British Journal of Pharmacology (2007) 152, 559–561
 0007–1188/07 $30.00
www.brjpharmacol.org

COMMENTARY

GPR55 and the vascular receptors for cannabinoids

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CB_1 and CB_2 receptors mediate most responses to cannabinoids but not some of the cardiovascular actions of endocannabinoids such as anandamide and virodhamine, or those of some synthetic agents, like abnormal cannabidiol (abn-cbd). These agents induce vasorelaxation which is antagonised by rimonabant but only at high concentrations relative to those required to block CB_1 receptors. Vasorelaxation to anandamide is sensitive to *Pertussis* toxin (though that to abn-cbd is not), and so is thought to be mediated by a G protein-coupled receptor through $G_{i/o}$. An orphan receptor, GPR55, apparently a cannabinoid receptor, is activated by abn-cbd, but is not the receptor mediating vasorelaxation to this agent, as the response persists in vessels from GPR55 knockout mice. However, the activity of anandamide in GPR55 knockout mice is not yet reported and so the role of GPR55 as a cannabinoid receptor mediating vascular responses has yet to be finalised.
British Journal of Pharmacology (2007) **152,** 559–561; doi:10.1038/sj.bjp.0707421; published online 20 August 2007

Keywords: cannabinoids; cannabinoid receptor; GPR55; abnormal cannabidiol; rimonabant; $G_{i/o}$ G protein; blood pressure; blood vessel; endocannabinoids

Abbreviations: abn-cbd, abnormal cannabidiol; O-1602, *trans*-4-[3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-methyl-1,3-benzenediol; O-1918, (−)-1,3-dimethoxy-2-(3-3,4-*trans*-*p*-menthadien-(1,8)-yl)-orcinol; PTX, *Pertussis* toxin; THC, Δ^9-tetrahydrocannabinol; WIN 55,212-2, R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]-pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)-methanone

Central and peripheral responses to cannabinoids are most often associated with actions at one or another of two cloned receptors, designated the CB_1 and the CB_2 receptors (Howlett *et al*., 2002). However, vasorelaxation in the rat mesenteric arterial system, either in response to the endocannabinoid, anandamide (White and Hiley, 1997; Wagner *et al*., 1999), or to the phytocannabinoid, Δ^9-tetrahydrocannabinol (THC; O'Sullivan *et al*., 2005a), is not consistent with action at these receptors. For example, the concentrations of rimonabant, a CB_1 receptor antagonist, required to block the actions of anandamide are much higher than those needed to block CB_1 receptors (White and Hiley, 1998) whereas the vasorelaxation to THC is insensitive to it (O'Sullivan *et al*., 2005a). Other blood vessels, such as the rat coronary artery (Ford *et al*., 2002) and rabbit aorta (Mukhopadhyay *et al*., 2002), also show vasorelaxant responses to cannabinoids that are not consistent with actions at CB_1 or CB_2 receptors although relaxation of rat aorta to THC is sensitive to CB_2, rather than CB_1, receptor blockade (O'Sullivan *et al*., 2005b).

Rather more substantial evidence for the existence of a novel cannabinoid receptor in the cardiovascular system has come from studies on abnormal cannabidiol (abn-cbd), a synthetic analogue of another phytocannabinoid, cannabidiol. It, like anandamide, evokes mesenteric vascular relaxation but is equally effective in wild-type and cannabinoid receptor knockout mice (Járai *et al*., 1999; Wagner *et al*., 1999). The responses to abn-cbd and anandamide have many similarities (Járai *et al*., 1999; Wagner *et al*., 1999; Ho and Hiley, 2003) and have been suggested to be due to actions on an additional cannabinoid receptor, the 'endothelial anandamide receptor' (Wagner *et al*., 1999; Offertáler *et al*., 2003). Identification of activity at this receptor is greatly aided by the fact that it is sensitive to antagonism by (−)-1,3-dimethoxy-2-(3-3,4-*trans*-*p*-menthadien-(1,8)-yl)-orcinol (O-1918), an analogue of abn-cbd (Offertáler *et al*., 2003). Since the actions of anandamide and abn-cbd are sensitive to inhibition by *Pertussis* toxin (PTX; White and Hiley, 1997; Járai *et al*., 1999), the novel receptor is thought to be G protein-coupled and act through $G_{i/o}$.

Abn-cbd and O-1918 therefore provide tools to evaluate candidate proteins as novel vascular cannabinoid receptors. The most promising field to search is the large number of orphan G protein-coupled receptors encoded within the human genome. One of these, GPR55, has been reported in patents to be activated by cannabinoid ligands (Baker *et al*., 2006), although rather little is otherwise known about it other than a brief report of its distribution in human brain (Sawzdargo *et al*., 1999). Its position as a cannabinoid receptor has been challenged on the basis of an *in silico* comparison of the 'functional fingerprint' of its putative binding pocket for agonists with those of the CB_1 and CB_2 receptors (Petitet *et al*., 2006) and so the appearance, in this

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Received 11 July 2007; accepted 20 July 2007; published online 20 August 2007

issue, of a report from the laboratories of GlaxoSmithKline by Johns *et al*. (2007) is especially welcome. This paper fills in some of the uncertainties concerning the candidacy of GPR55 as the vascular cannabinoid receptor for anandamide and abn-cbd but also leaves a number of tantalizing questions.

Johns *et al*. (2007) report that GPR55, when expressed in HEK293 cells, is activated in the low nanomolar concentration range by both abn-cbd and *trans*-4-[3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-methyl-1,3-benzenediol (O-1602), another cannabidiol analogue, as assessed by GTPγS binding. In contrast, a patent from AstraZeneca (Drmota *et al*., 2004) shows abn-cbd to be 1000-fold less potent than this in a similar assay. On the other hand both reports agree that R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)-methanone (WIN 55, 212-2), an agonist which is relatively non-selective between CB_1 and CB_2 receptors, has no effect in these assays. Together, these observations support the notion that GPR55 is a receptor for cannabinoid analogues with a distinct activation profile from that of the well-characterized CB_1 and CB_2 receptors.

A major strength of the paper by Johns *et al*. (2007) is the use of GPR55 knockout mice to characterize the cardiovascular role of this receptor. Interestingly, the knockout mice show no significant difference in basal heart rate and blood pressure but this conflicts with a report that GPR55 knockout mice are hypertensive which was given by Greasley from the AstraZeneca group in an unpublished talk to the Oxford Meeting of the British Pharmacological Society in December 2006. Johns *et al*. (2007) found that abn-cbd lowered systemic blood pressure in both the knockout and the wild-type mice, suggesting that this response is not mediated by GPR55. In the myograph, abn-cbd relaxed mesenteric arteries from both strains of mice with similar EC_{50} values (wild-type, 255 ± 88 nM; GPR55 knockout, 516 ± 176 nM) and sensitivity to O-1918 (K_B: wild-type, $1.8 \pm 1.3\,\mu M^{-1}$; GPR55 knockout, $2.8 \pm 2.0\,\mu M^{-1}$); the K_B values compare with one of $1.9\,\mu M^{-1}$ estimated from the data in Offertáler *et al*. (2003).

So, both Drmota *et al*. (2004) and Johns *et al*. (2007) show clearly that GPR55 is a receptor for cannabinoid analogues, but is it the 'endothelial anandamide receptor'? The GlaxoSmithKline group have studied abn-cbd and O-1602, but not anandamide itself either in the blood vessels from GPR55 knockout mice or in the GTPγS assay in cells transfected with GPR55. Furthermore, they have not shown whether or not either the GTPγS response or the vascular responses to abn-cbd are sensitive to PTX; this is a consensual observation for the vasorelaxation evoked by anandamide in rat mesenteric arteries (White and Hiley, 1997; Járai *et al*., 1999). On the other hand, in functional studies of rat mesenteric artery, vasorelaxation to abn-cbd is not sensitive to PTX (Ho and Hiley, 2003) whereas its actions on p42/44 mitogen-activated protein kinase and protein kinase B/Akt, in human umbilical vein endothelial cells are sensitive to toxin pretreatment (Offertáler *et al*., 2003). Perhaps these responses are mediated by different receptors, both of which are sensitive to antagonism by O-1918. One of these receptors could be the 'anandamide receptor', whereas the other is the receptor mediating the abn-cbd response in the GPR55 knockout mice. Alternatively, one receptor could couple to $G_{i/o}$ and to another signalling pathway to mediate the different responses. Indeed, a GlaxoSmithKline patent concerning GPR55 reports that it couples through G_{12} or G_{13} when investigated in a yeast and this would not be expected to be PTX-sensitive (Brown and Wise, 2001).

Johns *et al*. (2007) have also not shown if the GTPγS response in GPR55-expressing cells to abn-cbd is sensitive to the abn-cbd antagonist which would help show up further pharmacological similarity or dissimilarity between this receptor and the vascular responses. Responses to both anandamide and abn-cbd are antagonised by O-1918 but they show slightly different sensitivity; at a concentration of $1\,\mu M$, O-1918 induced a shift of approximately threefold against abn-cbd (Offertáler *et al*., 2003) but 10-fold against anandamide (O'Sullivan *et al*., 2004). This might be due to experimental variation, as not many results with O-1918 are yet reported, or it could be that the antagonist acts at two receptors for cannabinoids; further information about the actions of O-1918 will be welcome.

Johns *et al*. (2007) have clearly shown that GPR55 does not mediate the vascular responses to abn-cbd in the mouse mesenteric artery even though it is a potent agonist at the human receptor in a recombinant system. Although previous results with this agonist have been from the rat mesenteric bed, it seems unlikely that a species difference underlies this result. Therefore, investigations of GPR55 in the vasculature await the arrival of more ligands as well as more data from the AstraZeneca group to throw light on the apparent discrepancies between their results and those of Johns *et al*. (2007). The most prominent of these are the possible differences in blood pressure in GPR55 knockout mice and the large discrepancy in the reported potency of abn-cbd in cells expressing GPR55.

Conflict of interest

The authors state no conflict of interest.

References

Baker D, Pryce G, Davies WL, Hiley CR (2006). *In silico* patent searching reveals a new cannabinoid receptor. *Trends Pharmacol Sci* **27**: 1–4.

Brown AJ, Wise A (2001). GlaxoSmithKline. Identification of modulators of GPR55 activity. Patent WO01/86305.

Drmota T, Greasley P, Groblewski T (2004). AstraZeneca. Screening assays for cannabinoid-ligand type modulators of GPR55. Patent WO2004/074844.

Ford WR, Honan SA, White R, Hiley CR (2002). Evidence of a novel site mediating anandamide-induced negative inotropic and coronary vasodilatator responses in rat isolated hearts. *Br J Pharmacol* **135**: 1191–1198.

Ho WS, Hiley CR (2003). Vasodilator actions of abnormal-cannabidiol in rat isolated small mesenteric artery. *Br J Pharmacol* **138**: 1320–1332.

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA *et al*. (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* **54**: 161–202.

Járai Z, Wagner JA, Varga K, Lake KD, Compton DR, Martin BR *et al*. (1999). Cannabinoid-induced mesenteric vasodilation through an

endothelial site distinct from CB_1 or CB_2 receptors. *Proc Natl Acad Sci USA* **96**: 14136–14141.

Johns DG, Behm DJ, Walker D, Ao Z, Shapland EM, Daniels DA *et al.* (2007). The novel endocannabinoid receptor GPR55 binds atypical cannabinoids but does not mediate their vasodilatory effects. *Br J Pharmacol* **152**: 825–831 (this issue).

Mukhopadhyay S, Chapnick BM, Howlett AC (2002). Anandamide-induced vasorelaxation in rabbit aortic rings has two components: G protein dependent and independent. *Am J Physiol* **282**: H2046–H2054.

Offertáler L, Mo FM, Bátkai S, Liu J, Begg M, Razdan RK *et al.* (2003). Selective ligands and cellular effectors of a G protein-coupled endothelial cannabinoid receptor. *Mol Pharmacol* **63**: 699–705.

O'Sullivan SE, Kendall DA, Randall MD (2004). Heterogeneity in the mechanisms of vasorelaxation to anandamide in resistance and conduit rat mesenteric arteries. *Br J Pharmacol* **142**: 435–442.

O'Sullivan SE, Kendall DA, Randall MD (2005a). The effects of Δ^9-tetrahydrocannabinol in rat mesenteric vasculature, and its interactions with the endocannabinoid anandamide. *Br J Pharmacol* **145**: 514–526.

O'Sullivan SE, Kendall DA, Randall MD (2005b). Vascular effects of Δ^9-tetrahydrocannabinol (THC), anandamide and N-arachidonoyldopamine (NADA) in the rat isolated aorta. *Eur J Pharmacol* **507**: 211–221.

Petitet F, Donlan M, Michel A (2006). GPR55 as a new cannabinoid receptor: still a long way to prove it. *Chem Biol Drug Des* **67**: 252–253.

Sawzdargo M, Nguyen T, Lee DK, Lynch KR, Cheng R, Heng HH *et al.* (1999). Identification and cloning of three novel human G protein-coupled receptor genes GPR52, PsiGPR53 and GPR55: GPR55 is extensively expressed in human brain. *Brain Res Mol Brain Res* **64**: 193–198.

Wagner JA, Varga K, Járai Z, Kunos G (1999). Mesenteric vasodilation mediated by endothelial anandamide receptors. *Hypertension* **33**: 429–434.

White R, Hiley CR (1997). A comparison of EDHF-mediated and anandamide-induced relaxations in the rat isolated mesenteric artery. *Br J Pharmacol* **122**: 1573–1584.

White R, Hiley CR (1998). The actions of some cannabinoid receptor ligands in the rat isolated mesenteric artery. *Br J Pharmacol* **125**: 533–541.