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COMMENTARY

Angiogenesis: a new physiological role for *N*-arachidonoyl serine and GPR55?

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N-arachidonoyl serine (ARA-S) is one of a number of acyl amino acids recently identified in mammalian tissues. It has been referred to as an endocannabinoid-like lipid largely based on its structural similarities with the endocannabinoid, *N*-arachidonoyl ethanolamide (anandamide). However, little is known about its potential physiological functions and receptor targets. In this issue of the *British Journal of Pharmacology*, Zhang and colleagues show that ARA-S is a potent inducer of endothelial cell proliferation and migration, and angiogenesis *in vitro*. Furthermore, this pro-angiogenic action is mediated, at least partly, by activation of the poorly characterized, G-protein-coupled GPR55 receptor. ARA-S, via GPR55, increases phosphorylation of extracellular signal-regulated kinases and Akt, and vascular endothelial growth factor signalling. These exciting findings highlight the endothelium as an endogenous target for ARA-S and GPR55.
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Abbreviations: ARA-S, *N*-arachidonoyl serine; VEGF, vascular endothelial growth factor

Endocannabinoids, including *N*-arachidonoyl ethanolamide (anandamide), are novel lipid signalling molecules that activate cannabinoid CB_1 and/or CB_2 receptors, and, in some cases, the transient receptor potential vanilloid type1 (TRPV1) receptor (see Howlett *et al.*, 2002). They are believed to play a role, for example, in fine tuning neuronal activation, cardiovascular functions and inflammatory responses (Howlett *et al.*, 2002). While the endocannabinoid system continues to be a focus of much research effort, technological advances in detecting traces of cellular lipids have led to the identification of an increasing number of novel lipids that share structural similarities with endocannabinoids (Bradshaw and Walker, 2005). However, little information on their synthetic and degradation pathways, as well as biological activities, is currently available. One of these endogenous lipids, *N*-arachidonoyl serine (ARA-S) is an example of an acyl amino acid detected in mammalian tissues (Huang *et al.*, 2001). ARA-S induces arterial relaxation (EC_{50} ~1–10 μM; Milman *et al.*, 2006; Godlewski *et al.*, 2009; Parmar and Ho, 2010), and thus might play a role in the regulation of blood flow. Unlike anandamide, ARA-S has negligible activity at CB_1, CB_2 and TRPV1 receptors (Milman *et al.*, 2006). Instead, vasorelaxation may partly be mediated by a novel, $G_{i/o}$-coupled receptor in the endothelium, and partly through direct activation of large-conductance Ca^{2+}-activated K^+ channels in the vascular smooth muscle (Milman *et al.*, 2006; Godlewski *et al.*, 2009).

In this issue of the *British Journal of Pharmacology*, Zhang *et al.* (2010) provide convincing evidence that ARA-S acts as a pro-angiogenic factor, in addition to being a vasorelaxant. The authors show that ARA-S promotes proliferation and migration of primary human dermal microvascular endothelial cells, with maximal effect achieved at 1 μM. Furthermore, ARA-S also increases endothelial tube formation (by 50%) and neovascularization (by 100%) *in vitro*, suggesting a potent pro-angiogenic action. ARA-S appears to act by increasing the release of vascular endothelial growth factor C (VEGF-C), which is an important regulator of angiogenesis, and expression of VEGF-2 and VEGF-3 receptors. Interestingly, in contrast, cannabinoids have been reported to reduce endothelial cell survival and migration (Blázquez *et al.*, 2003). Anandamide, via CB_1 receptors, also suppresses VEGF and VEGF-1 receptor expression in tumour cells *in vivo* (Portella *et al.*, 2003), indicating that ARA-S and anandamide exert opposing effects on angiogenesis. To better understand the role of

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ARA-S as a pro-angiogenic factor, investigation into the synthetic and degradation pathways for ARA-S, and perhaps endogenous regulators of tissue contents of ARA-S (and anandamide) especially in cases of endothelial damage, is much needed. Notably, the relevant cellular source and (patho) physiological level of ARA-S remain unknown. *In vivo* effects of ARA-S on angiogenesis in wound healing and/or tumour growth should also be explored.

In an effort to elucidate the mechanisms of actions of ARA-S, Zhang *et al*. (2010) also reveal a new physiological function for the poorly characterized G-protein-coupled receptor, GPR55 (Ross, 2009). At present, the pharmacology of GPR55 is highly controversial; L-α-lysophosphatidylinositol is thought to be the endogenous agonist, whereas cannabinoid receptor ligands might also activate the receptor depending on the cellular system and functional assay used (see Ross, 2009). GPR55 has been implicated as a novel, $G_{i/o}$-coupled endothelial cannabinoid (CB_x) receptor which modulates vascular tone. This CB_x receptor is distinct from CB_1 and CB_2 receptors, but has been suggested to contribute to vasorelaxation to anandamide (Howlett *et al*., 2002) and cannabinoid receptor-inactive arachidonoyl amino acids (ARA-S, Milman *et al*., 2006; *N*-arachidonoyl glycine, Parmar and Ho, 2010). Unfortunately, research in this area has been hampered by the limited pharmacological tools available; cannabidiol and O-1918 are often used as GPR55 and CB_x antagonists, respectively, but their specificity is questionable (Lauckner *et al*., 2008; Godlewski *et al*., 2009; Parmar and Ho, 2010). Here, the authors overcome this hurdle by using siRNA technique to specifically knock down GPR55 expression in endothelial cells. They confirm the expression of GPR55 in the primary human endothelial cells used, and, more importantly, demonstrate that GPR55 knock-down partially inhibits the pro-angiogenic effects of ARA-S. Interestingly, knock-down of GPR55 alone also reduces basal endothelial tube formation in the absence of ARA-S, substantiating the role of GPR55 in the regulation of endothelial function and angiogenesis. In theory, GRP55 could therefore serves as a novel target for improving circulation in persistent wounds and ischaemic heart disease. It would also be beneficial to explore if GPR55 knock-out animals are more resistant to tumour growth and metastasis.

Previous studies suggest that GPR55 is coupled to $G_{12/13}$ (and probably G_q) proteins, and thus activates the small GTPase, RhoA, and causes Ca^{2+} mobilization (Ross, 2009). In contrast, ARA-S appears to act via $G_{i/o}$ proteins, and downstream phosphorylation of extracellular signal-regulated kinases and Akt, which have long been associated with cell proliferation and migration, supporting the proposal that GPR55 displays agonist-dependent signalling pathways (Ross, 2009). This is the first indication that ARA-S can activate GPR55. Importantly, however, a direct agonist action by ARA-S on GPR55 was not verified. Further studies will be needed to test if other intermediates are involved. It should also be borne in mind that signalling mechanisms independent of GPR55 might also be deployed by ARA-S.

Beyond the role of GPR55 and ARA-S in angiogenesis, Zhang *et al*. (2010) provide tantalizing evidence for GPR55 in vascular reactivity responses to ARA-S. The apparent coupling of endothelial GPR55 to $G_{i/o}$, instead of $G_{12/13}$, is consistent with the ability of *Pertussis* toxin, a $G_{i/o}$ uncoupler, to inhibit vasorelaxation attributed to CB_x receptor (e.g. ARA-S, Milman *et al*., 2006). In addition, O-1918, which has unproven action on GPR55, antagonizes both the vasorelaxation (Milman *et al*., 2006) and endothelial tube formation (Zhang *et al*., 2010) induced by ARA-S. This mirrors the observation that abnormal cannabidiol, a putative agonist for the vascular CB_x receptor, also enhances endothelial cell migration through $G_{i/o}$ and Akt activation (Mo *et al*., 2004). However, as Zhang and colleagues suggest, it still remains to identify definitively the GPR55 as the long sought-after CB_x receptor involved in vasorelaxation. Given the conflicting results obtained from studies using GPR55 over-expressed in cultured cells and the lack of established selective antagonists, molecular approaches that inhibit the function of native receptors, similar to those used in the current study, would seem more desirable. Of particular interest would be to examine the effects of siRNA knock-down of GPR55, or whole animal knock-out of GPR55, on the vasorelaxant responses to ARA-S and anandamide.

Zhang *et al*. (2010) have clearly shown that ARA-S and GPR55 regulate endothelial function and promote angiogenesis. This paper represents an exciting step forward in improving our understanding of the physiological roles of both the endogenous lipid and the receptor. It also raises intriguing questions that will undoubtedly be addressed in future studies.

Conflict of interest

The author states no conflict of interest.

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