J. Biochem. 2012;152(2):125–131 doi:10.1093/jb/mvs071

JB THE JOURNAL OF BIOCHEMISTRY

JB Review

Biology of the apelin-APJ axis in vascular formation

Received May 14, 2012; accepted June 12, 2012; published online June 28, 2012

Hiroyasu Kidoya[1,*] and Nobuyuki Takakura[1,2,†]

[1]Department of Signal Transduction, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan; and [2]Japan Science and Technology Agency, Core Research for Evolution Science and Technology, K's Gobancho, 7 Gobancho, Chiyoda-ku, Tokyo 102-0076, Japan

*Hiroyasu Kidoya, Department of Signal Transduction, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan. Tel: +81-66-879-8312, Fax: +81-66-879-8314, email: kidoya@biken.osaka-u.ac.jp

[†]Present address: Nobuyuki Takakura, Department of Signal Transduction, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan.

Apelin is a bioactive peptide with diverse physiological actions on many tissues mediated by its interaction with its specific receptor APJ. Since the identification of apelin and APJ in 1998, pleiotropic roles of the apelin/APJ system have been elucidated in different tissues and organs, including modulation of the cardiovascular system, fluid homeostasis, metabolic pathway and vascular formation. In blood vessels, apelin and APJ expression are spatiotemporally regulated in endothelial cells (ECs) during angiogenesis. *In vitro* analysis revealed that the apelin/APJ system regulates angiogenesis by the induction of proliferation, migration and cord formation of cultured ECs. Moreover, apelin seems to stabilize cell–cell junctions of ECs. In addition, genetically engineered mouse models suggest that apelin/APJ regulates vascular stabilization and maturation in physiological and pathological angiogenesis. In this review, we summarize the current understanding of the apelin/APJ system for vascular formation and maturation.

Keywords: apelin and APJ/development/regenerative medicine/tumour angiogenesis/vascular formation.

Abbreviations: bFGF, basic fibroblast growth factor; CAM, chorioallantoic membrane; DCs, dendritic cells; ECs, endothelial cells; ERK, extracellular-regulated kinases; HIF, hypoxia inducible factor; iNKT, invariant natural killer T; ISVs, intersomitic vessels; PI3K, phosphatidylinositol-3 kinase; VEGF, vascular endothelial growth factor.

Characteristics of Apelin and APJ

Apelin was initially identified as an endogenous ligand for the orphan G protein-coupled receptor with seven transmembrane domains, APJ, isolated from bovine stomach extracts in 1998 (*1*). Apelin is secreted as a 77 amino acid pre-proprotein, an immature peptide, which is cleaved by protease to form C-terminal products, including apelin-13, apelin-17 and apelin-36 (*2*). These isoforms have distinct activities, with the shorter isoform seeming to be the more potent activator for APJ. In mammals, the sequence of preproapelin is strongly conserved in different species, and it has complete identity for the last 23 residues of the C-terminal. Apelin (65–77) activates extracellular signal-regulated kinases through a pertussis toxin sensitive G protein (*3*). Apelin-13 and apelin-36 have different receptor binding affinity and cause different intracellular trafficking of APJ (*4*). The gene encoding the APJ receptor was identified by homology cloning in 1993 (*5*). APJ has high-sequence homology with the angiotensin II type I receptor, but it does not bind angiotensin II. Apelin is believed to be the only endogenous ligand for APJ.

Apelin and APJ mediate a wide range of physiological actions, including regulation of cardiovascular function, fluid homeostasis, adipo-insular axis and angiogenesis. Studies on APJ function have been focused on the cardiovascular system, because of its similarity to the angiotensin II receptor. It seems that intravenous injection of apelin induces a reduction in blood pressure (*6*). The hypotensive effect of apelin is a consequence of intracellular activation of nitric oxide synthase (*7*). Apelin also causes vasoconstriction because of contraction of the vascular smooth muscle cells (*8*). Plasma apelin levels and APJ expression are clearly modulated in patients with heart failure (*9*, *10*). It has been reported that apelin has positive inotropic effects in *in vitro* and *in vivo* studies (*11–14*). Apelin has been shown to be expressed and released from adipocytes by fasting and refeeding factors, such as insulin, and can act as an adipokine (*15*). For these reasons, apelin is now attracting attention in the context of metabolic disease. In patients with type 2 diabetes, plasma apelin concentrations are increased (*16*, *17*). It has also been suggested that apelin may have other effects, such as on fluid homeostasis (*18*), gastrin stimulation (*19*) and immune responses (*20*).

Distribution and Regulation of Expression of Apelin and APJ

Tissue and cellular distribution

In mammals, APJ expression is widely distributed in various peripheral tissues of adult and embryo. Highest levels of APJ are found in the lung and heart, and significant but lower levels of APJ mRNA are present in skeletal muscle, pituitary gland, kidney and ovary (*21*) (*22*). In these tissues, APJ expression was localized in the vascular and endocardial

endothelial cells (ECs) and smooth muscle cells (*23*). APJ expression is also observed in the brain, including the cerebral cortex, hypothalamus, hippocampus and pituitary gland (*6*, *22*). Apelin expression is also detected in a range of peripheral tissues, including heart, liver, kidney, adipose tissues and brain, with highest levels found in the lung and the mammary gland (*2*, *6*, *20*, *24*). Localization of apelin expression in tissue was observed in vascular ECs, adipose tissue and epithelial cells (*7*).

An important physiological role for apelin and APJ is suggested by the observation of widespread distribution of receptor and ligand expression in tissues, as described previously. Apelin and APJ were found to be abundantly expressed in various peripheral tissues, and localization was restricted to blood vessels (*2*, *7*, *8*, *20*, *22*–*26*), suggesting a role for apelin/APJ in angiogenesis and vascular formation.

Regulation of transcription

Many transcription factors that may regulate apelin gene expression have been reported. Rat and human apelin core promoter sequences contain putative binding sites for upstream stimulatory factor 1/2, and overexpression of USF upregulates apelin transcription (*27*). Multiple signal transducer and activator of transcription binding sites have been identified in the rat apelin promoter, and apelin expression is elicited by stimulation using inflammatory cytokines associated with binding of phospho-Stat3 (*28*). In white adipocytes, apelin is upregulated by the transcriptional co-activator peroxisome proliferator-activated receptor γ co-activator 1 α (*29*). Under hypoxic conditions, hypoxia inducible factor-1α (HIF1-α) binds to the hypoxia-responsive element (-813/-826) located within the first intron of the human apelin gene and increases apelin expression in vascular cells (*30*).

The molecular mechanism of APJ gene transcriptional regulation has not been well characterized so far. Analysis of the 5′-flanking region of a rat APJ genomic clone identified sites with the strongest promoter activity in a region between (-966/-165) where the Sp1 motif suggested to play a major role (*31*). Other investigations using gene-based single-nucleotide polymorphism analysis have also shown that both APJ and apelin genes are probably regulated by Sp1 (*32*).

Cell effects and intracellular responses

Apelin has been shown to induce the proliferation and migration of APJ-expressing ECs (*33*). The major signalling pathways of apelin are mediated initially by Gi-protein coupled to the APJ receptor and protein kinase C (Fig. 1). It has been reported that apelin causes concentration-dependent inhibition of forskolin-stimulated production of cAMP and increases the phosphorylation of extracellular-regulated kinases (ERK) or protein kinase B in umbilical ECs (*3*, *20*). Apelin/APJ-induced ERK activation is mediated by pertussis toxin sensitive G protein (*3*). On binding of apelin to APJ, the phosphatidylinositol-3 kinase (PI3K) pathway and the ERK pathway lead to the phosphorylation of p70S6K. It has been reported that the PI3K-protein kinase B pathway contributes to EC migration (*34*) and that the ERK/PI3K-p70S6K pathway regulates EC proliferation (*35*). Acceleration of cell motility by apelin was also reported using Chinese hamster ovary cells expressing APJ (*21*). Recently, it has been shown that APJ forms a heterodimer with κ-opioid receptors and leads to phosphorylation of ERK, resulting in increased cell proliferation (*36*). In addition, apelin inhibits the mouse pulmonary arterial EC apoptosis, observed in pulmonary arterial hypertension (*37*). It has been reported that apelin-induced anti-apoptosis is mediated by the induction of Bcl2 protein expression and activation of the PI3K/protein kinase B signalling pathway (*38*). Apelin-36 and apelin-13 can activate the same set of intracellular effectors, but they display some differences in their Gi-protein coupling and differ greatly in their desensitization pattern (*39*).

Role of Apelin in Blood Vessel Formation

Developmental stage

The establishment of vascular network is absolutely necessary for the growth and maintenance of tissues/organs. New blood vessel formation in vertebrates is known to occur by two different processes, vasculogenesis and angiogenesis (*40*). Vasculogenesis is the process of formation of *de novo* primitive vascular networks directly from angioblastic precursor cells. In contrast, angiogenesis is a process of formation of new vascular segments by sprouting from the pre-existing vessels. Vasculogenesis is normally observed in early embryogenesis, whereas angiogenesis occurs during development and in post-natal life.

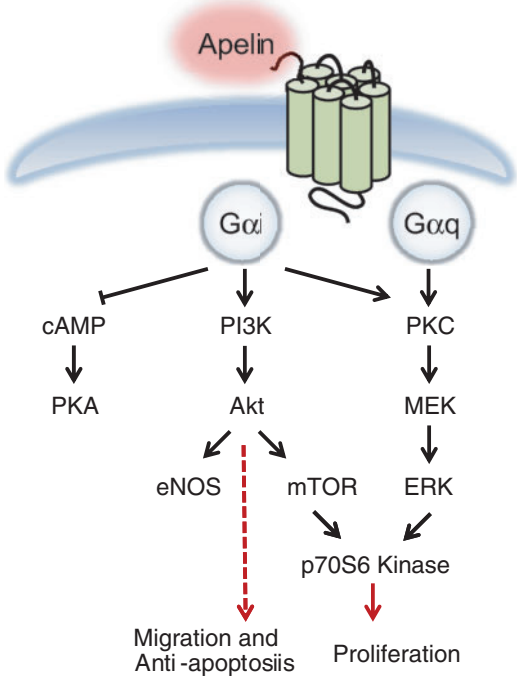

Fig. 1 Schematic of intracellular signal transduction pathways and cellular effects in the apelin/APJ system.

Apelin seems to have important roles in angiogenesis during embryogenesis. In *Xenopus laevis* embryos, expression of homologues of apelin and APJ are observed in developing vascular structures of the inter-somitic vein (*41*). Implantation of beads carrying apelin peptide stimulated prominent outgrowth of ECs and, conversely, morpholino-based translational inhibition of apelin and APJ suppressed inter-segmental angiogenesis (*42*). During segmentation of zebrafish embryos, expression of an ortholog of APJ is observed in epithelial structures, such as venous vasculature (*43*). Consistent with these results, APJ is expressed on ECs in newly formed blood vessels in mouse embryos. At E8.5, APJ expression was observed in ECs that had sprouted from the dorsal aorta, but not in those that were forming the dorsal aorta by the process of vasculogenesis. At E9.5, APJ expression was observed in the migrating end region of inter-somitic vessels sprouting from the dorsal aorta, gradually disappearing as the blood vessels matured. Furthermore, apelin protein was also detected in the somite region at E9.5 (*44*). These expression profiles suggest that the apelin/APJ system plays a spatiotemporal role in blood vessel formation by its transient expression on blood vessels ECs during angiogenesis. The role of apelin in vascular formation has been studied in our group using mouse embryos. We previously showed that the apelin/APJ system induces cell–cell assembly and the proliferation of vascular ECs. When the apelin gene was knocked out, the caliber of inter-somatic vessels in the embryo was narrower. These results indicated that the apelin/APJ system is involved in maturation of blood vessels by caliber size modification during angiogenesis (*44*) (Fig. 2).

In the neonatal mouse retina, APJ expression is upregulated in ECs of the radial vessels sprouting from the optic nerve head region, but attenuated after vessel stabilization. Expression of the apelin gene was also observed in the sprouts of extending vessels at the leading edge (*45*, *46*). During sprouting angiogenesis, growing vascular capillaries are spearheaded by specialized ECs, termed outgrowing tip cells, which act as a guide for the direction of migration of newly developed blood vessels. Behind the tip cells, proliferating ECs, termed stalk cells, induce elongation of the blood vessels, and tip and stalk cells are dynamically challenged and replaced alternately during sprouting angiogenesis (*47*, *48*). Recently, apelin was identified as one of the genes with high expression in tip cells and has been suggested to modulate proliferation of stalk cells expressing the APJ receptor (*49*) (Fig. 2); therefore, apelin-APJ signalling may have a role in tip-cells and stalk-cells characterization. Using apelin-deficient mice, it was suggested that the apelin/APJ system participates in retinal vascularization and ocular development by modulating the angiogenic response to vascular endothelial growth factor (VEGF) and/or basic fibroblast growth factor (*50*). Moreover, we recently proposed that apelin/APJ activation in ECs is a trigger for finalization of blood vessel formation, indirectly mediated by the induction of astrocyte maturation (*51*). During development of the retinal vasculature, APJ mRNA expression is specifically restricted to the venules and the associated capillaries (*46*), indicating a possible function of apelin signalling for venous vascular formation.

Tumour angiogenesis

Apelin has been reported to be broadly expressed in ECs of tumours of different origins. By comparing gene expression profiles in tumours versus normal endothelium, apelin has been identified as a tumour endothelial-specific gene (*52*). In human breast carcinoma, apelin expression was detected in the vascular ECs by immunohistochemical analysis (*53*). As described previously, apelin expression is physiologically modulated by tissue hypoxia and regulated by HIF-1α (*30*). The hypoxic tumour microenvironment may thus induce HIF1α-dependent apelin expression in tumour ECs.

It has been reported that the expression level of the APJ receptor is also increased in tumour endothelium. In glioblastoma, both APJ and apelin transcripts are highly upregulated within the microvasculature compared with blood vessels in normal brain tissue (*41*). Consistent with this result, we detected high-level expression of apelin and APJ mRNA in ECs from tumours generated by the inoculation of Lewis lung carcinoma and colon 26 adenocarcinoma cells into mice. In addition, immunohistochemical analysis of colon 26 tumour revealed that ~13% and 27% of the vessels were APJ-positive and apelin-positive, respectively, and most ECs co-expressed apelin and APJ (*54*). Of course, the ratios of apelin or APJ positivity in ECs may be different depending on the tumour size and tumour growth course. Observation of high APJ expression in angiogenic blood vessels in tumours was similar to that during normal development. Co-expression of ligand and receptor in ECs of the newly formed tumour blood vessels suggests the possibility that APJ in ECs is stimulated by autocrine and paracrine loops.

Several attempts have been made to determine the role of the apelin/APJ system in tumour angiogenesis, using apelin-overexpressing tumour cells. In a human non-small cell lung cancer xenograft model, apelin gene transfer significantly stimulated tumour growth and increased microvessel densities and diameters *in vivo* (*55*). Another group reported that mammary tumour cells stably overexpressing apelin cDNA also stimulated tumour growth *in vivo*, probably associated with enhanced angiogenesis in the tumours (*56*). However, in our own research, we found that overexpression of apelin in colon 26 tumours significantly suppressed tumour growth by inducing tumour vascular maturation (*54*). This difference might depend on the particular apelin/APJ signalling pathway, probably involved in individual tumours and different tumour models, i.e., syngeneic mouse model or allogeneic mouse model using immunodeficient mice. Recent reports indicate that anti-angiogenic cancer drugs, such as VEGF signalling inhibitors, cause 'normalization' of aberrant tumour vasculature and, thus, induce the formation of functional mature vasculature (*57*, *58*). One of the major therapeutic benefits of tumour

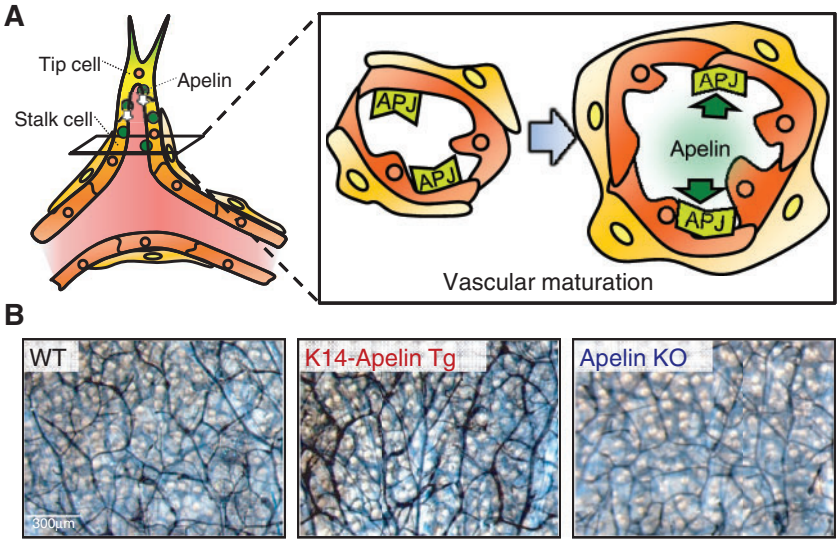

Fig. 2 (A) **Schematic representation showing how apelin/APJ signalling induces vascular maturation**. High levels of expression of apelin protein in tip cells probably activates APJ signalling in the neighbouring stalk cells. Subsequently, these cells will adopt proliferating and aggregating behaviour to form enlarged mature vessels. (B) Image of ear-skin blood vessels in apelin-transgenic mice under regulation of the K14 promoter, and apelin-deficient mice. Compared with wild-type mice, more enlarged mature vessels are observed in apelin-transgenic mice and more narrow immature vessels in apelin-deficient mice.

vascular normalization is enhancement of the effects of conventional anti-tumour therapies, such as chemotherapy and radiation therapy (*58*). In our study, apelin-mediated vascular maturation enhanced the effect of immunotherapy with dendritic cells. These therapeutic effects resulted from induction of tumour cell apoptosis by effective infiltration of activated invariant natural killer T cells (*54*).

Thus, regulation of APJ activity might lead to the development of new vascular normalization drugs, which should be more efficacious than anti-angiogenic agents because of their unique ability to induce vascular enlargement.

Vascular regeneration

Several lines of evidence indicate that apelin can significantly enhance migration, proliferation and capillary tube-like formation of cultured ECs (*30*, *33*, *35*, *44*, *59*). In *in vivo* Matrigel plug assays for angiogenesis, addition of apelin resulted in the formation of capillary-like structures (*33*). Moreover, apelin peptide stimulates angiogenesis in the chicken chorioallantoic membrane assay (*42*). Downregulation of apelin expression by the local delivery of apelin-targeting small interfering RNA into grafted adipose tissue leads to dramatic inhibition of angiogenesis (*59*). In the rat portal hypertension model, treatment with the APJ-specific antagonist F13A markedly reduced splanchnic neovascularization and formation of portosystemic collateral vessels (*60*). According to these reports, it is suggested that apelin can be used for therapeutic angiogenesis.

Analysis of transgenic mice expressing apelin in the epidermis under the transcriptional control of the K14 promoter revealed that apelin can induce the formation of enlarged capillaries, but not arteriola and venula in the dermis. Moreover, overexpression of apelin inhibited vascular leakage caused by VEGF or histamine. These results indicate that apelin can induce non-leaky larger blood vessels *in vivo* (*61*).

In cardiac failure, endothelial apelin expression correlates with other hypoxia-responsive genes, and apelin and APJ are upregulated in ECs of various tissues after systemic hypoxia (10% FIO_2) *in vivo*. It has been suggested that apelin expression in the endothelium of the heart is induced through the endothelial-specific HIF-2α pathway (*62*). Another group also reported that apelin expression was significantly increased in lungs of mice under hypoxic conditions (10% O_2) in an HIF-1α-dependent manner. Small interfering RNA-mediated apelin or APJ knockdown inhibited hypoxia-induced vessel regeneration in the caudal fin regeneration model in zebrafish (*30*). In accordance with these observations, we found that endogenous apelin is required for recovery of hind limb perfusion after induction of ischaemia (*58*). Using mouse hind limb ischaemia models produced by occlusion of the femoral artery, expression of APJ and apelin mRNA was significantly increased in ECs from the ischaemic muscle. In apelin-deficient mice, severe necrosis of the toes and delayed recovery of blood flow were observed when inducing ischaemia. These results suggested the involvement of the apelin/APJ system in collateral vessel formation during the process of recovery from ischaemia states. Thus, we found that APJ expression is induced after ischaemia treatment, and endogenous apelin is required for functional recovery.

Apelin gene transfer promotes formation of enlarged and non-leaky blood vessels in the hind limb ischaemia model. Simultaneous overexpression of apelin and VEGF by plasmid administration was superior to VEGF alone at restoring tissue integrity after ischaemia damage by improved generation of enlarged blood vessels in the ischaemic muscle (*61*). Moreover, apelin

induced vascular stabilization by inhibiting VEGF-mediated internalization of vascular endothelial cadherin resulted in suppression of hind limb oedema (*61*). In addition to its role in blood vessel formation, APJ is expressed in human lymphatic ECs, and apelin induces their migration and cord formation. Transgenic mice harbouring apelin in the dermis showed reduced development of oedema by promoting stabilization of lymphatic vessels (*63*). Taken together, these findings suggest that the apelin/APJ system represents a new therapeutic target for ischaemic disease.

Conclusions

Recent studies have revealed multiple roles of the apelin/APJ system in vascular formation in physiological and pathological situations, including during development, tissue regeneration and tumourigenesis. Apelin has a unique function as a regulator of vascular maturation and stabilization by increasing the caliber of newly formed blood vessels and strengthening barrier function between ECs. Moreover, expression of apelin and APJ genes is temporally upregulated during blood vessel development and downregulated in stabilized vasculature. Detailed understanding of the function of the apelin/APJ system and expression analysis in blood vessels will provide insights for improving the use of agonists and antagonists that modulate apelin signalling in different vascular diseases.

Funding

This work was supported by Grant-in Aid for Scientific Research on Innovative Areas (No. 23122511) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) and Grant-in Aid for Young Scientist B (No. 23701054) from the Japan Society for the Promotion of Science (JSPS).

Conflict of interest

None declared.

References

1. Tatemoto, K., Hosoya, M., Habata, Y., Fujii, R., Kakegawa, T., Zou, M.X., Kawamata, Y., Fukusumi, S., Hinuma, S., Kitada, C., Kurokawa, T., Onda, H., and Fujino, M. (1998) Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem. Biophys. Res. Commun.* **251**, 471–476
2. Kawamata, Y., Habata, Y., Fukusumi, S., Hosoya, M., Fujii, R., Hinuma, S., Nishizawa, N., Kitada, C., Onda, H., Nishimura, O., and Fujino, M. (2001) Molecular properties of apelin: tissue distribution and receptor binding. *Biochim. Biophys. Acta.* **1538**, 162–171
3. Masri, B., Lahlou, H., Mazarguil, H., Knibiehler, B., and Audigier, Y. (2002) Apelin (65-77) activates extracellular signal-regulated kinases via a PTX-sensitive G protein. *Biochem. Biophys. Res. Commun.* **290**, 539–545
4. Lee, D.K., Ferguson, S.S., George, S.R., and O'Dowd, B.F. (2010) The fate of the internalized apelin receptor is determined by different isoforms of apelin mediating differential interaction with beta-arrestin. *Biochem. Biophys. Res. Commun.* **395**, 185–189
5. O'Dowd, B.F., Heiber, M., Chan, A., Heng, H.H., Tsui, L.C., Kennedy, J.L., Shi, X., Petronis, A., George, S.R., and Nguyen, T. (1993) A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. *Gene* **136**, 355–360
6. Lee, D.K., Cheng, R., Nguyen, T., Fan, T., Kariyawasam, A.P., Liu, Y., Osmond, D.H., George, S.R., and O'Dowd, B.F. (2000) Characterization of apelin, the ligand for the APJ receptor. *J. Neurochem.* **74**, 34–41
7. Tatemoto, K., Takayama, K., Zou, M.X., Kumaki, I., Zhang, W., Kumano, K., and Fujimiya, M. (2001) The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. *Regul. Pept.* **99**, 87–92
8. Katugampola, S.D., Maguire, J.J., Matthewson, S.R., and Davenport, A.P. (2001) [(125)I]-(Pyr(1))Apelin-13 is a novel radioligand for localizing the APJ orphan receptor in human and rat tissues with evidence for a vasoconstrictor role in man. *Br. J. Pharmacol.* **132**, 1255–1260
9. Foldes, G., Horkay, F., Szokodi, I., Vuolteenaho, O., Ilves, M., Lindstedt, K.A., Mäyränpää, M., Sármán, B., Seres, L., Skoumal, R., Lakó-Futó, Z., deChâtel, R., Ruskoaho, H., and Tóth, M. (2003) Circulating and cardiac levels of apelin, the novel ligand of the orphan receptor APJ, in patients with heart failure. *Biochem. Biophys. Res. Commun.* **308**, 480–485
10. Ronkainen, V.P., Ronkainen, J.J., Hanninen, S.L., Leskinen, H., Ruas, J.L., Pereira, T., Poellinger, L., Vuolteenaho, O., and Tavi, P. (2007) Hypoxia inducible factor regulates the cardiac expression and secretion of apelin. *FASED J.* **21**, 1821–1830
11. Berry, M.F., Pirolli, T.J., Jayasankar, V., Burdick, J., Morine, K.J., Gardner, T.J., and Woo, Y.J. (2004) Apelin has in vivo inotropic effects on normal and failing hearts. *Circulation* **110 (11 Suppl. 1)**, II187–II193
12. Jia, Y.X., Pan, C.S., Zhang, J., Geng, B., Zhao, J., Gerns, H., Yang, J., Chang, J.K., Tang, C.S., and Qi, Y.F. (2006) Apelin protects myocardial injury induced by isoproterenol in rats. *Regul. Pept.* **133**, 147–154
13. Atluri, P., Morine, K.J., Liao, G.P., Panlilio, C.M., Berry, M.F., Hsu, V.M., Hiesinger, W., Cohen, J.E., and Joseph Woo, Y. (2007) Ischemic heart failure enhances endogenous myocardial apelin and APJ receptor expression. *Cell. Mol. Biol. Lett.* **12**, 127–138
14. Maguire, J.J., Kleinz, M.J., Pitkin, S.L., and Davenport, A.P. (2009) [Pyr1]apelin-13 identified as the predominant apelin isoform in the human heart: vasoactive mechanisms and inotropic action in disease. *Hypertension* **54**, 598–604
15. Boucher, J., Masri, B., Daviaud, D., Gesta, S., Guigne, C., Mazzucotelli, A., Castan-Laurell, I., Tack, I., Knibiehler, B., Carpéné, C., Audiqier, Y., Saulnier-Blanche, J.S., and Valet, P. (2005) Apelin, a newly identified adipokine up-regulated by insulin and obesity. *Endocrinology* **146**, 1764–1771
16. Heinonen, M.V., Purhonen, A.K., Miettinen, P., Paakkonen, M., Pirinen, E., Alhava, E., Akerman, K., and Herzig, K.H. (2005) Apelin, orexin-A and leptin plasma levels in morbid obesity and effect of gastric banding. *Regul. Pept.* **130**, 7–13
17. Li, L., Yang, G., Li, Q., Tang, Y., Yang, M., Yang, H., and Li, K. (2006) Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. *Exp. Clin. Endocrinol. Diabetes* **114**, 544–548

18. Roberts, E.M., Newson, M.J., Pope, G.R., Landgraf, R., Lolait, S.J., and O'Carroll, A.M. (2009) Abnormal fluid homeostasis in apelin receptor knockout mice. *J. Endocrinol.* **202**, 453–462
19. Lambrecht, N.W., Yakubov, I., Zer, C., and Sachs, G. (2006) Transcriptomes of purified gastric ECL and parietal cells: identification of a novel pathway regulating acid secretion. *Physiol. Genomics* **25**, 153–165
20. Habata, Y., Fujii, R., Hosoya, M., Fukusumi, S., Kawamata, Y., Hinuma, S., Kitada, C., Nishizawa, N., Murosaki, S., Kurokawa, T., Onda, H., Tatemoto, K., and Fujino, M. (1999) Apelin, the natural ligand of the orphan receptor APJ, is abundantly secreted in the colostrum. *Biochim. Biophys. Acta.* **1452**, 25–35
21. Hosoya, M., Kawamata, Y., Fukusumi, S., Fujii, R., Habata, Y., Hinuma, S., Kitada, C., Honda, S., Kurokawa, T., Onda, H., Nishimura, O., and Fujino, M. (2000) Molecular and functional characteristics of APJ. Tissue distribution of mRNA and interaction with the endogenous ligand apelin. *J. Biol. Chem.* **275**, 21061–21067
22. O'Carroll, A.M., Selby, T.L., Palkovits, M., and Lolait, S.J. (2000) Distribution of mRNA encoding B78/apj, the rat homologue of the human APJ receptor, and its endogenous ligand apelin in brain and peripheral tissues. *Biochim. Biophys. Acta.* **1492**, 72–80
23. Devic, E., Rizzoti, K., Bodin, S., Knibiehler, B., and Audigier, Y. (1999) Amino acid sequence and embryonic expression of msr/apj, the mouse homolog of Xenopus X-msr and human APJ. *Mech. Dev.* **84**, 199–203
24. Medhurst, A.D., Jennings, C.A., Robbins, M.J., Davis, R.P., Ellis, C., Winborn, K.Y., Lawrie, K.W., Hervieu, G., Riley, G., Bolaky, J.E., Herrity, N.C., Murdock, P., and Darker, J.G. (2003) Pharmacological and immunohistochemical characterization of the APJ receptor and its endogenous ligand apelin. *J. Neurochem.* **84**, 1162–1172
25. Edinger, A.L., Hoffman, T.L., Sharron, M., Lee, B., Yi, Y., Choe, W., Kolson, D.L., Mitrovic, B., Zhou, Y., Faulds, D., Collman, R.G., Hesselgesser, J., Horuk, R., and Doms, R.W. (1998) An orphan seven-transmembrane domain receptor expressed widely in the brain functions as a coreceptor for human immunodeficiency virus type 1 and simian immunodeficiency virus. *J. Virol.* **72**, 7934–7940
26. Kleinz, M.J. and Davenport, A.P. (2004) Immunocytochemical localization of the endogenous vasoactive peptide apelin to human vascular and endocardial endothelial cells. *Regul. Pept.* **118**, 119–125
27. Wang, G., Qi, X., Wei, W., Englander, E.W., and Greeley, G.H. Jr (2006) Characterization of the 5′-regulatory regions of the rat and human apelin genes and regulation of breast apelin by USF. *FASEB J.* **20**, 2639–2641
28. Han, S., Wang, G., Qi, X., Englander, E.W., and Greeley, G.H. Jr (2008) Involvement of a Stat3 binding site in inflammation-induced enteric apelin expression. *Am. J. Physiol. Gastrointest. Liver Physiol.* **295**, G1068–G1078
29. Mazzucotelli, A., Ribet, C., Castan-Laurell, I., Daviaud, D., Guigne, C., Langin, D., and Valet, P. (2008) The transcriptional co-activator PGC-1alpha up regulates apelin in human and mouse adipocytes. *Regul. Pept.* **150**, 33–37
30. Eyries, M., Siegfried, G., Ciumas, M., Montagne, K., Agrapart, M., Lebrin, F., and Soubrier, F. (2008) Hypoxia-induced apelin expression regulates endothelial cell proliferation and regenerative angiogenesis. *Circ. Res.* **103**, 432–440
31. O'Carroll, A.M., Lolait, S.J., and Howell, G.M. (2006) Transcriptional regulation of the rat apelin receptor gene: promoter cloning and identification of an Sp1 site necessary for promoter activity. *J. Mol. Endocrinol.* **36**, 221–235
32. Hata, J., Matsuda, K., Ninomiya, T., Yonemoto, K., Matsushita, T., Ohnishi, Y., Saito, S., Kitazono, T., Ibayashi, S., Iida, M., Kiyohara, Y., Nakamura, Y., and Kubo, M. (2007) Functional SNP in an Sp1-binding site of AGTRL1 gene is associated with susceptibility to brain infarction. *Hum. Mol. Genet.* **16**, 630–639
33. Kasai, A., Shintani, N., Oda, M., Kakuda, M., Hashimoto, H., Matsuda, T., Hinuma, S., and Baba, A. (2004) Apelin is a novel angiogenic factor in retinal endothelial cells. *Biochem. Biophys. Res. Commun.* **325**, 395–400
34. Hashimoto, Y., Ishida, J., Yamamoto, R., Fujiwara, K., Asada, S., Kasuya, Y., Mochizuki, N., and Fukamizu, A (2005) G protein-coupled APJ receptor signaling induces focal adhesion formation and cell motility. *Int. J. Mol. Med* **16**, 787–792
35. Masri, B., Morin, N., Cornu, M., Knibiehler, B., and Audigier, Y. (2004) Apelin (65-77) activates p70 S6 kinase and is mitogenic for umbilical endothelial cells. *FASEB J.* **18**, 1909–1911
36. Li, Y., Chen, J., Bai, B., Du, H., Liu, Y., and Liu, H. (2012) Heterodimerization of human apelin and kappa opioid receptors: roles in signal transduction. *Cell. Signal.* **24**, 991–1001
37. Alastalo, T.P., Li, M., Perez Vde, J., Pham, D., Sawada, H., Wang, J.K., Koskenvuo, M., Wang, L., Freeman, B.A., Chang, H.Y., and Rabinovitch, M. (2011) Disruption of PPARgamma/beta-catenin-mediated regulation of apelin impairs BMP-induced mouse and human pulmonary arterial EC survival. *J. Clin. Invest.* **121**, 3735–3746
38. Xie, H., Yuan, L.Q., Luo, X.H., Huang, J., Cui, R.R., Guo, L.J., Zhou, H.D., Wu, X.P., and Liao, E.Y. (2007) Apelin suppresses apoptosis of human osteoblasts. *Apoptosis* **12**, 247–254
39. Masri, B., Morin, N., Pedebernade, L., Knibiehler, B., and Audigier, Y. (2006) The apelin receptor is coupled to Gi1 or Gi2 protein and is differentially desensitized by apelin fragments. *J. Biol. Chem.* **281**, 18317–18326
40. Risau, W. (1997) Mechanisms of angiogenesis. *Nature* **386**, 671–674
41. Kalin, R.E., Kretz, M.P., Meyer, A.M., Kispert, A., Heppner, F.L., and Brandli, A.W. (2007) Paracrine and autocrine mechanisms of apelin signaling govern embryonic and tumor angiogenesis. *Dev. Biol.* **305**, 599–614
42. Cox, C.M., D'Agostino, S.L., Miller, M.K., Heimark, R.L., and Krieg, P.A. (2006) Apelin, the ligand for the endothelial G-protein-coupled receptor, APJ, is a potent angiogenic factor required for normal vascular development of the frog embryo. *Dev. Biol.* **296**, 177–189
43. Tucker, B., Hepperle, C., Kortschak, D., Rainbird, B., Wells, S., Oates, A.C., and Lardelli, M. (2007) Zebrafish angiotensin II receptor-like 1a (agtrl1a) is expressed in migrating hypoblast, vasculature, and in multiple embryonic epithelia. *Gene Expr. Patterns* **7**, 258–265
44. Kidoya, H., Ueno, M., Yamada, Y., Mochizuki, N., Nakata, M., Yano, T., Fujii, R., and Takakura, N. (2008) Spatial and temporal role of the apelin/APJ

system in the caliber size regulation of blood vessels during angiogenesis. *EMBO J*. **27**, 522–534

45. Saint-Geniez, M., Masri, B., Malecaze, F., Knibiehler, B., and Audigier, Y. (2002) Expression of the murine msr/apj receptor and its ligand apelin is upregulated during formation of the retinal vessels. *Mech. Dev*. **110**, 183–186
46. Saint-Geniez, M., Argence, C.B., Knibiehler, B., and Audigier, Y. (2003) The msr/apj gene encoding the apelin receptor is an early and specific marker of the venous phenotype in the retinal vasculature. *Gene Expr. Patterns* **3**, 467–472
47. Jakobsson, L., Franco, C.A., Bentley, K., Collins, R.T., Ponsioen, B., Aspalter, I.M., Rosewell, I., Busse, M., Thurston, G., Medvinsky, A., Schuttle-merker, S., and Gerhardt, H. (2010) Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. *Nat. Cell Biol*. **12**, 943–953
48. Arima, S., Nishiyama, K., Ko, T., Arima, Y., Hakozaki, Y., Sugihara, K., Koseki, H., Uchijima, Y., Kurihara, Y., and Kurihara, H. (2011) Angiogenic morphogenesis driven by dynamic and heterogeneous collective endothelial cell movement. *Development* **138**, 4763–4776
49. del Toro, R., Prahst, C., Mathivet, T., Siegfried, G., Kaminker, J.S., Larrivee, B., Breant, C., Duarte, A., Takakura, N., Fukamizu, A., Penninger, J., and Eichmann, A. (2010) Identification and functional analysis of endothelial tip cell-enriched genes. *Blood* **116**, 4025–4033
50. Kasai, A., Shintani, N., Kato, H., Matsuda, S., Gomi, F., Haba, R., Hashimoto, H., Kakuda, M., Tano, Y., and Baba, A. (2008) Retardation of retinal vascular development in apelin-deficient mice. *Arterioscler. Thromb. Vasc. Biol*. **28**, 1717–1722
51. Sakimoto, S., Kidoya, H., Naito, H., Kamei, M., Sakaguchi, H., Goda, N., Fukamizu, A., Nishida, K., and Takakura, N. (2012) A role for endothelial cells in promoting the maturation of astrocytes through the apelin/APJ system in mice. *Development* **139**, 1327–1335
52. Seaman, S., Stevens, J., Yang, M.Y., Logsdon, D., Graff-Cherry, C., and St Croix, B. (2007) Genes that distinguish physiological and pathological angiogenesis. *Cancer Cell* **11**, 539–554
53. Wang, Z., Greeley, G.H. Jr, and Qiu, S. (2008) Immunohistochemical localization of apelin in human normal breast and breast carcinoma. *J. Mol. Histol*. **39**, 121–124
54. Kidoya, H., Kunii, N., Naito, H., Muramatsu, F., Okamoto, Y., Nakayama, T., and Takakura, N. (2011) The apelin/APJ system induces maturation of the tumor vasculature and improves the efficiency of immune therapy. *Oncogene*, doi: 10.1038/onc.2011.489
55. Berta, J., Kenessey, I., Dobos, J., Tovari, J., Klepetko, W., Jan Ankersmit, H., Hegedus, B., Renyi-Vamos, F., Varga, J., Lorincz, Z., Paku, S., Ostoros, G., Rozsas, A., Timar, J., and Dome, B. (2010) Apelin expression in human non-small cell lung cancer: role in angiogenesis and prognosis. *J. Thorac. Oncol*. **5**, 1120–1129
56. Sorli, S.C., Le Gonidec, S., Knibiehler, B., and Audigier, Y. (2007) Apelin is a potent activator of tumour neoangiogenesis. *Oncogene* **26**, 7692–7699
57. Jain, R.K. (2005) Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* **307**, 58–62
58. Dickson, P.V., Hamner, J.B., Sims, T.L., Fraga, C.H., Ng, C.Y., Rajasekeran, S., Hagedorn, N.L., McCarville, M.B., Stewart, C.F., and Davidoff, A.M. (2007) Bevacizumab-induced transient remodeling of the vasculature in neuroblastoma xenografts results in improved delivery and efficacy of systemically administered chemotherapy. *Clin. Cancer Res*. **13**, 3942–3950
59. Kunduzova, O., Alet, N., Delesque-Touchard, N., Millet, L., Castan-Laurell, I., Muller, C., Dray, C., Schaeffer, P., Herault, J.P., Savi, P., Bono, F., and Valet, P. (2008) Apelin/APJ signaling system: a potential link between adipose tissue and endothelial angiogenic processes. *FASEB J*. **22**, 4146–4153
60. Tiani, C., Garcia-Pras, E., Mejias, M., de Gottardi, A., Berzigotti, A., Bosch, J., and Fernandez, M. (2009) Apelin signaling modulates splanchnic angiogenesis and portosystemic collateral vessel formation in rats with portal hypertension. *J. Hepatol*. **50**, 296–305
61. Kidoya, H., Naito, H., and Takakura, N. (2010) Apelin induces enlarged and nonleaky blood vessels for functional recovery from ischemia. *Blood* **115**, 3166–3174
62. Sheikh, A.Y., Chun, H.J., Glassford, A.J., Kundu, R.K., Kutschka, I., Ardigo, D., Hendry, S.L., Wagner, R.A., Chen, M.M., Ali, Z.A., Yue, P., Huynh, D.T., Connolly, A.J., Pelletier, M.P., Tsao, P.S., Robbins, R.C., and Quertermous, T. (2008) In vivo genetic profiling and cellular localization of apelin reveals a hypoxia-sensitive, endothelial-centered pathway activated in ischemic heart failure. *Am. J. Physiol. Heart Circ. Physiol*. **294**, H88–H98
63. Sawane, M., Kidoya, H., Muramatsu, F., Takakura, N., and Kajiya, K Apelin attenuates UVB-induced edema and inflammation by promoting vessel function. *Am. J. Pathol* **179**, 2691–2697