ELSEVIER

Therapeutic potential of interfering with apelin signalling

S. Caroline Sorli, Loïc van den Berghe, Bernard Masri, Bernard Knibiehler and Yves Audigier

IFR31, Unité INSERM U-589, CHU Rangueil, Bât. L3, BP84225, 1 avenue Jean-Poulhès, 31432-Toulouse Cédex 4, France

The apelin receptor is a G protein-coupled receptor activated by several apelin fragments. Its tissue distribution suggests that apelin signalling is involved in a broad range of physiological functions. Endothelial cells, which express high levels of apelin receptors, respond to apelin through the phosphorylation of key intracellular effectors associated with cell proliferation and migration. In addition, apelin is a mitogen for endothelial cells and exhibits angiogenic properties in matrigel experiments. This review focuses on the therapeutic potential of apelin signalling, which is associated with pathologies that result from decreased vascularisation (ischemias) or neovascularisation (retinopathies and solid tumors).

G protein-coupled receptors (GPCRs) belong to a large family of transmembrane proteins that recognize a variety of different ligands and regulate the activity of many intracellular effectors. Receptors from this family participate in a wide range of physiological functions in different tissues. The corollary of their physiological importance is that numerous pathologies are attributed to their signalling dysfunction. Thus, half of the drugs currently used for treating human diseases represent molecules that interact with members of this receptor family. Consequently, the discovery of new such receptors is likely to be followed by the design of new pharmacological agents that counteract pathological dysfunctions of their associated signalling pathways. The precise role of a particular receptor and the nature of its associated signalling pathway determine its pharmacological interest and its potential as a therapeutic agent. Based on our current understanding of the apelin receptor and its downstream signalling pathway, it is clear that this GPCR holds promise as a target for therapeutic development countering multiple pathologies.

Receptor expression and physiopathology of apelin signalling

Insight as to the existence of an apelin signalling pathway began with the cloning of the human and amphibian receptors [1,2]. Several years later, the endogenous apelin ligand was identified as encoding a preproprotein of 77 amino acids [3] that undergoes maturation generating at least two active peptide fragments: apelin (42–77) and apelin (65–77). Given that the expression of a receptor correlates with its physiological function, the determination of the various cell types that express the apelin receptor has been paramount in increasing our understanding of the role of the apelin signalling pathway (reviewed in Refs [4,5]) (Table 1).

The apelin receptor was first detected in endothelial cells during the embryonic formation of large vessels [2,6]. Its activation leads to phosphorylation of ERKs, Akt and p70 S6 kinase [7,8]. These transduction cascades constitute the basis for a dual function of apelin signalling at the endothelial level. First, activation of Akt induces the activation of endothelial NO synthase and NO release [9], which, in turn, relaxes smooth muscle cells and lowers blood pressure through peripheral vasodilatation [10]. Interestingly, knock out of the gene encoding the apelin receptor (msr/apj) results in an increased vasopressor response to angiotensin II, showing that apelin has a counter-regulatory role to that of angiotensin [11]. Accordingly, agonists of the apelin receptor might represent a pharmacological strategy for the treatment of hypertension. Second, activation of the apelin receptor promotes phosphorylation of several proteins (ERKs, Akt and p70S6kinase) that represent key intracellular

Corresponding author: Audigier, Y. (yves.audigier@toulouse.inserm.fr)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2006.10.011

TABLE 1

Physiological function and therapeutic implications of apelin signalling

Receptor expression	Cell response	Tissue effect	Therapeutic implications	Refs
Endothelial cell	NO production	Vasodilatation	Hypertension	[9,10]
	Activation of Akt, ERKs and p70S6K	Angiogenesis	Ilschemia; neovascularization	[2,7,8,12]
Neuron	Decrease of vasopressin release	Diuresis	Hypertension; diabetes insipidus?	[13,14]
Cardiomyocyte	Activation of PLC and PKC	Inotropic effect	Heart failure	[16–21]
Enterochromaffin-like gastric cell	ERK activation	Increase of CCK release	Ulcer?	[23]
	Decrease of intracellular calcium	Increase in tissue levels of histamine	Ulcer?	[25]
		Inhibition of gastrin-induced acid secretion	Ulcer?	[25]
Pancreatic islet cell	?	Inhibition of insulin secretion	Diabetes?	[26]
Osteoblast	Activation of PI3K and Akt	Bone formation	Bone diseases	[28]
T Lymphocyte	?	Cytokine secretion	HIV infection	[29–34]

effectors linked to cell migration and proliferation. Apelin has been reported to be a mitogen for endothelial cells [7] and to display angiogenic properties in matrigel experiments [12]. Consequently, apelin signalling represents an interesting therapeutic target in ischemic states and pathological neovascularisation.

Apelin receptor transcripts were originally detected in several regions of the brain [1], with the highest density in the hypothalamus [13,14]. The apelin receptor is expressed in a subset of neurons that are responsible for the central regulation of hormone release, body fluid homeostasis, water and food intake and circadian rhythms. Such a role in the central nervous system emphasizes the therapeutic potential of the apelin receptor for the treatment of some hormonal dysfunctions and pathologies linked to an altered homeostasis of body fluids.

High expression of apelin receptor mRNA has also been observed in cardiac tissue from various organisms [2,6,15]. The presence of the corresponding protein has been confirmed by the demonstration that high-affinity binding sites for iodinated apelin exist in this tissue [16]. The first evidence for a direct action of apelin on the heart was revealed by the potent inotropic effect of apelin on isolated heart [17], which is likely to result from an activation of the apelin receptor that is expressed at the surface of cardiomyocytes [18]. Interestingly, several reports suggest that apelin signalling is altered in heart failure [19]. Chronic treatment with apelin has cardioprotective effects by reducing cardiac loading without inducing ventricular hypertrophy [5,20,21]. Accordingly, apelin signalling is a promising target for the treatment of heart failure and ischemic heart disease (reviewed in Ref. [22]).

Although apelin was initially isolated from stomach extracts [3], its expression was only recently assigned to several gastric cell types [23], including parietal cells [24]. In addition, the gene encoding the apelin receptor is highly expressed in gastric enterochromaffin-like cells [25], suggesting that apelin signalling decreases histamine release and downregulate acid secretion of parietal cells. Apelin signalling could therefore represent a new target in the treatment of gastric ulcers.

More recently, the apelin receptor was shown to be present in pancreatic islet cells, where apelin inhibits the rapid insulin response of glucose [26]. This inhibition is linked to the interplay between apelin and insulin-regulated pathways, as seen in adipocytes, where insulin stimulates apelin production [27]. In addition, tumor necrosis factor (TNF)-α was recently shown to upregulate apelin expression from adipocytes and to induce an increase in apelin plasma levels [28]. The emerging role of apelin signalling in the adipoinsular axis reveals its participation in the pathological mechanisms associated with insulin resistance and obesity-related disorders, and suggests its therapeutic potential in the treatment of obesity (reviewed in Ref. [22]).

Proliferation of human osteoblasts, which also express the apelin receptor, is induced upon apelin treatment through activation of a PI3 kinase/Akt transduction cascade [29]. The mitogenic effect of apelin on osteoblasts could be a starting point for the treatment of various bone diseases.

In preliminary investigations on its activity, apelin was found to modulate the production of some cytokines by spleen cells [30]. Such an effect might be linked to the demonstration that the human apelin receptor acts as a co-receptor for the entry of some strains of the immunodeficiency virus [31,32] and that apelin peptides can block entry of the virus [33–35].

In view of this broad range of physiological functions, apelin signalling has high therapeutic potential. This review focuses on the prospective applications of apelin signalling in blood vessel formation and vascular plasticity.

Apelin signalling and vascular plasticity

The endothelial cell is intimately involved in vessel formation and is the integration center of vascular plasticity. Indeed, it represents the first cell type that emerges at the beginning of the primitive network and acts as the organization center for the other cell layers. In addition, it integrates the signals provided by various environmental factors and this integration defines both the cellular response (proliferation or apoptosis) and the corresponding modification of the vascular network (extension or regression).

The expression of the apelin receptor is high during the formation of embryonic vessels [2,6], and during the postnatal formation of retinal vessels [36] (Figure 1a). This link between receptor expression and vessel formation correlates with the mitogenic properties of apelin on endothelial cells [7] and its angiogenic activity in matrigel experiments [12]. In addition, apelin signalling

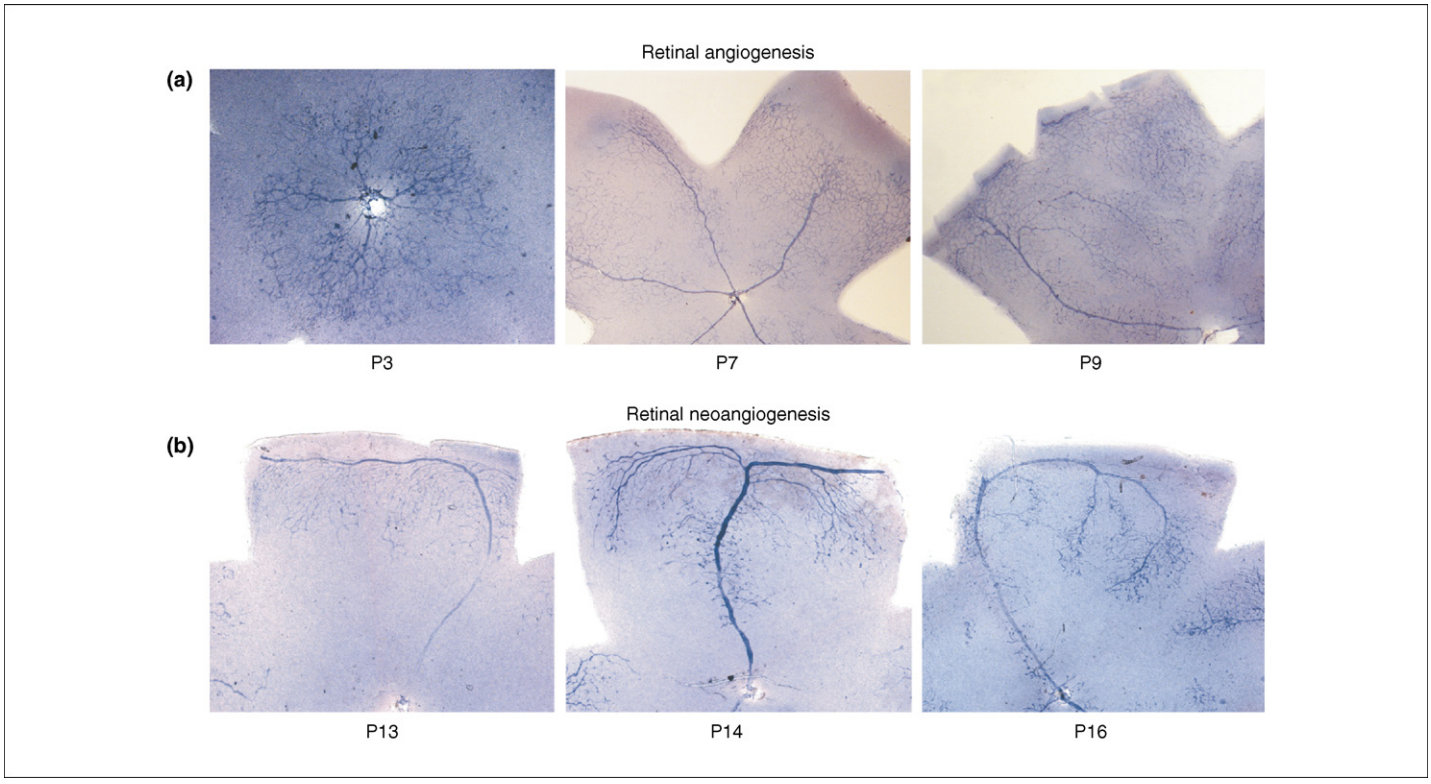

FIGURE 1

Expression of the apelin receptor is upregulated in retinal vessels during physiological and pathological angiogenesis. (a) Retinal angiogenesis. Expression of the apelin receptor was determined during the formation of retinal vessels by *in situ* hybridisation performed on whole-mount retinas at postnatal days P3, P7 and P9. The mRNA transcripts were localized in endothelial cells and their expression traces the centrifugal extension of the retinal network from the optic disk to the periphery of the retina (S.C.S., unpublished). **(b)** Retinal neoangiogenesis. Expression of the apelin receptor was determined during the hyperoxia-induced formation of new retinal vessels in the mouse model of retinopathy of the prematurity (ROP) by *in situ* hybridisation performed at postnatal days P13, P14 and P16. The mRNA transcripts were localized in endothelial cells and their expression traces the centripetal extension of the retinal network from the periphery of the retina to the optic disc (L.v.d.B., unpublished). .

was recently shown to be important during embryonic blood vessel and heart development in lower vertebrates [37,38].

Given that apelin signalling might also have a similar role during pathological angiogenesis, the apelin receptor represents an interesting therapeutic target. Receptor agonists could be pharmacological tools used in therapeutic angiogenesis against ischemic diseases, and antagonists could be applied in antiangiogenic strategies to block neovascularisation associated with tumor growth and ischemic retinopathies.

Apelin signalling and ischemic diseases

The mitogenic and angiogenic properties of apelin on endothelial cells suggest a therapeutic potential in the treatment of ischemic pathologies concerning the two primary target organs of angiogenic therapy: the heart (see above) and leg muscle. Although there are presently no published data on this topic, it can be anticipated that the various approaches already performed with vascular endothelial growth factor (VEGF) (reviewed in Ref. [39]) will be soon applied to apelin. Indeed, the delivery of apelin peptides, or the gene encoding them, can be initiated in animal models and then followed by preclinical studies and controlled clinical trials. Accordingly, non-peptidic agonists of the apelin receptor represent attractive molecules for a therapeutic stimulation of blood vessel growth.

Apelin signalling and retinal neovascularisation

The retinal vasculature is a good model system used for the study of blood vessel development. In the mouse, the formation of retinal vessels begins at birth and corresponds to a centrifugal extension of the retinal vessels from the optic disc to the periphery of the retina. Interestingly, *in situ* hybridization on pup retinas reveals that expression of the apelin receptor is upregulated during the formation of retinal vessels and traces the centrifugal extension of the superficial vasculature (Figure 1a) [36].

Hyperoxia treatment of premature babies induces the abnormal formation of new retinal vessels leading to cecity. A mouse model of retinal neovascularisation [40] reproduces the primary features associated with retinopathy of prematurity (and also ischemic retinopathies) and enables the study of the molecular mechanisms and cellular events associated with pathological neoangiogenesis. Seven-day-old mice are exposed to hyperoxia for five days, which induces endothelial apoptosis and vasoobliteration and generates ischemic territories. In these territories, the return to normoxia is akin to a relative form of hypoxia, which promotes the expression of VEGF and the formation of new vessels. As ischemia is located in the mid-retina, it reverses the direction of the forming vessels from a physiological and centrifugal extension to a pathological and centripetal extension. During the formation of these new vessels between postnatal days P12 and P17, apelin receptor expression is

selectively upregulated in these vessels and traces the reversed extension of the retinal network (Figure 1b). Accordingly, apelin signalling might also be involved in the endothelial proliferation associated with the pathological formation of new retinal vessels. Therefore, the apelin receptor represents a promising target in the fight against this form of pathological neoangiogenesis. In addition, the potential implications of apelin in diabetes suggest that apelin antagonists will treat not only the 'primary effects' of apelin in diabetes, but will also ultimately counteract its secondary effects in the retina at the vascular level.

Apelin signalling and choroïdal neovascularisation

In view of the important role of VEGF in retinal and choroïdal neovascularisation (reviewed in Ref. [41]), it is tempting to speculate that apelin signalling participates in the physiological formation of choroïdal vessels as well as in the pathological formation of excedentary vessels observed in age-related macular degeneration (AMD). Whether the apelin receptor is expressed by endothelial cells lining the choroïdal vessels is currently being investigated.

Apelin signalling and tumor neovascularisation

To extend the previously mentioned studies on retinal neovascularisation, it has been hypothetized that apelin signalling is involved in another form of pathological neovascularisation, such as that associated with solid tumor growth. As formulated by Folkman [42], tumor-driven neoangiogenesis is a prerequisite for the survival and growth of solid tumors [43]. To characterize the neoangiogenic activity of apelin, different mouse models of tumor neovascularisation have been developed.

Expression of the murine apelin receptor (msr/apj) was not detected at the mRNA level by real-time PCR, and the absence of protein expression was deduced from the lack of adenylylcyclase inhibition induced by apelin (Figure 2a; S.C.S. *et al.*, unpublished). Then, as the basal expression level of apelin measured by real-time PCR was low, stable B16 clones overexpressing the gene encoding apelin were created, and the effects on proliferation analysed *in vitro*. All the clones overexpressing apelin did not show, *in vitro*, any alteration in their proliferation rate when compared with that of mock-transfected cells (Figure 2a). These findings therefore rule out possible autocrine effects of apelin overexpression on tumor cells. The consequences of apelin overexpression *in vivo* were then investigated by comparing the growth and vascularisation of tumors following subcutaneous injection of B16-mock or B16-apelin clones (Figure 2b). Apelin overexpression increased tumor growth *in vivo*, even in the context of a tumor cell line that has a high proliferation rate, such as the B16 melanoma cell line. Interestingly, the slopes of the growth curves were similar, but the curve of the apelin tumors was shifted to the left. In agreement with the data obtained *in vitro*, apelin overexpression increases *in vivo* tumor growth without altering the proliferation rate of tumoral cells and acts on the vascular component of the tumor by a paracrine action on the endothelial cells of the host vessels. Indeed, an identical effect on another tumor cell type was also observed (S.C.S. *et al.*, unpublished), which was associated with an increased expression of the endothelial marker CD31/PECAM and the presence of large tumor vessels. Taken together, apelin would primarily accelerate the angiogenic switch and potently activate tumor neoangiogenesis, which in turn increases the *in vivo* growth of the tumor. The pathological relevance of this neoangiogenic activity is strongly corroborated by the demonstration that the apelin gene is strongly induced by gazeous hypoxia and upregulated in one-third of human tumors (S.C.S. *et al.*, unpublished).

Spatio-temporal specificity of pharmacological targetting to apelin signalling

The classic pharmacological target of a signalling pathway is the receptor. In this context, specific agonists have to be designed for the treatment of ischemic diseases, whereas specific antagonists can be used to abrogate the abnormal development of new vessels. However, to inhibit apelin signalling, many alternative strategies could be developed that either act at a different level or use a different tool, as described for VEGF (reviewed in Ref. [44]). Accordingly, antibodies against apelin or the apelin receptor could be used, as well as RNAi (reviewed in Ref. [45]) and aptamers (reviewed in Ref. [46]).

Interestingly, apelin signalling displays unique properties in the spatio-temporal expression of its components. Both apelin and the apelin receptor are selectively upregulated during the formation of retinal vessels [36] as well as during the formation of new retinal vessels in the mouse model of retinopathy (Figure 1). These observations suggest that apelin signalling is specifically involved in the proliferative phase linked to the extension of the vascular network. Pharmacological targetting of a pathway that is specifically activated in proliferating cells represents an interesting therapeutic advantage, especially given that antagonists would not act on the quiescent endothelium of the mature vascular network.

In contrast to VEGF, apelin is expressed by endothelial cells [36]. In addition, a different expression pattern of apelin and its receptor has been observed inside the endothelium: endothelial cells that express the apelin gene were localized at the leading edge of the vascular network, whereas the receptor was mainly expressed by endothelial cells lining primary and secondary vessels [36]. Interestingly, recent works have revealed that the formation of retinal vessels involves a morphological and functional specialisation of two endothelial populations [47], as described for the formation of the respiratory system and the nervous network. Indeed, endothelial cells at the edge, called tip cells, form cell protrusions (filipodia) that decode the gradient of chemotactic signals and direct the polarised extension of the network. Downstream, endothelial cells, referred to as stalk cells, proliferate to increase the number of endothelial cells required for extension of the vascular network.

Using isolectin B4 conjugated to fluorescein for labelling endothelial cells on whole-mount retinas, high expression levels of apelin in tip cells have been observed, easily recognized at the edge of the vascular network by the presence of their filopodia (Figure 3a; S.C.S., unpublished). Interestingly, apelin receptor transcripts were not detected in this first row of endothelial cells (tip cells), but were observed in the cells underneath (stalk cells) as well as in most endothelial cells of the vascular network (Figure 3b). These observations confirm and extend the previous results already described [36] and demonstrate that the two genes are expressed in distinct endothelial populations. Thus, the differential spatial expression of apelin and its receptor could be linked to the functional specialisation of the two endothelial subpopula-

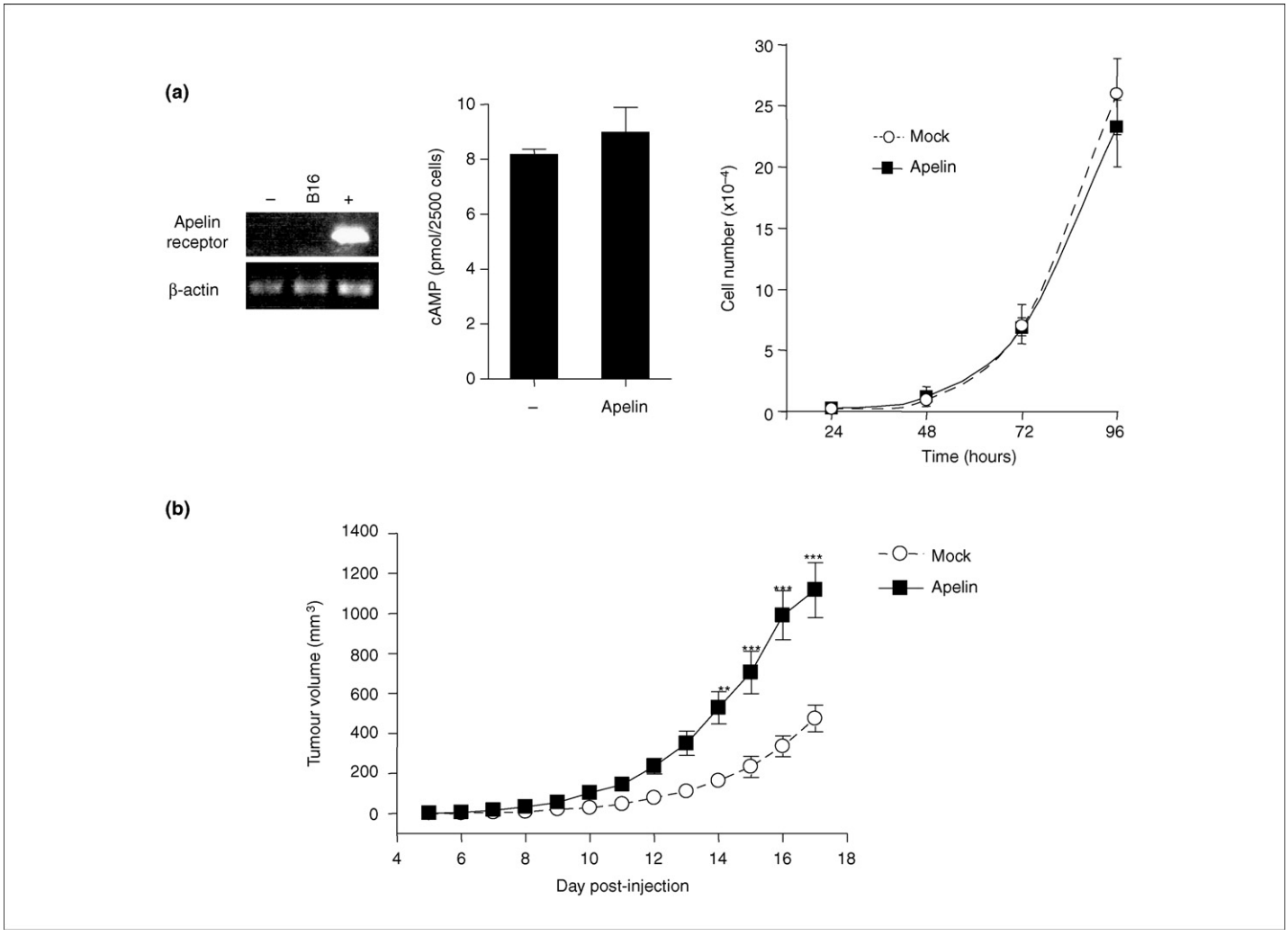

FIGURE 2

Apelin is a potent activator of tumor neoangiogenesis. (a) Expression of the apelin receptor is not detected in B16 melanoma cells. Left panel: no transcript of the apelin receptor was detected. Total RNA from B16 cells was isolated, reverse-transcribed and amplified for the indicated gene products by PCR. The negative (−) and the positive (+) controls contained cDNAs from CHO wild-type cells and a CHO cell line stably transfected with the mouse apelin receptor cDNA, respectively. Middle panel:a: apelin (65–77) does not inhibit forskolin-stimulated adenylylcyclase. B16 melanoma cells were incubated without (−) or with apelin (65–77), and cAMP levels were measured on cell lysates by radioimmunoassay. Right panel: B16 melanoma cells were stably transfected with the empty plasmid vector (B16-mock clones) or the plasmid vector containing the murine apelin cDNA (B16-apelin clones). Apelin overexpression does not alter the *in vitro* proliferation of B16 melanoma cells. The *in vitro* proliferation of cultured B16-mock and B16-apelin clones were determined by counting the number of cells at the times indicated. Values represent the mean ± SEM of triplicate determinations obtained in five separate experiments for three clones.**(b)** Apelin overexpression increases tumor growth *in vivo*. B16-mock and B16-apelin clones (1.5×10^5 cells) were injected in nine different animals and tumor size was measured at the days indicated. Values represent the mean ± SEM of the results from nine animals (S.C.S., unpublished).

tions. Thus, pharmacological targetting of apelin signalling might lead to a different effect on physiological and pathological angiogenesis than do approaches that target VEGF signalling. In this context, a combination of antagonists acting on the activity of several angiogenic factors might be useful, at the level not only of the proliferation of endothelial cells but also of signal integration of angiogenic factors that might differentially activate subpopulations of endothelial cells.

Clinical potential of apelin signalling in neovascular diseases

The link between ocular neovascularization and tumor-induced angiogenesis is the formation of excedentary and pathological vessels. Thus, as far as ocular neovascularisation is concerned, an antagonist of the apelin receptor would be useful for the treatment of the excedentary vessels that are formed by retinal vasculature (diabetic retinopathies) or choroïdal vasculature (AMD).

Given that tumor growth depends on the formation of a neovascular supply, inhibition of tumor-induced neoangiogenesis represents an effective strategy to treat human cancers [42]. Indeed, antiangiogenic strategies are currently being performed at the clinical stage, either with antibodies against the VEGF receptor [48] or with angiostatic agents, such as endostatin [49]. These strategies are advantageous because they act on endothelial cells that are genetically stable, rather than on tumoral cells, the instability of which can generate resistances to therapy [50]. In addition, physiological angiogenesis rarely occurs at the adult stage (except in the ovary and uterus and during wound repair).

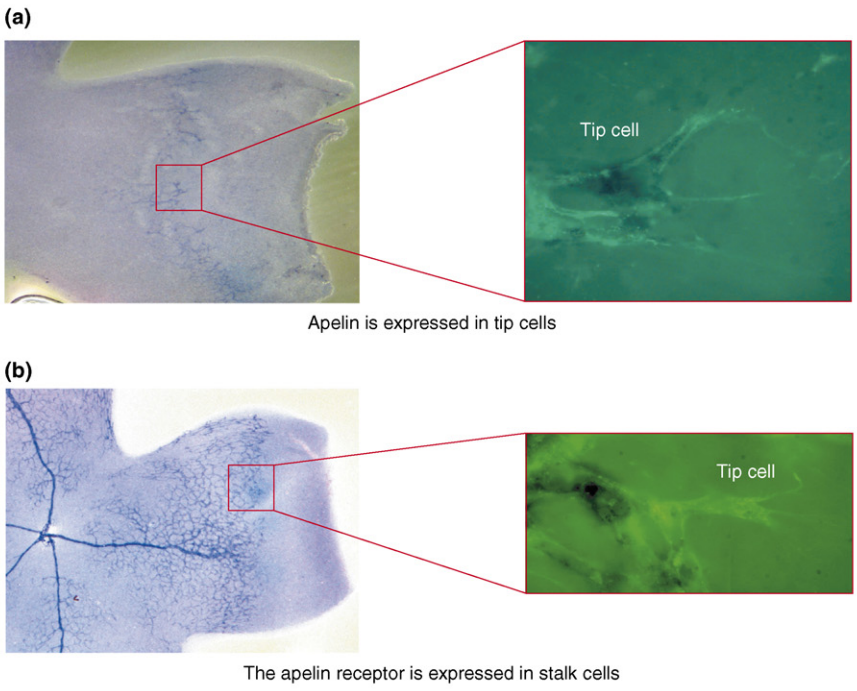

FIGURE 3

The expression of apelin and its receptor localize to different endothelial subpopulations. (a) Expression of the gene encoding apelin was detected by *in situ* hybridisation and endothelial cells were labelled with *Bandeiraea simplicifolia* isolectin B4 conjugated to fluorescein. Apelin expression is high in endothelial cells that are extending filopodia (tip cells). **(b)** Expression of apelin receptor gene was detected by *in situ* hybridisation and endothelial cells were labelled with *Bandeiraea simplicifolia* isolectin B4 conjugated to fluoresceine. Apelin receptor expression was not detected in tip cells but was high in the other endothelial cells (stalk cells) (L.v.d.B., unpublished).

Finally, these strategies, in combination with antitumoral strategies, enable the inhibition of solid tumor growth by acting on both the vascular component and the tumoral component. A similar approach can therefore be applied to apelin signalling, either alone as an antiangiogenic strategy or in combination with cytotoxic compounds in antiangiogenic chemotherapy.

In view of its participation in a range of physiological functions, it could be inferred that interfering with apelin signalling would generate a lot of undesirable side effects. However, knockout of the gene encoding the apelin receptor produces neither embryonic defects nor histological effects in various tissues [11]. By contrast, even though regulation of apelin signalling could lead to side effects, it could be possible to design approaches that are limited to a precise target tissue. Interestingly, a blockade of apelin signalling that inhibits pathological angiogenesis can be expected to target specifically the endothelial tissue owing to the administration route. In addition, the correlation between endothelial proliferation and upregulation of the apelin receptor gene suggests that interfering with apelin signalling in the adult is restricted to sites of neoangiogenesis. Indeed, the vascular activation of apelin signalling can be envisioned as a rescue or plastic function, which becomes activated mainly in pathological situations. Such a hypothesis is corroborated by the phenotype of apelin receptor-deficient mice, which is associated with no change in basal arterial pressure but with an increased vasopressor response with angiotensin II [11]. Taken together, the apelin receptor constitutes an interesting target for the design of new drugs for treating many diseases, including the prime causes of human mortality: cancer and cardiovascular diseases.

Acknowledgements

We thank J. Iacovoni for helpful discussions and critical reading of the article. S.C.orliS. is the recipient of a postdoctoral fellowship from the Association pour la Recherche sur le Cancer.

References

1 O'Dowd, B.F. *et al.* (1993) A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. *Gene* 136, 355–360
2 Devic, E. *et al.* (1996) Expression of a new G protein-coupled receptor X-msr is associated with an endothelial lineage in *Xenopus laevis*. *Mech. Dev.* 59, 129–140
3 Tatemoto, K. *et al.* (1998) Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem. Biophys. Res. Commun.* 251, 471–476
4 Masri, B. *et al.* (2005) Apelin signalling: a promising pathway from cloning to pharmacology. *Cell. Signal.* 17, 415–426
5 Audigier, Y. (2006) Apelin Receptor. *AfCS-Nat. Mol. Pages* doi:10.1038/mp.a000304.01
6 Devic, E. *et al.* (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
7 Masri, B. *et al.* (2004) Apelin (65-77) activates p70 S6 kinase and is mitogenic for umbilical endothelial cells. *FASEB J.* 18, 1909–1911

8 Masri, B. *et al.* (2006) The apelin receptor is coupled to gi1 or gi2 protein and is differentially desensitized by apelin fragments. *J. Biol. Chem.* 281, 18317–18326
9 Tatemoto, K. *et al.* (2001) The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. *Regul. Pept.* 99, 87–92
10 Lee, D.K. *et al.* (2000) Characterization of apelin, the ligand for the APJ receptor. *J. Neurochem.* 74, 34–41
11 Ishida, J. *et al.* (2004) Regulatory roles for APJ, a seven-transmembrane receptor related to AT1, in blood pressure *in vivo*. *J. Biol. Chem.* 279, 26274–26279
12 Kasai, A. *et al.* (2004) Apelin is a novel angiogenic factor in retinal endothelial cells. *Biochem. Biophys. Res. Commun.* 325, 395–400
13 De Mota, N. *et al.* (2000) Cloning, pharmacological characterization and brain distribution of the rat apelin receptor. *Neuroendocrinology* 72, 400–407
14 O'Carroll, A.M. *et al.* (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
15 Hosoya, M. *et al.* (2000) Molecular and functional characteristics of APJ. Tissue distribution of mRNA and interaction with the endogenous ligand apelin. *J. Biol. Chem.* 275, 21061–21067
16 Katugampola, S.D. *et al.* (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
17 Szokodi, I. *et al.* (2002) Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. *Circ. Res.* 91, 434–440
18 Kleinz, M.J. *et al.* (2005) Immunocytochemical localisation of the apelin receptor, APJ, to human cardiomyocytes, vascular smooth muscle and endothelial cells. *Regul. Pept.* 126, 233–240
19 Chen, M.M. *et al.* (2003) Novel role for the potent endogenous inotrope apelin in human cardiac dysfunction. *Circulation* 108, 1432–1439
20 Ashley, E.A. *et al.* (2005) The endogenous peptide apelin potently improves cardiac contractility and reduces cardiac loading *in vivo*. *Cardiovasc. Res.* 65, 73–82
21 Berry, M.F. *et al.* (2004) Apelin has *in vivo* inotropic effects on normal and failing hearts. *Circulation* 110 (Suppl. 1), II187–II193
22 Lee, D.K. *et al.* (2006) Unravelling the roles of the apelin system: prospective therapeutic applications in heart failure and obesity. *Trends Pharmacol. Sci.* 27, 190–194
23 Wang, G. *et al.* (2003) Apelin, a new enteric peptide, localization in the gtogastrointestinal tract, ontogeny, stimulation of gcgastric cell proliferation and of cholecystokinin secretion. *Endocrinology* 145, 1342–1348
24 Susaki, E. *et al.* (2005) Apelin cells in the rat stomach. *Regul. Pept.* 129, 37–41
25 Lambrecht, N.W. *et al.* (2006) Transcriptomes of purified gastric ECL and parietal cells: identification of a novel pathway regulating acid secretion. *Physiol. Genomics* 25, 153–165
26 Sorhede Winzell, M. *et al.* (2005) The apj receptor is expressed in pancreatic islets and its ligand, apelin, inhibits insulin secretion in mice. *Regul. Pept.* 131, 12–17
27 Boucher, J. *et al.* (2005) Apelin, a newly identified adipokine up-regulated by insulin and obesity. *Endocrinology* 146, 1764–1771
28 Daviaud, D. *et al.* (2006) TNFalpha up-regulates apelin expression in human and mouse adipose tissue. *FASEB J.* 20, 1528–1530
29 Xie, H. *et al.* (2006) Apelin and its receptor are expressed in human osteoblasts. *Regul. Pept.* 134, 118–125
30 Habata, Y. *et al.* (1999) Apelin, the natural ligand of the orphan receptor APJ, is abundantly secreted in the colostrum. *Biochim. Biophys. Acta* 1452, 25–35
31 Edinger, A.L. *et al.* (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
32 Choe, H. *et al.* (1998) The orphan seven-transmembrane receptor apj supports the entry of primary T-cell-line-tropic and dualtropic human immunodeficiency virus type 1. *J. Virol.* 72, 6113–6118
33 Cayabyab, M. *et al.* (2000) Apelin, the natural ligand of the orphan seven-transmembrane receptor APJ, inhibits human immunodeficiency virus type 1 entry. *J. Virol.* 74, 11972–11976
34 Puffer, B.A. *et al.* (2000) Expression and coreceptor function of APJ for primate immunodeficiency viruses. *Virology* 276, 435–444
35 Zou, M.X. *et al.* (2000) Apelin peptides block the entry of human immunodeficiency virus (HIV). *FEBS Lett.* 473, 15–18
36 Saint-Geniez, M. *et al.* (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
37 Cox, C.M. *et al.* (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
38 Inui, M. *et al.* (2006) Xapelin and Xmsr are required for cardiovascular development in *Xenopus laevis*. *Dev. Biol.* 298, 188–200
39 Springer, M.L. (2006) A balancing act: therapeutic approaches for the modulation of angiogenesis. *Curr. Opin. Investig. Drugs* 7, 243–250
40 Smith, L.E. *et al.* (1994) Oxygen-induced retinopathy in the mouse. *Invest. Ophthalmol. Vis. Sci.* 35, 101–111
41 Campochiaro, P.A. (2000) Retinal and choroidal neovascularization. *J. Cell. Physiol.* 184, 301–310
42 Folkman, J. (1971) Tumor angiogenesis: therapeutic implications. *N. Engl. J. Med.* 285, 1182–1186
43 Verheul, H.M. *et al.* (2004) Are tumours angiogenesis-dependent? *J. Pathol.* 202, 5–13
44 Ferrara, N. and Kerbel, R.S. (2005) Angiogenesis as a therapeutic target. *Nature* 438, 967–974
45 Campochiaro, P.A. (2006) Potential applications for RNAi to probe pathogenesis and develop new treatments for ocular disorders. *Gene Ther.* 13, 559–562
46 Ng, E.W. *et al.* (2006) Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. *Nat. Rev. Drug Discov.* 5, 123–132
47 Gerhardt, H. *et al.* (2003) VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J. Cell Biol.* 161, 1163–1177
48 Klement, G. *et al.* (2000) Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity. *J. Clin. Invest.* 105, R15–R24
49 Black, W.R. and Agner, R.C. (1998) Tumour regression after endostatin therapy. *Nature* 391, 450
50 Boehm, T. *et al.* (1997) Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. *Nature* 390, 404–407