

PERSPECTIVE

A New Endothelial Target for Cannabinoids

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The article by Offertaler and colleagues in this issue contains evidence pointing to a novel vascular site of action of cannabinoids. The precise vascular actions of endogenous cannabinoids have been surprising complex and controversial (see Hillard, 2000; Ralevic et al., 2002; Randall et al., 2002). In 1995, Ellis et al. demonstrated that anandamide caused cerebrovascular vasodilatation in the rat via the release of prostanoids. The following year, Randall et al. (1996) demonstrated that anandamide was a relaxant in the rat mesenteric vasculature but that this did not involve cyclooxygenase products. Since these initial reports, a variety of mechanisms has been proposed for the vascular actions of anandamide. These have included the release of nitric oxide (Deutsch et al., 1997), activation of potassium channels (Randall et al., 1996, 1997), conversion to metabolites of arachidonic acid (Pratt et al., 1998), the activation of gap junctions and presumed mediation via the endothelium-derived hyperpolarizing factor (EDHF) (Chaytor et al., 1999), blockade of calcium channels (Gebremedhin et al., 1999), and the involvement of prostanoids (Fleming et al., 1999). Perhaps most intriguing was the 1999 proposal that anandamide was in fact an endogenous vanilloid that acted via sensory nerves to release transmitters such as calcitonin gene-related peptide, which mediated the vasorelaxation (Zygmunt et al., 1999). However, almost without exception each proposal or demonstration has been accompanied by subsequent reports that did not support the initial findings, and this may reflect both species and methodological differences between studies. Two particular areas of uncertainty were the involvement of the endothelium and the role of currently recognized cannabinoid CB receptors. Much of the latter stems from the uncertainty surrounding the selectivity of the “classic” cannabinoid CB₁ receptor antagonist SR141716A. Having said that, the strongest evidence for the involvement of cannabinoid CB₁ receptors came from the absence of cardiovascular responses to anandamide in CB₁ knockout mice (Ledent et al., 1999).

In 1999, Wagner and colleagues (Jarai et al., 1999) pro-

posed that anandamide acted, in part, via an endothelial anandamide receptor in rat mesenteric vessels. This was based on the observation that relaxation to anandamide was partly sensitive to both removal of the endothelium and SR141716A, but when the endothelium was removed, the sensitivity to the antagonist was lost. This led to the proposal that anandamide acted on a cannabinoid receptor that was sensitive to SR141716A but was not the CB₁ receptor and was termed the “anandamide receptor”. An additional observation was that the exogenous cannabinoid, Δ^9 -tetrahydrocannabinol, did not cause vasorelaxation. Subsequent work by that group, demonstrated that the endothelial cannabinoid receptor was also activated by the neurobehaviorally inactive “abnormal cannabidiol” (abn-cbd), which caused vasorelaxation (Jarai et al., 1999). One possibility to arise from the identification of the SR141716A-sensitive endothelium-dependent component is that anandamide acts in part via EDHF and that SR141716A is acting via inhibition of EDHF activity (e.g., through blockade of myoendothelial gap junctions) (Chaytor et al., 1999). However, Mukhopadhyay et al. (2002) demonstrated that the endothelium-dependent component was G-protein-coupled and mediated via nitric oxide whereas the endothelium-independent component was due to activation of vanilloid receptors, at least in rabbit aortic rings.

In the present issue, Kunos and colleagues (Offertaler et al., 2003) provide further characterization of the “endothelial anandamide” receptor. Specifically they report that a novel cannabidiol analogue, O-1918, opposes the relaxant effects of anandamide and abn-cbd, the hypotensive effects of abn-cbd and the phosphorylation of p42/44 MAP kinase induced by abn-cbd in endothelial cells. These actions of O-1918 are independent of CB₁ and CB₂ receptors, and this led the authors to conclude that O-1918 was a selective antagonist of the endothelial anandamide receptor. The authors point out that it is unlikely that O-1918 is acting at a different recognition site on CB₁ or CB₂ receptors because previous studies have shown that abn-cbd causes relaxation in vessels from

ABBREVIATIONS: EDHF, endothelium-derived hyperpolarizing factor; SR141716A, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboximide hydrochloride; abn-cbd, abnormal cannabidiol; MAP, mitogen-activated protein.

CB₁/CB₂ knockout mice (Jarai et al., 1999). The findings in this article, therefore, go a long way in confirming the existence of a novel cannabinoid receptor. Indeed, this has been proposed by other authors to account for actions of cannabinoids that are not blocked by currently available CB₁, CB₂, or vanilloid receptor antagonists. Therefore, O-1918 may provide an important pharmacological tool for others to investigate the contribution of the novel receptor to other effects attributable to endogenous and exogenous cannabinoids.

There is of course a note of caution. Although this article points to O-1918 opposing the vascular effects of anandamide, there is no demonstration of its selectivity. The ability of increasing micromolar concentrations of O-1918 to oppose vasorelaxation to abn-cbd in an apparently competitive manner certainly points to selectivity, but further experiments are clearly required to confirm that O-1918 does not interfere with putative distal sites of action such as potassium channels. This point is particularly important because the use of SR141716A was confused by non-cannabinoid effects.

A further question is raised by the previous identification of CB₁ receptors in human endothelial cells by Liu et al. (2000). In that previous study, mRNA for the CB₁ receptor was identified in human endothelial cells, and the receptors were shown to be coupled to MAP kinase. This may suggest that CB₁ receptors exist alongside the novel O-1918-sensitive receptor or may point to species differences. Clearly, there is now a need to examine whether O-1918 also opposes responses to anandamide and abn-cbd in human vessels. Similarly, how are the present findings resolved with the lack of cardiovascular effects of anandamide in CB₁ knockout mice (Lederent et al., 1999)? Once again, this is a controversial point; Jarai et al. (1999) demonstrated that abn-cbd caused vasorelaxation in mesenteric vessels from CB₁ and CB₁/CB₂ knockout mice, consistent with a non-CB₁/CB₂ action.

The findings with O-1918 provide new insights into cannabinoid pharmacology. Furthermore, the subsequent data also provide important information regarding the vascular actions of anandamide. In brief, the data suggest that the endothelium-dependent relaxation to abn-cbd and anandamide is G-protein-coupled to MAP kinase activation and charybdotoxin-sensitive potassium channels but not to nitric oxide. Taken together, the authors propose that the novel receptor may be coupled to the release of EDHF. The effects of abn-cbd were also shown to be independent of vanilloid receptors. Given this latter finding, it remains to be deter-

mined what mediates the substantial endothelium-independent relaxation to abn-cbd? If this is not due to sensory nerves, what is it due to? Do anandamide and abn-cbd have identical actions?

In summary, the article by Offertaler et al. provides us with a potential antagonist of a novel cannabinoid receptor but caution must be exercised as its pharmacological selectivity has yet to be defined. The authors also go some way to supporting a role for EDHF in mediating responses to anandamide but the mechanisms that underlie the endothelium-independent relaxation have yet to be defined.

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