

Cannabinoid Receptors: Where They are and What They do

K. Mackie

Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN, USA

Journal of Neuroendocrinology

Correspondence to:
Ken Mackie, Department of
Psychological and Brain Sciences,
Indiana University, 1101 E. 10th
Street, Bloomington, IN 47401, USA
(e-mail: kmackie@indiana.edu).

The endocannabinoid system consists of the endogenous cannabinoids (endocannabinoids), cannabinoid receptors and the enzymes that synthesise and degrade endocannabinoids. Many of the effects of cannabinoids and endocannabinoids are mediated by two G protein-coupled receptors (GPCRs), CB₁ and CB₂, although additional receptors may be involved. CB₁ receptors are present in very high levels in several brain regions and in lower amounts in a more widespread fashion. These receptors mediate many of the psychoactive effects of cannabinoids. CB₂ receptors have a more restricted distribution, being found in a number of immune cells and in a few neurones. Both CB₁ and CB₂ couple primarily to inhibitory G proteins and are subject to the same pharmacological influences as other GPCRs. Thus, partial agonism, functional selectivity and inverse agonism all play important roles in determining the cellular response to specific cannabinoid receptor ligands.

Key words: agonist trafficking, partial agonism, protean agonism.

doi: 10.1111/j.1365-2826.2008.01671.x

The endocannabinoid system

The endocannabinoid system consists of endogenous cannabinoids (endocannabinoids), cannabinoid receptors and the synthetic and degrading enzymes responsible for synthesis and degradation of endocannabinoids (1–3). Endocannabinoids are so named because they were first identified as activating the same receptors as cannabinoids, the primary psychoactive components of cannabis. The first endocannabinoid identified was arachidonoyl ethanolamide (anandamide; from the Sanskrit for 'internal bliss') (2). Anandamide is only one of a large family of related bioactive acyl ethanolamides (4). The second endocannabinoid identified was 2-arachidonoyl glycerol (2-AG) (2).

The synthesis, cellular transport and degradation of endocannabinoids are tightly regulated processes (2). A feature that distinguishes endocannabinoids from many other neuromodulators is that they are not synthesised in advance and stored in vesicles. Rather, their precursors exist in cell membranes and are cleaved by specific enzymes. This form of synthesis is often referred to as 'on demand'. As the discussion of these processes is beyond the scope of this paper, the interested reader is referred to several recent papers on the topic (5–8).

The identification of cannabinoid receptors grew out of a desire to understand the psychoactive effects of Δ^9 -tetrahydro-

cannabinol (THC), the principal psychoactive component of cannabis. Although several experiments hinted at the existence of specific protein receptors for Δ^9 -THC, Allyn Howlett *et al.* (2) provided definitive proof for a cannabinoid receptor. Their work established that cannabinoids activated a G protein-coupled receptor (GPCR) that inhibited adenylyl cyclase. Furthermore, they developed a binding assay for this receptor and showed that quite high levels of this receptor were present in certain brain regions (2).

The development of high affinity cannabinoid receptor agonists (a by-product of pharmaceutical research) permitted the mapping of cannabinoid receptor distribution in the brain, by both Herkenham *et al.* (9) and Jansen *et al.* (10). Interestingly, cannabinoid receptors are among the most abundant GPCRs. These initial autoradiographic studies also established that cannabinoid binding sites are highest in the brain regions implicated in the actions of cannabis (9, 10).

The cloning of a cannabinoid receptor by Matsuda *et al.* (11), provided the final evidence for the existence of a cannabinoid receptor and permitted the identification of cannabinoid receptor-expressing neurones. This cloning was swiftly followed by the cloning of a second cannabinoid receptor, designated CB₂, from a promyelocytic cell line (12). (Of course, the first cannabinoid receptor was then designated as CB₁).

Although endocannabinoids clearly activate CB₁ and CB₂ receptors, they also interact with other GPCRs and ion channels. The best known and characterised of these ion channel interactions is the activation of vanilloid receptor-type 1 (TRPV1) channels by anandamide (13), but endocannabinoids also interact with several types of potassium channels, alpha7 nicotinic receptors and 5-HT₃ receptors, among others (14). A major challenge in this field is to understand those interactions that are physiologically relevant, those that are merely a consequence of physical interactions between these lipophilic molecules and hydrophobic membrane proteins, and those that do not occur under relevant patterns of endocannabinoid release. In addition to their interactions with ligand-gated channels, endocannabinoids can also activate GPCRs in addition to CB₁ and CB₂ receptors. The best known of these is GPR55. Recent studies indicate that anandamide and, possibly, 2-AG activates GPR55 (15).

Cannabinoid receptor localisation

Much can be learned about the role of a receptor by determining its localisation. Cannabinoid receptors are a particularly good example of this. CB₁ receptor localisation has been determined using quantitative autoradiography, *in situ* hybridisation and immunocytochemistry (16). Each of these techniques possesses both advantages and disadvantages and offers its own unique insight into the biology of the system under study. The autoradiographic studies performed by Herkenham *et al.* (9) were highly significant for the field for three reasons. First, they demonstrated that CB₁ receptors were expressed at high levels in the brain regions expected from the psychoactive effects of Δ⁹-THC. Conversely, CB₁ receptor expression was low in brain regions unaffected by cannabinoids, such as the respiratory centres of the medulla. Second, they allowed precise quantitation of CB₁ receptor levels and demonstrated that CB₁ receptors are expressed more abundantly than most other GPCRs. Finally, in combination with *in situ* hybridisation (see below), they suggested CB₁ receptors might be concentrated on axon terminals.

The cloning of the CB₁ receptor led to several *in situ* hybridisation studies (17, 18). These established that the pattern of CB₁ receptor mRNA expression in the forebrain was dichotomous. On the one hand, a small number of forebrain neurones expressed very high levels of CB₁ receptor mRNA. On the other, a large number of neurones expressed CB₁ receptor mRNA at more modest levels. In the case of laminar structures (e.g. hippocampus) or nuclei with distant axonal projections (e.g. caudate putamen), the combination of *in situ* hybridisation and autoradiography suggested the CB₁ receptors are preferentially present on axons and their terminals.

Immunocytochemical studies have provided a very high-resolution description of CB₁ receptor expression (16). High-quality immunocytochemical studies require a favourable combination of specific antibodies and abundant protein. Fortunately, CB₁ receptors have a surfeit of highly antigenic domains and the receptor is often present at high levels. High-resolution anatomical studies in the forebrain revealed that CB₁ receptors are expressed at very high levels in a subset of GABAergic interneurons, the cholecystikinin (CCK) containing basket cells (19). Quantitative electron microscopic

studies showed that most CB₁ receptors were found on the pre-terminal axonal segment and the axons themselves (20). Very little CB₁ receptor is present on more proximal axons, dendrites, or the cell body. In addition to high levels of CB₁ receptors on CCK positive basket cells, these receptors are found at lower levels on many glutamatergic terminals throughout the brain (21).

CB₁ receptors have also been found in a number of peripheral tissues. Various studies provide evidence (either immunocytochemical or mRNA) for CB₁ receptor expression in fat (adipocytes) (22), liver (23), pancreas (24) and skeletal muscle (25). Although the function of these receptors is under investigation, it is possible that they play a role in the metabolic consequences of CB₁ receptor blockade.

Determination of the distribution of CB₂ receptors has lagged considerably behind that for CB₁ receptors. There are many reasons for this, including the lower abundance of CB₂ receptors relative to CB₁ receptors and the difficulty in raising highly selective CB₂ receptor antibodies. Nonetheless, strong evidence supports the notion that CB₂ receptors are expressed on a number of immune cells (particularly those derived from macrophages, such as microglia, osteoclasts and osteoblasts) and neurones, under certain conditions (26–28).

Signalling of cannabinoid receptors

Both CB₁ and CB₂ receptors primarily signal through the inhibitory G proteins G_i and G_o (2). However, under certain conditions and with certain agonists, coupling via G_s and G_{q/11} has been demonstrated. The implications of inhibitory G protein activation is that stimulation of CB₁ receptors leads to the inhibition of adenylyl cyclase, the activation of mitogen-activated protein kinases, the inhibition of certain voltage-gated calcium channels and the activation of G protein-linked inwardly rectifying potassium channels (2). Stimulation of CB₂ receptors has similar consequences, except the modulation of ion channels by CB₂ receptors is more variable (2).

The activation of these signalling pathways by CB₁ receptors and the high levels of these receptors on presynaptic terminals means that endocannabinoid stimulation of CB₁ receptors suppresses neuronal excitability and inhibits neurotransmission (1). Substantial evidence indicates that endocannabinoids play a major role in several forms of neuronal plasticity, including short-term (spike time-dependent plasticity, depolarisation-induced suppression of inhibition/excitation and metabotropic-induced suppression of inhibition/excitation) and long-term (long-term depression) plasticity (29). Thus, even though endocannabinoids are produced in a transient fashion, their effects can be quite long lasting. Although most studies examining endocannabinoid effects on neurones have focused on neurotransmission, there is functional evidence for somatic CB₁ receptors, whose activation hyperpolarises neurones (30).

Integration of endocannabinoid physiology and the pharmacology of cannabinoid receptor ligands

Assessment of the various pharmacological strategies targeting cannabinoid receptors requires a thorough understanding of how

these potential drugs interact with cannabinoid receptors. Thus, the simple conceptual framework of agonists and antagonists needs to be refined to account for more complex interactions between receptors and their ligands. The sections below consider the concepts of partial agonism, functional selectivity and inverse agonism.

Partial agonism

Not all agonists will activate a receptor to the same extent. This can easily be visualised with dose-response curves. Figure 1 compares the actions of a partial agonist with a full agonist for a hypothetical drug. What makes a drug a partial agonist? Simplistically, a receptor can be considered to exist in an active and an inactive conformation. A full agonist will favour the active conformation. By contrast, a partial agonist does not distinguish as strongly between the two conformations and, even when all of the receptors are occupied by the partial agonist, only a fraction of them will be in the active conformation and thus be able to signal. The implication of this depends on the density of receptors relative to downstream signalling molecules. If receptors are limiting, then the biological response of a partial agonist will be less than that of a full agonist. On the other hand, if receptors are in excess, the

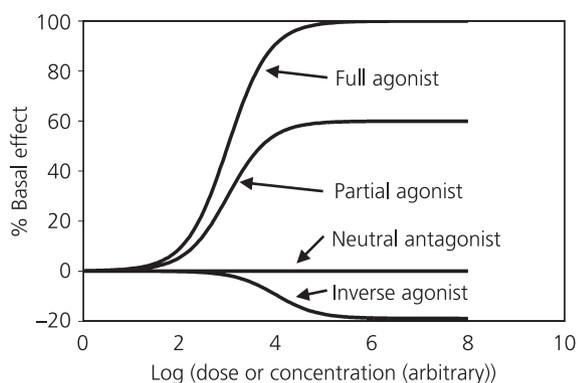


Fig. 1. Schematic of possible responses to various receptor ligands. A full (efficacious) agonist maximally stimulates a cellular response. A partial agonist (lower intrinsic efficacy) produces an intermediate response. A neutral antagonist has no detectable effect on the cellular response. Finally, an inverse agonist produces an effect opposite to that of an agonist.

maximal biological response will not be limited by the fraction of receptors occupied, but by downstream signalling molecules, and the maximal responses to a full agonist and partial agonist may be identical. An example of this is shown in Fig. 2 in an experiment investigating the activation of inwardly rectifying potassium channels by CB₁ receptors expressed in *Xenopus* oocytes. Here, when high amounts of CB₁ receptors are expressed (by injecting more CB₁ receptor mRNA) the maximal responses produced by WIN55,212-2, anandamide and AM356 (methanandamide, a metabolically stable anandamide analogue) are all similar, although WIN55,212-2 is more potent. By contrast, when less CB₁ receptor is expressed (by injecting less CB₁ receptor mRNA), the maximal responses to anandamide and AM356 are considerably less than to WIN55,212-2. Because the phenomenon of partial agonism depends on the relative quantities of signalling proteins, it is most proper to think of partial agonism as a property of a particular system, and not the ligand. Thus, the preferred term for an agonist that shows partial agonism is that it has low intrinsic activity. Anandamide and Δ^9 -THC are low intrinsic efficacy agonists at CB₁ receptors, whereas 2-AG is a high intrinsic efficacy agonist (31). This has potential implications for the interactions of Δ^9 -THC with 2-AG (32).

Functional selectivity

Accumulating evidence suggests that the classical concept of all agonists activating the same signalling pathways is too simplistic. Rather, it appears that certain agonists can more efficaciously activate one signalling pathway over another. This phenomenon is widespread among G protein coupled receptors, and has received the term agonist-directed trafficking or functional selectivity (33). An example of this for CB₁ receptor signalling is the regulation of tyrosine hydroxylase (TH) expression. In this example, the CB₁ receptor agonist HU210 stimulates the transcription of TH mRNA, a property shared by other CB₁ receptor agonists, such as Δ^9 -THC and WIN55,212-2 (34). Conversely, the CB₁ receptor agonist CP55,940 inhibits the transcription of TH mRNA, as do the structurally related analogues CP55,244 and CP47,947 (34). Functional selectivity has also been found for CB₂ receptor agonists. Here, adenylyl cyclase is inhibited by low concentrations of CP55,940 relative to those needed to stimulate ERK1/2 phosphorylation (35). By contrast, the order of potency is switched for 2-AG: low concentrations

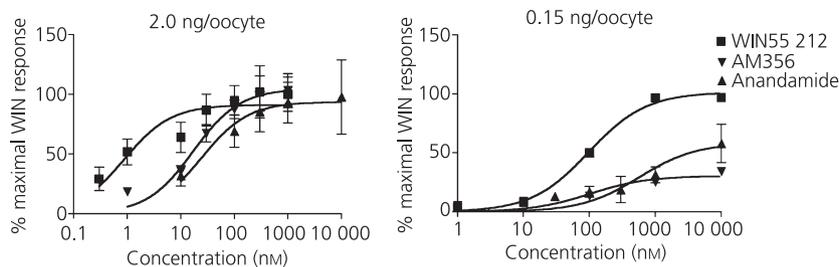


Fig. 2. Decreasing receptor expression unmasks partial agonism. *Xenopus* oocytes were injected with either 2 ng (left) or 0.15 ng (right) CB₁ receptor cRNA and a constant amount of cRNA for inwardly rectifying potassium channels (GIRK1). Potassium currents in response to the indicated concentrations of cannabinoid agonists were determined using a two-electrode voltage clamp.

of 2-AG efficaciously stimulate ERK1/2 activity, but higher concentrations are needed to inhibit adenylyl cyclase (35). Mechanistically, these results can be explained if different agonists favour different receptor conformations, and these different conformations may be more efficacious in activating distinct signalling pathways. Evidence that this occurs has been obtained for both CB₁ and CB₂ receptors where different agonists favour the coupling to different G proteins (36–38). Whatever the mechanism, the practical implications of these studies are considerable: investigations carried out with synthetic compounds, an approach often favoured in the cannabinoid field because of the practical difficulties inherent in using endocannabinoids, may not activate the same repertoire of signalling pathways as the endogenous activators of cannabinoid receptors. Thus, such experiments need to be evaluated with a skeptical eye, or at least their general findings need to be confirmed with directed experiments conducted using endocannabinoids.

Inverse agonism

Another important concept in receptor pharmacology is that of inverse agonism (39). Inverse agonism can be best explained by a model that assumes receptors exist in equilibrium between active and inactive states, with a fraction of the receptors being in the active state, even in the absence of agonist. Thus, in the absence of agonist, there will be a low level of signalling mediated by the receptor: 'basal signalling'. Agonists will shift more of the receptors to the active state, increasing signalling. A neutral antagonist will prevent agonists from binding but shows no preference in binding to either the active or inactive state of the receptor. Thus, it will not shift the equilibrium between active and inactive receptor, and will not change the basal level of signalling. However, an inverse agonist preferentially binds to the inactive state of the receptor decreasing the fraction of active receptor and suppressing basal signalling. This is shown schematically in Fig. 1. A neutral antagonist does not perturb signalling from its basal state. However, an inverse agonist can decrease signalling to less than basal levels; hence the use of the term 'inverse' agonist as the effect is in a direction opposite to that of an agonist.

Since the level of basal signalling is typically proportional to the number of active receptors, inverse agonism is usually most noticeable under conditions of high receptor expression, such as occurs in over expression systems. However, the high level of CB₁ receptor expression in the central nervous system also raises the possibility that inverse agonism may be relevant for CB₁ receptors *in vivo*. Another implication of inverse agonists is that G proteins preferentially bind to inactive receptors. Thus, at least theoretically, an inverse agonist can attenuate signalling by other GPCRs by sequestering of G proteins that these other GPCRs require for their signalling (40). Inverse agonism and partial agonism can also interact to create protean agonism (41). A protean agonist (which necessarily has a relatively low intrinsic efficacy) will show (partial) agonism in one system and inverse agonism in a second. This can come about if basal activity is high in the second system, so that the lower intrinsic efficacy of the protean agonist is manifested as a reduction from basal activity. Thus, protean agonism is very dependent

on the system being used to measure activity. AM1241 is a CB₂ ligand that can be a protean agonist (42).

Although it is possible to demonstrate inverse agonism in cell culture systems, it is much more difficult to distinguish between inverse agonism and antagonism of an endogenous neuromodulator in an intact animal. This is because it is virtually impossible to eliminate synthesis of a modulator, particularly in the case of endocannabinoids because there are multiple pathways for their synthesis (8, 43, 44).

Conflicts of interest

The author has received consulting fees from Abbott laboratories, Cara Therapeutics and sanofi-aventis. His research is supported by the NIH and the Gill Center at Indiana University.

Received: 29 November 2007,
accepted 8 February 2008

References

- 1 Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev* 2003; **83**: 1017–1066.
- 2 Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* 2002; **54**: 161–202.
- 3 Mackie K. Cannabinoid receptors as therapeutic targets. *Annu Rev Pharmacol Toxicol* 2006; **46**: 101–122.
- 4 Tan B, Bradshaw HB, Rimmerman N, Srinivasan H, Yu YW, Krey JF, Mon MF, Chen JSC, Hu SSJ, Pickens SR, Walker JM. Targeted lipidomics: discovery of new fatty acyl amides. *AAPS J* 2006; **8**: E461–E465.
- 5 Alexander SP, Kendall DA. The complications of promiscuity: endocannabinoid action and metabolism. *Br J Pharmacol* 2007; **152**: 602–623.
- 6 Basavarajappa BS. Critical enzymes involved in endocannabinoid metabolism. *Protein Pept Lett* 2007; **14**: 237–246.
- 7 Okamoto Y, Wang J, Morishita J, Ueda N. Biosynthetic pathways of the endocannabinoid anandamide. *Chem Biodivers* 2007; **4**: 1842–1857.
- 8 Simon GM, Cravatt BF. Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for alpha/beta-hydrolase 4 in this pathway. *J Biol Chem* 2006; **281**: 26465–26472.
- 9 Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC. Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J Neurosci* 1991; **11**: 563–583.
- 10 Jansen EM, Haycock DA, Ward SJ, Seybold VS. Distribution of cannabinoid receptors in rat brain determined with aminoalkylindoles. *Brain Res* 1992; **575**: 93–102.
- 11 Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 1990; **346**: 561–564.
- 12 Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993; **365**: 61–65.
- 13 Szallasi A, Di Marzo V. New perspectives on enigmatic vanilloid receptors. *Trends Neurosci* 2000; **23**: 491–497.
- 14 Oz M. Receptor-independent effects of endocannabinoids on ion channels. *Curr Pharm Des* 2006; **12**: 227–239.
- 15 Ryberg E, Larsson N, Sjogren S, Hjorth S, Hermansson NO, Leonova J, Elebring T, Nilsson K, Drmota T, Greasley PJ. The orphan receptor

- GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* 2007; **152**: 1092–1101.
- 16 Mackie K. Distribution of cannabinoid receptors in the central and peripheral nervous system. *Handb Exp Pharmacol* 2005; **168**: 299–325.
 - 17 Marsicano G, Lutz B. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. *Eur J Neurosci* 1999; **11**: 4213–4225.
 - 18 Matsuda LA, Bonner TI, Lolait SJ. Localization of cannabinoid receptor mRNA in rat brain. *J Comp Neurol* 1993; **327**: 535–550.
 - 19 Bodor AL, Katona I, Nyiri G, Mackie K, Ledent C, Hajos N, Freund TF. Endocannabinoid signaling in rat somatosensory cortex: laminar differences and involvement of specific interneuron types. *J Neurosci* 2005; **25**: 6845–6856.
 - 20 Nyiri G, Cserep C, Szabadits E, Mackie K, Freund TF. CB1 cannabinoid receptors are enriched in the perisynaptic annulus and on preterminal segments of hippocampal GABAergic axons. *Neuroscience* 2005; **136**: 811–822.
 - 21 Katona I, Urban GM, Wallace M, Ledent C, Jung KM, Piomelli D, Mackie K, Freund TF. Molecular composition of the endocannabinoid system at glutamatergic synapses. *J Neurosci* 2006; **26**: 5628–5637.
 - 22 Cota D, Marsicano G, Tschop M, Grubler Y, Flachskamm C, Schubert M, Auer D, Yassouridis A, Thöne-Reineke C, Ortmann S, Tomassoni F, Cervino C, Nisoli E, Linthorst ACE, Pasquali R, Lutz B, Stalla GK, Pagotto U. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest* 2003; **112**: 423–431.
 - 23 Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Bátkai S, Harvey-White J, Mackie K, Offertáler L, Wang L, Kunos G. Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest* 2005; **115**: 1298–1305.
 - 24 Cota D. CB1 receptors: emerging evidence for central and peripheral mechanisms that regulate energy balance, metabolism, and cardiovascular health. *Diabetes Metab Res Rev* 2007; **23**: 507–517.
 - 25 Cavuoto P, McAinch AJ, Hatzinikolas G, Janovska A, Game P, Wittert GA. The expression of receptors for endocannabinoids in human and rodent skeletal muscle. *Biochem Biophys Res Commun* 2007; **364**: 105–110.
 - 26 Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P, Bouaboula M, Shire D, Le Fur G, Casellas P. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem* 1995; **232**: 54–61.
 - 27 Ofek O, Karsak M, Leclerc N, Fogel M, Frenkel B, Wright K, Tam J, Attar-Namdar M, Kram V, Shohami E, Mechoulam R, Zimmer A, Bab I. Peripheral cannabinoid receptor, CB2, regulates bone mass. *Proc Natl Acad Sci USA* 2006; **103**: 696–701.
 - 28 Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, Stella N, Makriyannis A, Piomelli D, Davison JS, Marnett LJ, Di Marzo V, Pittman QJ, Patel KD, Sharkey KA. Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* 2005; **310**: 329–332.
 - 29 Chevaleyre V, Takahashi KA, Castillo PE. Endocannabinoid-mediated synaptic plasticity in the CNS. *Annu Rev Neurosci* 2006; **29**: 37–76.
 - 30 Bacci A, Huguenard JR, Prince DA. Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids. *Nature* 2004; **431**: 312–316.
 - 31 Luk T, Jin W, Zvonok A, Lu D, Lin XZ, Chavkin C, Makriyannis A, Mackie K. Identification of a potent and highly efficacious, yet slowly desensitizing CB1 cannabinoid receptor agonist. *Br J Pharmacol* 2004; **142**: 495–500.
 - 32 Straiker A, Mackie K. Cannabinoids, electrophysiology, and retrograde messengers: challenges for the next 5 years. *AAPS J* 2006; **8**: E272–E276.
 - 33 Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H, Javitch JA, Roth BL, Christopoulos A, Sexton PM, Miller KJ, Spedding M, Mailman RB. Functional selectivity and classical concepts of quantitative pharmacology. *J Pharmacol Exp Ther* 2007; **320**: 1–13.
 - 34 Bosier B, Tilleux S, Najimi M, Lambert DM, Hermans E. Agonist selective modulation of tyrosine hydroxylase expression by cannabinoid ligands in a murine neuroblastoma cell line. *J Neurochem* 2007; **102**: 1996–2007.
 - 35 Shoemaker JL, Ruckle MB, Mayeux PR, Prather PL. Agonist-directed trafficking of response by endocannabinoids acting at CB2 receptors. *J Pharmacol Exp Ther* 2005; **315**: 828–838.
 - 36 Breivogel CS, Childers SR. Cannabinoid agonist signal transduction in rat brain: comparison of cannabinoid agonists in receptor binding, G-protein activation, and adenylyl cyclase inhibition. *J Pharmacol Exp Ther* 2000; **295**: 328–336.
 - 37 Mukhopadhyay S, Howlett AC. Chemically distinct ligands promote differential CB1 cannabinoid receptor-Gi protein interactions. *Mol Pharmacol* 2005; **67**: 2016–2024.
 - 38 Prather PL, Martin NA, Breivogel CS, Childers SR. Activation of cannabinoid receptors in rat brain by WIN 55212-2 produces coupling to multiple G protein alpha-subunits with different potencies. *Mol Pharmacol* 2000; **57**: 1000–1010.
 - 39 Kenakin T. Functional selectivity through protean and biased agonism: who steers the ship? *Mol Pharmacol* 2007; **72**: 1393–1401.
 - 40 Vasquez C, Lewis DL. The CB1 cannabinoid receptor can sequester G-proteins, making them unavailable to couple to other receptors. *J Neurosci* 1999; **19**: 9271–9280.
 - 41 Kenakin T. Inverse, protean, and ligand-selective agonism: matters of receptor conformation. *FASEB J* 2001; **15**: 598–611.
 - 42 Yao BB, Mukherjee S, Fan Y, Garrison TR, Daza AV, Grayson GK, Hooker BA, Dart MJ, Sullivan JP, Meyer MD. In vitro pharmacological characterization of AM1241: a protean agonist at the cannabinoid CB2 receptor? *Br J Pharmacol* 2006; **149**: 145–154.
 - 43 Leung D, Saghatelian A, Simon GM, Cravatt BF. Inactivation of N-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. *Biochemistry* 2006; **45**: 4720–4726.
 - 44 Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, Chan AC, Zhou Z, Huang BX, Kim H-Y, Kunos G. A biosynthetic pathway for anandamide. *Proc Natl Acad Sci USA* 2006; **103**: 13345–13350.