavior) or in the promotion of obesity-associated disorders (type 2 diabetes and cardiovascular dysfunctions). In this regard, expression of apelin gene in adipose tissue is increased by insulin and TNFα. This review will consider the main roles of apelin in physiopathology with particular attention on its role in energy balance regulation and in obesity-associated disorders.

Key words: Adipocytes, Adipokines, Diabetes, Obesity, Insulin.

The "apelin story" began in 1993, when a putative receptor protein related to the angiotensin receptor was cloned during the search of novel orphan G protein-coupled receptors having a 7-transmembrane domain (67). The human gene of this angiotensin receptor-like protein (AGTRL1) was designated APJ and its

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transcripts were detected in many regions of the brain. Moreover, the APJ gene was mapped to chromosome 11 and later sublocalized to the locus 11q12. As for many cloned orphan receptors, the pharmacological and physiological relevance of APJ receptor was poorly defined at this stage. One major characteristic was that angiotensin II was unable to interact with APJ. Once cloned, the murine homolog of APJ receptor appeared to emerge during embryogenesis, especially in the primary blood vessels and the forming heart (18).

In 1998, Tatemoto and coworkers purified from bovine stomach extracts a protein that bound to the APJ receptor. Based on peptide sequences, the members of the same group cloned the corresponding bovine and human cDNA. The gene encodes a 77-amino acid polypeptide that includes a secretory signal sequence. The ligand of the orphan receptor APJ consisted in the C terminal of this polypeptide and was called "apelin", for APJ endogenous ligand (91). More precisely, the 36 residues of C-terminal preproapelin constituted the apelin-36, which was able to produce electrophysiological responses in cells expressing transfected APJ but not other receptors. The Japanese group of Fujino, Tatemoto and Onda then extended its pioneering observations by reporting that apelin is highly expressed in the mammary gland of pregnant rats and can be detected in milk (30). After having stated that apelin is an endogenous ligand for the APJ receptor, the same authors reported that apelin suppressed cytokine production by mouse spleen cells and was able to release cholecystokinin from intestinal endocrine cells. The apelin gene was then localized on the X chromosome at Xq25-q26.3 by several mapping studies, including one reported by the group of O'Dowd who first discovered the APJ

receptor (53). Thus, at the beginning of the 21st century, APJ was not an orphan receptor anymore and constituted – together with its own endogenous ligand – what is called the apelin/APJ system, which has been investigated beyond the gastro-intestinal, neuroendocrine and cardiovascular aspects of physiopathology. This review will consider these aspects before focusing on a more recent consideration of apelin as an adipocyte secreted factor, involved in energy balance regulation and in obesity-associated disorders.

Biology of APJ receptor

The human APJ receptor has a 7-transmembrane domain and shares a 30 % amino-acid sequence identity with angiotensin II type 1 receptor (AT1), another G protein-coupled receptor (53, 67). APJ receptor is expressed in many peripheral tissues and in brain, with endothelial cells being the more positive cell type. The rat APJ receptor, which presents 90 % homology with the human form, also shares 30 % homology with angiotensin receptor AT1A and has been demonstrated to be negatively coupled to adenylyl cyclase: once activated by apelin, it inhibits forskolin-induced cAMP accumulation in transfected CHO cells (16, 70). The coupling of APJ to Gi/o protein was confimed by pertussin toxin-sensitive ERK activation or adenylyl cyclase inhibition in cellular models transfected with the mouse or frog receptor (59, 63). In spite of this widely accepted coupling of APJ to Gi/o proteins, it remains relevant to note that this coupling was demonstrated mainly in transfected systems. More complex coupling and desensitization patterns were reported between APJ, Gα(i1) and Gα(i2) subunits and the effectors ERK and Akt in human umbilical

endothelial cells which naturally express all these partners (60). However, apelin-induced inhibition of cAMP accumulation has not been described yet in a native cell type, at least to our knowledge. Such a response of apelin/APJ system warrants further demonstration to be classified as relevant. APJ receptor is not only present at the cell surface but also exhibits nuclear localization in cells derived from human brain (54). Being stated to be agonist-independent, this nuclear localization is distinct from the rapid internalization of the APJ receptor induced by apelin stimulation in neuronal cells or in CHO cells expressing an APJ tagged form (20, 104) and its consequences are not elucidated yet.

Biology of apelin

Apelin gene is expressed in many peripheral tissues as well as in different brain areas. Its product, namely pre-proapelin, was found to exist in tissues under a high molecular weight form consisting in a dimer owing to disulfide linkages (24, 55). At present, it is accepted that there exist at least three biologically active forms of apelin, consisting of 13, 17, or 36 amino acids, all originating from a common 77-amino-acid pre-propeptide precursor (Fig. 1). The sequencing of the human, bovine, rat and mouse pre-proapelins has shown that there is a high sequence homology among the four species and a perfect identity for the last 22 C-terminal amino acids (30, 53, 91). The predominant molecular forms of endogenous hypothalamic and plasma apelins were found to be apelin-13 and, to a lesser extent, apelin-17, at least in mice (17). The researchers of the french Llorens-Cortes' team claimed that apelin-17 is one of the most potent apelin peptides regarding APJ stimulation and desensitization in hypothalamus (20, 72) while it was also reported that apelin-13 can activate APJ receptor more potently that the originally identified apelin-36 (80). In addition, the hydrophobic residues of apelin-13 play important roles in interactions with the APJ receptor (22). Due to its suspected higher resistance to degradation, the pyroglutamate form of apelin-13 (Pyr(1)-apelin-13) has been largely used to study *in vivo* or *in vitro* responses and is considered to be a physiologically relevant APJ ligand (49). The current use of Pyr(1)-apelin-13 is nevertheless in apparent contradiction with previous studies showing that its effects were more transient than those of apelin-36 (37), and that apelin-36 displaced better the binding of apelin than apelin-13 (47). An important role of the carboxyl-terminal phenylalanine in the action of apelin-
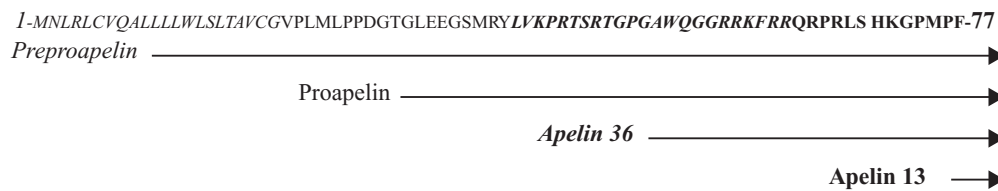
*1-MNLRLCVQALLLLWLSLTAVCG*VPLMLPPDGTGLEEGSMRY**LVKPRTSRTGPGAWQGGRRKFRR**QRPRLS HKGPMPF-**77**

Preproapelin ————————————————————————————————————→

Proapelin ————————————————————————————————→

Apelin 36 ————————————————————→

Apelin 13 ——→

Fig. 1: *Peptidic sequence of murine preproapelin.*
The preproprotein of 77 amino acids has been proposed to have a cleavage site which leads to proapelin (55 amino acids), then other peptides could generated, among them apelin-36, apelin-17 or apelin-13, all able to activate APJ receptor.

13 was demonstrated when analogs were generated and tested. Injections of apelin-13 (15 µg/kg) lowered mean arterial blood pressure of hypertensive rats by approximately 60 % while apelin-12 resulted in 15 % decrease only. Moreover, a mutation (F13A) in apelin-13 revealed a loss of function. Lastly, concomitant administration of apelin-13(F13A) blocked hypotensive effects of apelin-13 and revealed that apelin-13(F13A) behaved as an apelin-specific antagonist (55). Further mutations, substitutions or research of nonpeptidic stable analogues is a challenge for future insight on the apelin pharmacology.

Apelin bioavailability also deserves to be better defined, especially regarding the predominent form(s) circulating in plasma since numerous clinical studies have reported a very wide range of apelin plasma levels, not only under various physiopathological conditions, but also in healthy controls. This indicates that the immunoreactivity of apelin(s) varies from one study to another or that the proteolysis of secreted apelin(s) is a limiting factor, influenced by soluble or vessel-bound catabolizing enzyme(s). In this context, it is worth mentioning that alongside the similarities existing between angiotensin and apelin at the level of ligand, receptor sequence and tissue distribution, these two systems also share an enzyme involved in the degradation of their active peptides: the angiotensin-converting enzyme 2 (ACE2), a recently discovered homologue of the angiotensin-converting enzyme. ACE2 not only cleaves angiotensin I and II into the inactive Ang 1-9 and Ang 1-7, respectively, but is also involved in the deletion of the C-terminus of apelin-13. Therefore, since ACE is known as an essential regulator of the Renin-Angiotensin-Aldosterone system,

ACE2 has to be considered in the interplay between angiotensin and apelin, and to participate with ACE to the regulation of cardiovascular and renal functions (69). To pour more complexity, ACE2 is an integral membrane protein also hydrolyzing several other peptides, and described as a receptor for the severe acute respiratory syndrome coronavirus (96). ACE2 is neither inhibited by benzylsuccinate, a carboxypeptidase inhibitor, nor by other ACE inhibitors (32). Fortunately, specific ACE2 inhibitors are already under development owing to their potential beneficial effects in cardiovascular diseases, and attention must be paid to the impact of ACE2-related pharmacological agents on apelin/APJ system. Lastly, there are many other peptidases and enzymes potentially involved in apelin synthesis, maturation, secretion and degradation. Therefore, further examination of the molecular forms present in tissues and plasma could probably lead to reassess the apelin-like immunoreactivity quantifications published so far. In this regard, the observation of atrial and plasma levels of high molecular weight apelin, reported to be markedly higher than those of mature apelin-36 itself (24), is of interest.

Apelin/APJ system in the gastro-intestinal tract

Compelling evidence of the apelin/APJ system influence on digestive process has been reported, as a logical continuation of the pioneering purification of apelin from bovine stomach and its discovery as an APJ receptor ligand (91). Apelin is expressed in both endocrine and gastric exocrine cells, but not in the muscle layer of the stomach (86). An inhibitory feedback loop within this organ has been proposed: it is initiated by apelin release from

parietal cells, then apelin inhibits the gastrin-induced production of histamine by entero-chromaffin-like cells, resulting in a lower histaminergic stimulation of acid secretion by the parietal cell (52). In addition to this impairment of gastrin-induced acid secretion, apelin promotes cholecystokinin secretion, which in turn inhibits acid secretion (94). In the colon, apelin immunoreactivity was found in epithelial cells and its expression found to be increased during inflammatory reactions (31). Apelin has been reported to stimulate proliferation of gastric cells (94) or colonic epithelial cells (31). However, its proliferative activity is also effective in endothelial or myocardial cells, as reported below, and in osteoblasts (89, 98).

Apelin/APJ in the central nervous system

Many peptides secreted from the gastrointestinal tract also transmit to the brain signals for initiating or terminating food intake and energy expenditure. Accordingly, apelin has been found to play a role in neuroendocrine regulation of food intake. Intracerebroventricular injection of nanomolar doses of apelin resulted in a reduction in food intake in rats in two independent studies (68, 85). However, intravenous administration of apelin was devoid of such effect (85) and a lack of apelin action on food intake has been reported in diverse experimental conditions (88), or animal models, including an absence of change in food intake in mice genetically invalidated for apelin gene (51). Thus, an integrative role between gastro-intestinal functions and central control of feeding has not been clearly evidenced for apelin, as it is the case for ghrelin.

The term apelinergic can be used due to the presence of APJ receptor on neurones and the distribution of its ligand in brain (73), especially in hypothalamus, pituitary gland and caudate nucleus (1, 4, 71). In fact, apelin can be considered as a neuropeptide with potent diuretic actions since its intracerebroventricular administration limits vasopressin neuron activity, inhibits vasopressin release and reduces plasma levels of the antidiuretic arginine vasopressin (17, 70). Moreover, the changes in water intake repeatedly observed after central or peripheral apelin administration allow to assess that apelin increases drinking behaviour (53, 88).

The interaction between apelin/APJ system and the central control of drinking behaviour has been further evidenced by the observation of an increased expression of APJ mRNA in paraventricular nuclei of rats after prolonged water deprivation (64). On a physiological point of view, this APJ upregulation during water deprivation, corresponds to a reduced synthesis and release of apelin in brain, leading to reduced inhibitory effect of apelin on vasopressin release, thus preventing additional water loss at the kidney level (57). It can be concluded from these approaches that apelin counteracts the effects of vasopressin in the maintenance of body fluid homeostasis and has opposite regulations than the antidiuretic neuropeptide in response to changes in blood osmolarity, water intake or diuresis.

It is worth mentionning two other central actions of apelin: one is related to its neuroendocrine properties while the other allows to consider apelin as a neuroprotective agent. First, the capacity of apelin to promote adrenocorticotropin release has been documented by at least two independent studies (7, 72). Second, apelin has been reported to activate phosphorylation

of survival kinases Akt and Raf/ERK-1/2 and to protect hippocampal neurons from exitotoxic injury (66).

However, delineation of central actions of apelin remains incomplete and the actions of intracerebroventricular (ICV) administration of apelin(s) have been in several occasions, different from - or opposite to - those obtained with intravenous injection. For instance, while ICV injection of apelin-13 has been observed to increase mean arterial pressure and heart rate (44), its intravenous injection transiently decreases blood pressure in rats (9, 39, 53, 55, 62).

Apelin/APJ in the cardiovascular system

Apelin is strongly expressed in the heart together with APJ, and both are expressed in vessels, especially in endothelial cells. Several evidence for the role for apelin in pressure/volume home-ostasis and in the pathophysiology of cardiovascular diseases will be developed here, with an emphasis on the capacity of apelin to interfere with nitric oxide (NO) biovailability via APJ stimulation. Indeed, immediately after its discovery, the anti-hypertensive effect of apelin was found to be blocked by NO synthase inhibition (92). Then, numerous reports demonstrated that apelin/APJ activates nitric oxide synthases (NOS), exerts a hypotensive effect and plays a counterregulatory role against the pressor action of angiotensin II: in rat vein (29), rat aorta (42), mouse arteries (102), and human arteries (76). Noteworthy, the use of mice lacking the gene encoding APJ generated by a Japanese group (39) has definitively demonstrated that apelin lowers blood pressure via NO-dependent mechanism since

hypotensive response to apelin was abolished and the apelin-induced phosphorylation of endothelial NOS disappeared in APJ-deficient mice. Nonetheless, the baseline blood pressure of APJ-deficient mice was equivalent to that of wild-type, indicating that apelin/APJ system was solely modulating several of the multiple factors involved in blood pressure home-ostasis.

A slightly different conclusion was raised from the study of mice with targeted deletion of apelin, generated by an Austrian research team (51). Apelin-KO mice appeared to be healthy since they exhibited normal body weight, water and food intake, heart rate and morphology. However, with ageing, the mice develop cardiac dysfunction and pressure overload-induced heart failure. Therefore, apelin-KO mice showed that endogenous apelin is crucial to maintain cardiac contractility. This paradigm was in perfect agreement with the conclusions established from clinical and basic investigations on the cardiac effects of apelin. Apelin not only exhibits direct positive inotropic actions on the heart (2, 14, 23, 34, 87) but also exerts direct cardioprotective activity against ischemia/reperfusion injury (43, 80, 100). Regarding cell signalling, the inotropic action of apelin appears to be mediated by Gi/o protein, PKC and myosin light chain activation (34). In the spontaneously hypertensive rat, or after experimental myocardial injury, there is a decreased expression of apelin (Table I) and APJ (Table II) in heart together with a decreased apelin immuno-reactivity in plasma and ventricular and aortic tissues (40, 100, 101). The same pattern was observed in numerous clinical trial conducted on patients with cardiomyopathies (24), such as chronic heart failure (24, 28), coronary arterial disease

Table I. *Regulation of apelin (at the level of mRNA/protein) in diverse cell types and tissues.*

Agent or factor	Effect	Model	Reference
Insulin	+	3T3-F442A adipocytes	(Boucher *et al*., 2005)
"	+	3T3-L1 preadipocytes	(Wei *et al*., 2005)
"	+	3T3-L1	(Kralisch *et al*., 2007)
Growth hormone	+	3T3-L1	(Kralisch *et al*., 2007)
TNFα	+	3T3-F442A, mouse WAT	(Daviaud *et al*., 2006)
Trans-retinoic acid	+	rat heart and aorta	(Zhong *et al*., 2005)
Angiotensin blocker	+	failing heart	(Iwanaga *et al*., 2006)
Hypoxia	+	vascular cell line	(Cox *et al*., 2006)
"	+	rat cardiomyocytes	(Ronkainen *et al*., 2007)
"	+	cardiac endothelium	(Sheikh *et al*., 2007)
"	+	tumours	(Sorli *et al*., 2007)
"	+	3T3L1 adipocytes	(Glassford *et al*., 2007)
PGC-1α	+	adenovirus-infected WAT	(Valet P *et al*., unpublished)
Adipogenesis	+	3T3-F442A adipocytes	(Boucher *et al*., 2005)
Obesity and diabetes	+	WAT in humans or mice	(Boucher *et al*., 2005)
"	+	WAT in rats	(García-Díaz *et al*., 2007)
Chronic heart failure	+	human ventricle	(Foldes *et al*., 2003)
Inflammation	+	experimental colitis	(Han *et al*., 2007)
Vasculogenesis	+	mouse retinal vasculature	(Saint-Geniez *et al*., 2003)
Glucocorticoids	–	3T3-L1	(Wei *et al*., 2005)
Fasting	–	mouse WAT	(Boucher *et al*., 2005)
Hypertension	–	rat heart	(Zhong *et al*., 2005)
"	–	SHR heart	(Zhang *et al*., 2006)
Angiotensin	–	cardiac cells	(Iwanaga *et al*., 2006)
Vitamin C	–	cafeteria-fed rats	(García-Díaz *et al*., 2007)

"+" denotes a stimulatory effect of the mentioned factor on apelin gene expression; "–" denotes a down-regulation; TNFα, tumor necrosis factor α; WAT, white adipose tissue; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; SHR, spontaneously hypertensive rat.

(58), or atrial fibrillation (plasma apelin fell from 648 to 307 pg/ml) (21). The regulations of apelin and its receptor in cardiac dysfunction have been reviewed elsewhere (6) and will be not further detailed here. However, several controversies have emerged (8) (Tables I and II) and the changes of apelin/APJ in cardiovascular diseases, although being substantial, are not currently considered as good diagnostic parameters. For instance, recent Finnish and Dutch clinical trials have simultaneously shown that the alterations in plasma apelin levels found in patients with heart failure (idiopathic dilated cardiomyopathy) were minimal when compared to the changes in brain natriuretic peptide or tumour necrosis factor α (TNFα), and could no be used as an index to reflect the severity of heart failure (61, 93). Very recently, it was reported that the uremic status was a major determinant for plasma apelin, which decreased more in hemodialysis patients than in those with more severe heart involvement (12). Therefore plasma apelin can decrease regardless of the severity of heart failure and its immunoassay is not on the verge to become rapidly a useful biomarker for cardiopathies.

Table II. *Regulation of APJ receptor (at the level of mRNA/protein) in diverse cell types and tissues.*

Agent or factor	Effect	Model	Reference
Insulin	+	3T3-F442A adipocytes	(Boucher *et al.*, 2005)
NO/L-Arginine	+	rat caudate nucleus	(Bai *et al.*, 2007)
Vasculogenesis	+	mouse retinal vasculature	(Saint-Geniez *et al.*, 2003)
Heart failure	+	human heart	(Chen *et al.*, 2003)
Chronic heart failure	–	human atrium and ventricle	(Foldes *et al.*, 2003)
Diabetes	–	db/db mouse aorta	(Zhong *et al.*, 2007)
Hypertension	–	SHR heart and aorta	(Zhong *et al.*, 2005)
”	–	SHR heart and aorta	(Zhang *et al.*, 2006)

Same abbreviations as in Table I.

Nonetheless, apelin appears crucial for heart and vessel development and it has been repeatedly demonstrated that apelin acts as a mitogen in endothelial and cardiac cells, at least in animal models (38, 75, 77, 99). Apelin can also be considered as angiogenic agent (46, 84). More precisely, apelin is a potent activator of tumour neoangiogenesis by a paracrine effect on host vessels. In other words, cancer cells can secrete apelin for a paracrine action (95), without expressing themselves APJ receptors. This has a pathological relevance since the hypoxia-induced upregulation of apelin gene (Table I) and its overexpression appear to occur in one-third of human tumours (45, 83). Hypoxia has been reported to increase apelin/APJ in endothelial cells (Table I), therefore participating to angiogenesis (11, 13). Similarly, hypoxia increases apelin/APJ in cardiac cells (74, 78) and in fat cells (27), a cell-type which exhibits impressive features regarding apelin/APJ system, as detailed below.

Apelin/APJ in atherosclerosis

The pathogenesis of atherosclerosis is complex and closely associated with increased LDL-cholesterol, inflammation, endothelial dysfunction and subsequent events, such as expression of adhesion molecules and impairment in NO production. It has been shown that the circulating levels of several adipokines are altered in hypercholesterolemia (79). However, the influence of this lipid disturbance on serum apelin levels has been poorly studied. Recently, Tasci and associates (90) have examined circulating apelin concentrations in a specifically selected group of patients with elevated LDL-cholesterol, without additional disorders, such as obesity, systemic inflammation, diabetes or hypertension, but showing insulin resistance. In these patients, apelin levels were significantly lower than those found in controls. In spite of this, LDL-cholesterol did not negatively correlate with apelin. On the contrary, the HDL-cholesterol levels were positively correlated with plasma apelin. Moreover, a weak but significant negative correlation was established between insulin resistance and plasma apelin. Further studies are needed to analyse more deeply the association between plasma apelin and lipoprotein levels.

The role of apelin/APJ system has been also investigated in a study performed in APJ and apolipoprotein E double-knockout (APJ-/-ApoE-/-) mice, fed a high-

cholesterol diet (33). Atherosclerotic lesions were dramatically reduced in these mice when compared with APJ+/+ApoE-/- mice. Production of superoxide radicals and NADPH oxidase expression were decreased in vascular smooth muscle cells from mice APJ-/-ApoE-/- compared with mice APJ+/+ApoE-/-. Moreover, immunohistochemical detection of smooth muscle cells was also reduced in mice lacking APJ. Smooth muscle cells are known as a major source of reactive oxygen species which are involved in the initiation and progression of atherosclerosis through their ability to reduce NO biovailablity in the vasculature. Therefore, the reduction in smooth muscle cells observed in mice APJ-/-ApoE-/- probably impairs reactive oxygen species formation. Hashimoto and coworkers concluded that the apelin/APJ system is a mediator of oxidative stress in vascular tissue and that APJ deficiency is preventive against oxidative stress-linked atherosclerosis. They proposed a possible therapeutic use of apelin/APJ system inhibition for patients with atherosclerosis.

Apelin/APJ in adipose tissue and obesity-associated disorders

The presence of apelin in adipose tissue was originally described by the group of Tatemoto (92). Later, Valet and coworkers (3) demonstrated a direct production of apelin by isolated murine and human adipocytes. However, in different human tissues where adipocytes are present, immunocytochemical approaches showed that apelin was much more abundant in endothelial than in fat cells (48). Nowadays, apelin is considered as an adipokine, essentially as the result of its increased expression during adipocyte differentiation (3, 97) and its release by differentiat-

ed adipose cells into the medium culture. Moreover, apelin mRNA levels of cultured 3T3F442A adipocytes are increased by insulin (3). Another significant induction of apelin mRNA synthesis and protein secretion was evidenced in response to growth hormone (50) (Table I). On the contrary, glucorticoides decrease the apelin mRNA levels in 3T3-L1 adipocytes (97) while they stimulate angiotensin II production. Thus, the resulting reduced counter-regulatory activity of apelin against the pressor action of angiotensin II might partly be involved in the development of obesity-related hypertension (97). However, the apelin repression by dexamethasone is difficult to understand since: 1) dexamethasone is present in the differentiating milieu of 3T3L1 pre-adipocytes, and 2) it has been reported, in the rat genome, that DNA-motifs capable of binding glucocorticoid receptor are present in the APJ promoter and that there is a constitutive transcriptional regulation of this promoter by oestrogen and glucocorticoid receptors (65).

The regulation of apelin production in adipose tissue by insulin has been also observed *in vivo*. Indeed, apelin exhibit the same variations than insulin in mice subjected to fasting - refeeding experiments and in different animal models with significant increased (high-fat fed mice) or decreased (streptozotocin-treated mice) plasma insulin levels (3). TNFα has also been reported to increase *in vivo* apelin expression in adipose tissue and to elevate apelin plasma levels in mice (15). The tight correlation between apelin and TNFα expression in adipose tissue of lean and obese humans, together with the direct positive effect of TNFα on apelin expression and secretion in differentiated 3T3F442A adipocytes let suppose that TNFα may participate in the regulation of

circulating apelin (15). Diverse studies conducted on the apelin/APJ regulation in preadipocyte cultures have confirmed the insulin positive effect on apelin expression in 3T3L1 adipocytes (50, 97), but not that of TNFα (50), originally found to depend on PI3-kinase, c-Jun NH2-terminal kinase (JNK), and MAPK activation (15). Additional regulations will certainly be reported in a next future, at least owing to the presence of DNA motifs for Sp1 and CCAAT enhancer binding protein (C/EBP)α transcription factors that have been characterized in the apelin promoter and which let suppose a positive regulation by the cAMP-dependent signal cascade (65). In this view, the increase of apelin mRNA in adipose tissue of cafeteria fed rats (Table I) was partially reversed by dietary vitamin C supplementation (25), suggesting a putative sensitivity to oxidative stress that deserves to be further investigated.

In plasma from obese humans, there is an increase of both insulin and apelin (3). The increase of plasma apelin in human obesity has been confirmed (736 *vs.*174 pg/ml) and shown to be concomittent of an increase in leptin, orexin-A (35) or visfatin (56). However circulating apelin has not been correlated with body mass index (BMI) in all the studies published so far. It was the case for patients with chronic heart failure (10), indicating that obesity is not the sole factor influencing apelin circulating levels. In this view, not all the animal models of obesity exhibited increased apelin expression, which was demonstrated to be better linked with hyperinsulinemia (3).

Dysregulations of apelin/APJ system have also been documented in diabetic states (Tables I and II). Plasma apelin levels were significantly increased in type 2 diabetic subjects compared with glucose tolerant controls. Again, fasting plasma apelin levels correlated positively with fasting plasma insulin and BMI, but also with biomarkers of glucose intolerance (56) and the apelin levels of 2-h post-glucose load were significantly higher than the basal levels in every studied group. In diabetic rodents, there was also an increase of the ligand apelin in plasma and a lower expression of APJ receptor (mRNA and protein) in aortas, (102), suggesting some desensitization mechanism as already reported for *in vitro* systems (60). Very interestingly, twenty minutes treatment with 1-100 nM apelin was sufficient to reduce the exagerated contractile response of diabetic aortic rings to angiotensin II and to reverse the altered vasodilatory response to acetylcholine. Apelin also restored high levels of phosphorylation of Akt and eNOS, in a NO-dependent manner (103). A better definition of the link between apelin and pathogenesis of insulin resistance is therefore deemed. The recently evidenced correlation between apelin expression and malonyldihaldehyde levels in liver (a marker of lipid peroxidation), and leptin or IL-1 receptor in fat pads suggests a possible role for apelin in excessive adiposity, insulin-resistance, liver oxidative stress and inflammation (26). In this regard, the scarce studies on the metabolic effects of apelin bring new insights that will surely be expanded in the next years.

Repeated i.p. injection of apelin (0.1 μmol/kg /d for 14 days) decreased the weight of adipose tissue and lowered insulin and triglycerides in mice fed a normal or a hight-fat diet without influencing food intake. Apelin increased adiponectin expression and lowered that of leptin. It also increased the expression of uncoupling proteins UCP1 and 3 and resulted in an increase of energy expenditure and a

decrease of respiratory quotient, traducing an increased fat oxidation (36). Although these observations are in agreement with a previous study reporting that ICV administration of apelin (1-10 µg) increase rat core temperature in a manner which depends on corticotropin-releasing hormone (41), the signalling pathways of this energy-dissipating effects of apelin have to be deciphered. Meanwhile, these anti-obesity effects of apelin could lead to be considered it as a beneficial peptide, as recently proposed (5) and may explain its increase in obesity states as an adaptive response while under the same physiopathological conditions, regarding insulin sensitivity, the deleterious TNFα is increased and the beneficial adiponectin decreased. Therefore, it remains to demonstrate whether apelin is deleterious or not regarding the insulin secretion and effects, like other adipokines such as TNFα, IL-6, leptin, visfatin, adipsin or resistin. There is no convincing argument to currently state on this point. In fact, there is no apelin expression in the pancreas (94), but APJ receptor is present on pancreatic islets and pharmacological administration of apelin-36 partially inhibits *in vivo* the insulin secretion stimulated by intravenous glucose in control or obese and insulin-resistant high-fat fed mice. Apelin also inhibits insulin secretion *in vitro*, but only at 1 µM (82). In spite of this putative negative control of insulin release, apelin exerts direct metabolic effects in human and mouse adipocytes via APJ receptor activation, which appears to reinforce insulin action (19). Since there are strong evidences of apelin-induced phosphorylation of Akt, at least in heart (80, 81) and vessels (60, 103), one could expect that this signaling molecule is also an apelin target in adipocyte.

Conclusion

The diverse cardiovascular functions of the apelin/APJ system described so far can be briefly summarized as an endothelium-dependent vasodilatation, a vasoconstriction by direct action on the smooth muscle and a positive inotropism. Owing to all its effects on fluid homeostasis and to its cardiovascular actions, apelin appears to have a physiological role in counter-regulation of the angiotensin and vasopressin systems. The vasodilator action of the apelin/APJ system could therefore counterbalance the vasoconstrictor actions of the angiotensinII/AT1 receptor system and could play an important role in the vascular pathophysiology and in diabetes-associated cardiovascular diseases. Nevertheless, growing evidence of apelin interactions with energy balance and metabolic control will extend the properties of this novel adipokine to interplay with other defects beyond the cardiovascular system but dealing with insulin resistance and glucose or lipid utilisation, especially in obesity or dyslipidemias.

C. CARPÉNÉ, C. DRAY, C. ATTANÉ, P. VALET, M.P. PORTILLO, I. CHURRUCA, F.I. MILAGRO y I. CASTAN-LAURELL. *Implicación del sistema apelina/APJ en procesos fisiopatológicos* (minirrevisión)*.* J. Physiol. Biochem., 63 (4), 359-374, 2007.

La apelina es un péptido que actúa como ligando del receptor acoplado a proteína G APJ. La apelina puede ejercer su función bajo la forma de diferentes péptidos activos, de 13,

17 o 36 aminoácidos, que se originan a partir de un precursor común de 77 aminoácidos. Tanto la apelina como su receptor APJ se expresan en numerosos tejidos, tanto en roedores como en el humano, y ejercen sus funciones en el sistema nervioso central y en los tejidos periféricos. Así, la apelina está implicada en la regulación de las funciones cardiovasculares, la homeostasis hídrica, la angiogénesis y la proliferación celular. Más recientemente, se ha descrito que la apelina es también secretada como una adipoquina por los adipocitos, y aparece sobreexpresada en situaciones de obesidad. Actuando como hormonas circulantes o como factores paracrinos, las adipoquinas están involucradas en la regulación de muy diversas funciones fisiológicas (adipogénesis, almacenamiento y gasto de energía, apetito) o en la aparición de los trastornos asociados a la obesidad (diabetes tipo 2, disfunciones cardiovasculares, hipertensión). En este contexto, se ha observado que la expresión génica de apelina en el tejido adiposo esta aumentada por insulina y TNFα. Esta revisión analiza el papel fisiológico de la apelina, prestando especial atención a su importancia en la regulación del balance energético y en los trastornos asociados a obesidad.

Palabras clave: Adipocitos, Adipoquinas, Obesidad, Diabetes, Insulina.

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