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COMMENTARY

Tuning the endocannabinoid system: allosteric modulators of the CB_1 receptor

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Cannabinoid CB_1 receptor antagonists are novel therapeutics with potential for the treatment of a number of conditions including obesity, nicotine addition and metabolic syndrome. In 2005, Price *et al.* demonstrated that the cannabinoid CB_1 receptor contains an allosteric-binding site which binds synthetic small molecules. In this issue of the *British Journal of Pharmacology,* Horswill *et al.* have extended these observations. They demonstrate that a structurally similar small molecule allosterically modulates the cannabinoid CB_1 receptor and reduces body weight and food intake in an acute feeding model. Allosteric modulation now contends as a new strategy in the therapeutic exploitation of cannabinoid receptors that may offer certain advantages over the more familiar small molecules targeting the orthosteric site.
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The most studied endocannabinoids are arachidonoylethanolamide (anandamide) and 2-arachidonoyl glycerol (2-AG). These eicosanoids have a neuromodulatory role and are key signalling molecules involved in a range of physiological processes including appetite regulation, pain and memory processing, and addiction. 2-AG is a retrograde signalling molecule and modulates synaptic transmission by interaction with presynaptic cannabinoid CB_1 receptors to inhibit neurotransmitter release. Within the brain, the distribution of CB_1 receptors is heterogeneous; the cerebral cortex, hippocampus, lateral caudate-putamen, substantia nigra pars reticulata, globus pallidus, entopeduncular nucleus and the molecular layer of the cerebellum are all highly populated with CB_1 receptors (Christopoulos and Kenakin, 2002). CB_1 receptor antagonists are being developed directed towards the treatment of obesity (Horvath, 2003; Duffy and Rader, 2007), osteoporosis (Idris *et al.*, 2005), nicotine addiction (Le Foll and Goldberg, 2004) and mental illness (Muccioli and Lambert, 2005).

A few years ago, Price *et al.* (2005) identified three novel allosteric modulators of the cannabinoid CB_1 receptor. These compounds, synthesized at Organon research, display a number of features that are characteristic of allosteric modulators. Firstly, enhancement of the equilibrium binding of the orthosteric ligand, [^{3}H]CP55950, to mouse brain membranes. Secondly, a ligand-dependent effect, as demonstrated by distinct effects on the equilibrium binding of the different orthosteric radioligands to mouse brain membranes. Thirdly, a slowing of the dissociation rate constant(s) for [^{3}H]CP55940 from the occupied CB_1 receptor. Finally, non-competitive inhibition of orthosteric agonist efficacy: as demonstrated by the effect of the compounds on the E_{max} of CB_1 receptor agonists in stimulation of [^{35}S]GTPγS binding to mouse brain membranes, inhibition of electrically evoked contractions of the mouse vas deferens and in a hCB_1 receptor luciferase reporter assay. In this issue of the *British Journal of Pharmacology*, Horswill *et al*. (2007) report a patently similar profile for PSNCBAM-1; in HEK293-hCB_1 membranes, this compound increased the equilibrium binding of [^{3}H]CP55940, but produced a noncompetitive functional antagonism of agonist-stimulated [^{35}S]GTPγS binding and agonist-induced inhibition of cAMP production. The prosidion (PSN) and the organon (Org) compounds display a ligand-dependent effect, enhancing the specific binding of the CB_1/CB_2 receptor agonist [^{3}H]CP55940 but inhibiting the binding of the inverse agonist [^{3}H]SR141716A.

Clearly, these compounds display an intriguing dichotomy of effect on orthosteric ligand affinity versus efficacy; they are allosteric *enhancers* of agonist-binding *affinity* and allosteric *inhibitors* of agonist-signalling *efficacy*. Both studies converge to demonstrate a complex scenario for the *in vitro* pharmacology of these compounds. A key finding in the Horswill *et al*. paper is the first demonstration of *in vivo* activity of a CB_1 receptor allosteric modulator in an acute food-intake model. The CB_1 receptor inverse agonist, SR141716A (Acomplia) has recently been approved for treatment of obesity in Europe. Furthermore, a number of other therapeutic indications may be targets for compounds of this class, including nicotine addiction (Le Foll and Goldberg, 2004), the metabolic syndrome (Horvath, 2003;

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Duffy and Rader, 2007) and inflammation (Costa, 2007). The demonstration that a functional allosteric antagonist of the CB_1 receptor has a hypophagic effect *in vivo* announces a new strategy of drug discovery that may offer advantages over existing approaches for targeted manipulation of the endocannabinoid system.

Numerous CB_1 receptor antagonists have been synthesized that display a significant degree of subtype selectivity versus the CB_2 receptor (Alexander *et al.*, 2007). However, recently, it has been shown that certain well-established cannabinoid receptor ligands, including the inverse agonists, have affinity for an orphan receptor, GPR55 (Baker *et al*., 2006; Alexander *et al.*, 2007). Furthermore, certain cannabinoid ligands may interact also with TRPV1 receptors (Ross, 2003). As has been previously demonstrated for other GPCRs, synthesis of receptor subtype-selective ligands can be fraught by high sequence homology within the orthosteric-binding domain between receptor subtypes (Rees *et al*., 2002; Christopoulos and Kenakin, 2002). Invoking a CB_1 receptor allosteric site may provide a higher degree of subtype selectivity.

Currently, the CB_1 receptor antagonists advancing through clinical trials appear to be inverse agonists (Pertwee, 2005). Inverse agonists of G protein coupled receptor (GPCRs) are known to exert long-lasting effects on receptor function (Greasley and Clapham, 2006) that may be of consequence to patients on certain treatment regimes. Prolonged exposure to CB_1 receptor inverse agonists may prove to be detrimental, beneficial or inconsequential as compared to exposure to a neutral antagonist. The Org compounds do not behave as inverse agonists, having no effect on CB_1 receptor constitutive activity (Price *et al.*, 2005). Horswill *et al*. report that the PSN compound has no effect on constitutive activity in hCB_1 yeast report assays as compared to SR141716A. However, in the [^{35}S]GTPγS-binding assay, the PSN compound produced an inverse effect, albeit displaying significantly lower inverse efficacy than SR141716A.

The discovery of an allosteric modulator of the cannabinoid CB_1 receptor that has a hypophagic effect *in vivo* represents a new addition to the existing variety of ligands that recognize the cannabinoid receptors. Allosteric modulation now contends as a novel strategy in the therapeutic exploitation of these receptors that may offer certain advantages over the more familiar small molecules targeting the orthosteric-binding site. Future research will undoubtedly address the question of whether allosteric modulators of the CB_1 receptor are advantageous in certain pathophysiological scenarios by comparison with the currently available orthosteric ligands.

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