REVIEW

Meta-analysis of cannabinoid ligand binding affinity and receptor distribution: interspecies differences

JM McPartland[1], M Glass[2] and RG Pertwee[3]

[1]*Department of Molecular Biology, GW Pharmaceuticals, Salisbury, Wiltshire, UK;* [2]*Department of Pharmacology, University of Auckland, Auckland, New Zealand and* [3]*School of Medical Sciences, University of Aberdeen, Aberdeen, UK*

A meta-analysis, unlike a literature review, synthesizes previous studies into new results. Pooled data from 211 studies measured ligand binding affinities at human (*Hs*) or rat (*Rn*) cannabinoid receptors CB_1 and CB_2. Cochrane methods were modified for this non-clinical analysis. Meta-regression detected data heterogeneity arising from methodological factors: use of sectioned tissues, lack of PMSF and choice of radioligand. Native brain tissues exhibited greater affinity (lower nM) than transfected cells, but the trend fell short of significance, as did the trend between centrifugation and filtration methods. Correcting for heterogeneity, mean K_i values for Δ^9-tetrahydrocannabinol differed significantly between $HsCB_1$ and $RnCB_1$ (25.1 and 42.6 nM, respectively) but not between $HsCB_1$ and $HsCB_2$ (25.1 and 35.2). Mean K_d values for $HsCB_1$, $RnCB_1$ and $HsCB_2$ of CP55,940 (2.5, 0.98, 0.92) and WIN55,212-2 (16.7, 2.4, 3.7) differed between $HsCB_1$ and $RnCB_1$ and between $HsCB_1$ and $HsCB_2$. SR141716A differed between $HsCB_1$ and $RnCB_1$ (2.9 and 1.0 nM). Anandamide at $HsCB_1$, $RnCB_1$ and $HsCB_2$ (239.2, 87.7, 439.5) fell short of statistical differences due to heterogeneity. We consider these K_d and K_i values to be the most valid estimates in the literature. Sensitivity analyses did not support the numerical validity of cannabidiol, cannabinol, 2-arachidonoyl glycerol and all ligands at $RnCB_2$. Aggregate rank order analysis of CB_1 distribution in the brain (pooled from 119 autoradiographic, immunohistochemical and *in situ* hybridization studies) showed denser $HsCB_1$ expression in cognitive regions (cerebral cortex) compared to $RnCB_1$, which was relatively richer in movement-associated areas (cerebellum, caudate-putamen). Implications of interspecies differences are discussed.
British Journal of Pharmacology (2007) **152**, 583–593; doi:10.1038/sj.bjp.0707399; published online 16 July 2007

Introduction

Δ^9-Tetrahydrocannabinol (THC) primarily affects animals via cannabinoid receptors type 1 and 2 (CB_1 and CB_2). CB_1 and CB_2 activate intracellular G-proteins, which transduce signal to a variety of effectors, such as ion channels, adenylyl cyclase, phospholipase C and the mitogen-activated protein kinase cascade (Howlett, 2002). The first step in this signal transduction involves the binding of a ligand to a receptor. Receptor–ligand binding is measured as affinity, expressed in molar units, usually in a nanomolar range (Pertwee, 2005). Researchers studying cannabinoid often perform experiments with an underlying null hypothesis (implicit, if

unstated) that receptors are functionally equivalent between species. Method sections in published papers often make statements such as 'Chinese Hamster Ovary (CHO) cells transfected with CB_1 receptors,' without identifying the CB_1 species. Publications regarding rodent receptors are frequently generalized to human orthologues. A few studies have compared human and rodent receptors, but the studies lacked statistical power. Given the molecular divergences between human and rodent orthologues (McPartland *et al.*, 2006), the null hypothesis seems flawed.

In the absence of an adequate cross-species comparison, we conducted a meta-analysis. Broadly defined, meta-analysis includes any methodology that combines data from independent studies. A valid meta-analysis identifies sources of heterogeneity between studies and manages hetero-geneity by placing defined limits upon data selection (Glass *et al.*, 1981). Heterogeneity in cannabinoid receptor–ligand

binding studies may arise from methodological factors, such as the use of native tissues versus transfected cells, use of enzyme inhibitors and choice of radioligand. These factors can be explored in a subgroup meta-regression analysis. Meta-regression may determine why affinity values for 2-AG (*sn*-2-arachidonoyl glycerol), for example, 'look like a random compilation of numbers' (R Mechoulam, personal communication).

The Cochrane Collaboration (www.cochrane.org) leads meta-analysis research worldwide, although they focus upon healthcare interventions (Higgins and Green, 2005). The Cochrane 'effect size' or 'standardized mean difference' pertains to randomized, controlled trials (for instance to the size of a treatment effect between experiment and control) and is not applicable to our literature, where direct interspecies comparisons were rarely measured and controls or placebos were not tested. Non-medical researchers have emphasized the metric for meta-analysis should not be limited to effect sizes (Boyce *et al*., 2005), which agrees with the theory set out by Cochrane himself (Cochran, 1954). We adopted all relevant aspects of the Cochrane protocol, because of its proven validity and robustness.

This meta-analysis focused on *in vitro* studies of radioligand binding affinities, as well as the distribution of CB_1 receptors in the brain as measured by radioligand, immunohistochemistry and *in situ* hybridization methods. We have described the studies that met inclusion criteria, explored methodological heterogeneities among the studies, pooled data from appropriate studies and concluded with *post hoc* sensitivity analyses of our models and methods. From these results, we can draw conclusions regarding phenotypic divergences between orthologues.

Methods

Search strategy and study selection
We briefly describe the analysis here. For details regarding inclusion criteria, heterogeneity tests, subgroup analysis, quality assessment and publication bias, see Supplementary file, Extend Methods at the *British Journal of Pharmacology* website (http://www.nature.com/bjp). MEDLINE was searched for articles published in any language through December 2006, using the following keywords: affinity, anandamide (AEA), cannabinoid, endocannabinoid, tetrahydrocannabinol, tritiated and 2-arachidonoyl glycerol. Retrieved articles were screened for supporting citations and antecedent sources were retrieved. Investigators of original studies were contacted to obtain clarifications and missing data. Three groups of reviewers independently considered studies for inclusion, and resolved disagreements by consensus. To be accepted for analysis, an article had to meet the following criteria: (1) the study examined CB_1 or CB_2 of human (*Homo sapiens, Hs*) or rat (*Rattus norvegicus, Rn*) origin. For sensitivity analyses, we also included studies of rhesus macaque (*Macaca fascicularis, Mf*) and mouse (*Mus musculus, Mm*). Data were limited to normal wild types. Studies of chimeric cell lines were excluded (for example, F-11 and NG108-15 cells), as were studies of tissues with unidentified receptors (for example, foetal lung fibroblasts),

(2) studies were limited to nine frequently tested cannabinoids: *N*-arachidonoyl ethanolamine (AEA), cannabidiol (CBD), cannabinol (CBN), CP55,940, HU210, SR141716A, THC, WIN55,212-2 and 2-AG, (3) studies were limited to two aspects of cannabinoid ligand binding:

(a) CB_1 and CB_2 affinity studies that reported ligand K_d or K_i, measured in molar units. Fifteen IC_{50} studies were also included after investigators provided information that enabled conversion of IC_{50} to K_i using the Cheng–Prusoff equation.
(b) CB_1 receptor distribution studies, limited to: (i) CB_1 studies of the brain, excluding spinal cord, retina and the peripheral nervous system; (ii) studies of adult animals; (iii) studies that used radioligand binding, immunohistochemistry or *in situ* hybridization methods.

Data extraction
We conducted a prospective meta-analysis, where studies were identified and determined to be eligible before the results were synthesized. Reviewers used piloted, standardized data extraction sheets. The following data were extracted from affinity studies: the species of CB_1 or CB_2 receptor, each ligand and its mean K_d and/or K_i, sample size, sample variance and methodological covariates. Five methodological covariates were selected for data extraction, chosen by *a priori* hypotheses: (1) use of brain homogenates versus brain sections; (2) use of phenylmethylsulphonyl fluoride (PMSF) to prevent the breakdown of AEA by catabolic enzymes; (3) use of centrifugation versus rapid filtration for the separation of free and bound radioligand; (4) differences between K_d and K_i values, and K_i variance, due to the use of dissimilar radioligands; (5) use of native tissues versus heterologously expressed systems (transfected cells).

For the meta-analysis of CB_1 distribution studies, extracted data included the following: the species of CB_1, the mean receptor density per brain region, study size and several methodological covariates: (1) whether the study used radioligand binding, immunohistochemistry or *in situ* hybridization; (2) which probe was used (that is, which radioligand, tagged antibody or labelled oligonucleotide); (3) whether the study measured receptor density from autoradiographs or from dissected brain regions. Decisions regarding data extraction met consensus before data were extracted for synthesis. When more than one publication described the results of a single experiment, we extracted data from the publication with the most complete information regarding that experiment.

Data synthesis
Data were synthesized quantitatively or qualitatively. Quantitatively, cannabinoid affinity at four receptors ($HsCB_1$, $RnCB_1$, $HsCB_2$, $RnCB_2$) was synthesized twice, as a pooled mean and as a pooled weighted mean. The weighted mean adjusted each study's mean by its inverse variance, because larger studies with less variance should carry more 'weight' in a meta-analysis. To determine whether pooling was statistically appropriate, the coefficient of variation (CV) was determined for each pooled mean. CV measures data dispersion of a probability distribution, defined as the ratio

Ligand affinity and receptor distribution of CB1
JM McPartland *et al*
585

of the standard deviation to the mean (Reed *et al.*, 2002). We applied the Cochrane 'skew test' to the CV (Higgins and Green, 2005): a skewed mean with excessive heterogeneity was defined by a CV $\geqslant 1$ (that is, standard deviation $\geqslant$ mean). For means with excessive heterogeneity, we applied a random effects model (Higgins and Green, 2005), and performed meta-regressions upon subgroups, based on methodological covariates described above. Sources of heterogeneity identified via meta-regressions were removed from synthesis. To these adjusted means, we reapplied the CV-skew test. Persistently skewed means were submitted to Grubb's test, using an outlier calculator (GraphPad, www.graphpad.com/quickcalcs/Grubbs1.cfm). Studies with data outliers were inspected for methodological irregularities, and in some cases were also removed from synthesis, as noted in Supplementary Table 1.

The distribution data for CB_1 receptors in brain were synthesized as scalar transformations (ranked orders) or as narrative comparisons. Scalar transformations were performed upon every study that reported CB_1 density in $\geqslant 2$ brain regions. The relative density of each brain region was ranked (arrayed) from highest value to lowest value. Rank orders from individual studies were then aggregated using a bubble-sort algorithm (Sese and Morishita, 2001). Narrative comparisons were used to describe aspects of CB_1 distribution that were not quantified in original studies.

Sensitivity analysis

A series of analyses were performed to judge *a priori* hypotheses and the robustness of the results. We began by identifying additional methodological covariates based upon *post hoc* observations. The validity and numerical precision of pooled K_i and K_d values were tested, using three approaches: (1) K_i and K_d values in original studies were transformed into aggregate rank orders. For every study that examined $\geqslant 2$ ligands per receptor, ligands were ranked from highest to lowest affinity; individual studies were then aggregated using a bubble-sort algorithm, (2) pooled K_i and K_d values for *Hs* and *Rn* were compared to studies in the literature that made direct interspecies comparisons, (3) affinity values for *Rn* were compared to affinity values from *Mm* studies. *Rn* and *Mm* cross-species differences undoubtedly exist, but the rodents share similarity on a molecular level—RnCB$_1$ and MmCB$_1$ are 99.8% identical (diverging at one residue out of 473 amino acids), compared with RnCB$_1$ and HsCB$_1$ differing at 12 residues plus a codon deletion.

Sensitivity analyses for CB_1 brain distribution data also utilized cross-species comparisons. Regional rank orders for RnCB$_1$ were examined for consistency with MmCB$_1$ rank orders, and HsCB$_1$ rank orders were examined for consistency with two other primates, rhesus macaque (*Macaca fascicularis, Mf*) and baboon (*Papio hamadryas, Ph*).

Results of data extraction and synthesis

Receptor–ligand affinity

A flow diagram of article selection for receptor–ligand affinity meta-analysis is shown in Figure 1. A total of 211 affinity studies concerning *Hs* and *Rn* orthologues met the inclusion criteria, and collectively reported 676 affinity values, presented in Supplementary Table 1. Most studies measured affinity at one receptor, but some studies compared ligand affinities between orthologues or paralogues (Figure 2). Thirty studies of *Mm* orthologues met the inclusion criteria, presented in Supplementary Table 2. No affinity studies of *Mf* were identified that met inclusion criteria.
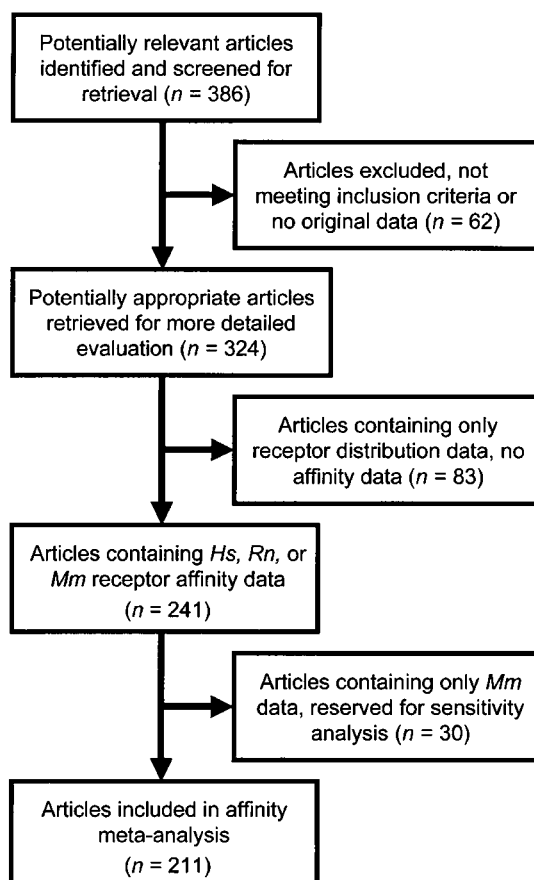

Figure 1 Flow diagram of article selection for ligand affinity meta-analysis.
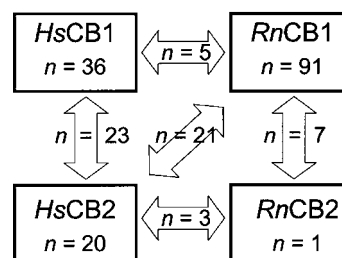

Figure 2 Diagram showing all the 211 articles included in the receptor–ligand affinity meta-analysis. The numbers of articles that tested ligand affinity at one receptor are indicated in boxes. The numbers of articles that compared ligand affinity at two receptors are indicated in arrows. Additionally, four articles compared all four receptors (not illustrated).

Weighted means were compromised by missing data. Many studies omitted information regarding sample size or sample variance, or the studies did not state whether their variances were s.d.s or s.e.s (see notes for each study in Supplementary Tables 1 and 2). Therefore, we conducted heterogeneity (CV-skew) tests on unweighted means. Results from the CV-skew tests (Supplementary Table 3) indicated a significant degree of heterogeneity among studies, making them inappropriate to pool in a fixed-effects model. This supported our decision to employ subgroup meta-regression.

Meta-regression demonstrated statistically significant differences between studies that used brain sections versus studies that used brain homogenates (Table 1). Native tissues produced lower K_i values than transfected cells, but differences fell short of significance. Centrifugation assays produced lower K_i values than filtration assays, but differences were not significant (except for AEA, see Discussion). K_d values were significantly different from K_i values, and the difference was primarily due to K_i studies that used [³H]SR141716A. The use of PMSF significantly lowered the K_i of AEA in brain tissues, but PMSF did not change K_i values in transfected cells.

Based on meta-regression results, we withdrew heterogeneous data derived from studies that used sectioned brain tissue, studies of AEA in brain homogenates that lacked PMSF and K_i studies that used [³H]SR141716A. Adjusted means were submitted to Grubb's test and several outliers were removed from synthesis (Supplementary Table 1). The remaining data were utilized for calculating pooled means. Mean K_d values for [³H]CP55,940, [³H]WIN55212-2 and [³H]SR141716A are presented in Table 2. Mean K_d values presented in bold typeface had numerical validities confirmed by sensitivity analyses (described below). Differences between validated means were tested for statistical significance, accompanied by K_d ratios. A K_d ratio <1 indicated that ligand affinity was greater for the *Hs* orthologue (a small K_d value indicating high affinity). Conversely, a K_d ratio >1 indicated that ligand affinity was greater for the *Rn* orthologue. Several interspecies differences were statistically significant. Mean K_i values for THC, CBD, CBN, AEA, 2-AG and HU210 are presented in Table 3. Robust data at $HsCB_1$ and $RnCB_1$ receptors demonstrated interspecies differences for THC, whereas AEA was not significantly different between $HsCB_1$ and $RnCB_1$ (despite a relatively large K_d ratio of 2.7), due to remaining heterogeneity among studies. Data regarding CBD, CBN, 2-AG, and all the comparisons at $RnCB_2$ were less robust, hampered by heterogeneity and small sample sizes. We also compared paralogues $HsCB_1$ and $HsCB_2$ and recovered significant differences (Table 3); comparisons between paralogues $RnCB_1$ and $RnCB_2$ were hampered again by small samples sizes at $RnCB_2$.

Table 1 Subgroup meta-regression: effects of methodological factors upon mean affinity values

Methodological factor[a]	Affinity mean (nM), standard error, sample size	Difference between means
Brain homogenates versus Brain sections	3.4±1.1 (*n*=77) 20.8±3.1 (*n*=10)	Statistical difference, *P*=0.0001
Native tissues versus Transfected cells	1.6±0.76 (*n*=13) 3.2±0.45 (*n*=38)	No difference, *P*=0.12
Filtration of radioligand versus Centrifugation of radioligand	1.0±0.10 (*n*=56) 0.87±0.10 (*n*=20)	No difference, *P*=0.28
K_d versus K_i	0.98±0.12 (*n*=51) 8.2±1.5 (*n*=26)	Statistical difference, *P*=0.0001
K_i values of CP55,940 obtained using: [³H]CP55,940 [³H]SR141716A [³H]WIN55212-2 [³H]HU243	1.1±4.55 (*n*=14) 19.4±3.41 (*n*=25) (a) 0.89±4.55 (*n*=14) 1.3±7.62 (*n*=5)	(a)=statistical difference to all other ligands, *P*=0.002
Brain tissue with PMSF versus Brain tissue without PMSF	205.8±301.9 (*n*=30) 2500.9±413.3 (*n*=16)	Statistical difference, *P*=0.0001
CB₁-transfected cells with PMSF versus CB₁-transfected cells without PMSF	196.5±123.1 (*n*=6) 396.4±100.5 (*n*=9)	No difference, *P*=0.29
CB₂-transfected cells with PMSF versus CB₂-transfected cells without PMSF	477.9±385.5 (*n*=9) 916.1±348.7 (*n*=11)	No difference, *P*=0.45

Abbreviation: PMSF, phenylmethylsulphonyl fluoride.
[a]All meta-regressions were performed on the single ligand and receptor that provided the greatest amount of extractable data; CP55,940 at $RnCB_1$ data were used for all methodological factors, except the three PMSF versus no PMSF meta-regressions. For the PMSF versus no PMSF analyses, the brain tissue comparison used AEA at $RnCB_1$ data, the CB₁-transfected cells used AEA at $HsCB_1$ data and the CB₂-transfected cells used AEA at $HsCB_2$ data. Statistical significances (*P*-values) were calculated with Mann–Whitney *U*-test (Systat; Evanston, IL, USA).

Table 2 Meta-analytic K_d values for radioligands at human (*Hs*) and rat (*Rn*) cannabinoid receptors

Radioligand	HsCB₁ $K_d \pm s.e.$	RnCB₁ $K_d \pm s.e.$	HsCB₂ $K_d \pm s.e.$	RnCB₂ $K_d \pm s.e.$	HsCB₁ $K_d \pm s.e.$	HsCB₂ $K_d \pm s.e.$	RnCB₁ $K_d \pm s.e.$	RnCB₂ $K_d \pm s.e.$
[³H]CP55,940	**2.5**±0.39 (*n*=28)	**0.98**±0.12 (*n*=51)	**0.92**±0.16 (*n*=18)	**0.84**±0.30 (*n*=8)	**2.5**±0.39 (*n*=28)	**0.92**±0.16 (*n*=18)	**0.98**±0.12 (*n*=51)	**0.84**±0.30 (*n*=8)
	Difference between species, *P*=0.001; K_d ratio = 2.6		No difference, *P*=0.32; K_d ratio = 1.1		Difference between CB₁ and CB₂, *P*=0.001; K_d ratio = 2.7		No difference, *P*=0.19; K_d ratio = 1.2	
[³H]WIN55212	**16.7**±1.71 (*n*=6)	**2.4**±0.348 (*n*=13)	**3.7**±1.19 (*n*=7)	35.6 (*n*=2)	**16.7**±1.71 (*n*=6)	**3.7**±1.19 (*n*=7)	**2.4**±0.348 (*n*=13)	ND
	Difference between species, *P*=0.001; K_d ratio = 7.0				Difference between CB₁ and CB₂, *P*=0.003; K_d ratio = 4.5			
[³H]SR141716A	**2.9**±0.54 (*n*=10)	**1.0**±0.22 (*n*=19)	ND	ND	**2.9**±0.54 (*n*=10)	ND	**1.0**±0.22 (*n*=19)	ND
	Difference between species, *P*=0.002; K_d ratio = 2.9							

Abbreviations: *Hs, Homo sapiens*; *Rn, Rattus norvegicus*.
The results in the table are expressed as $K_d \pm s.e.$ (nM) and the number of studies extracted for determination of pooled means. Means in bold typeface had their numerical validity confirmed by sensitivity analyses. Differences between validated means were tested for statistical significance with Mann–Whitney *U*-test (Systat; Evanston, IL, USA). ND = no K_d data in Supplementary Table 1.

Table 3 Meta-analytic K_i values for ligands at human (*Hs*) and rat (*Rn*) cannabinoid receptors

Ligand	HsCB₁ $K_i \pm s.e.$	RnCB₁ $K_i \pm s.e.$	HsCB₂ $K_i \pm s.e.$	RnCB₂ $K_i \pm s.e.$	HsCB₁ $K_i \pm s.e.$	HsCB₂ $K_i \pm s.e.$	RnCB₁ $K_i \pm s.e.$	RnCB₂ $K_d \pm s.e.$
THC	**25.1**±5.54 (*n*=16)	**42.6**±5.01 (*n*=18)	**35.2**±5.86 (*n*=16)	13.0±7.70 (*n*=3)	**25.1**±5.54 (*n*=16)	**35.2**±5.86 (*n*=16)	**42.6**±5.01 (*n*=18)	13.0±7.70 (*n*=3)
	Difference between species, *P*=0.010; K_i ratio = 0.59				No difference between CB₁ and CB₂, *P*=0.12; K_i ratio = 0.71			
CBD	ND	2210.5±558.08 (*n*=6)	2860 (*n*=1)	1000 (*n*=1)	ND	2860 (*n*=1)	2210.5±558.08 (*n*=6)	1000 (*n*=1)
CBN	525.3±308.1 (*n*=3)	368.0±121.1 (*n*=8)	168.2±32.16 (*n*=7)	ND	525.3±308.1 (*n*=3)	168.2±32.16 (*n*=7)	368.0±121.1 (*n*=8)	ND
AEA	**239.2**±61.77 (*n*=16)	**87.7**±11.32 (*n*=26)	**439.5**±95.89 (*n*=22)	267.8±67.94 (*n*=5)	**239.2**±61.77 (*n*=16)	**439.5**±95.89 (*n*=22)	**87.7**±11.32 (*n*=26)	267.8±67.94 (*n*=5)
	No difference between species, *P*=0.23; K_i ratio = 2.7				No difference between CB₁ and CB₂, *P*=0.06; K_i ratio = 0.54			
2-AG	3423.6±3288.24 (*n*=3)	1180.5±538.59 (*n*=4)	1193.8±327.71 (*n*=9)	1900.0±1800.0 (*n*=2)	3423.6±3288.24 (*n*=3)	1193.8±327.71 (*n*=9)	1180.5±538.59 (*n*=4)	1900.0±1800.0 (*n*=2)
HU210	**0.25**±0.069 (*n*=9)	**0.34**±0.102 (*n*=7)	**0.40**±0.095 (*n*=15)	ND	**0.25**±0.069 (*n*=9)	**0.40**±0.095 (*n*=15)	**0.34**±0.102 (*n*=7)	ND
	No difference between species, *P*=0.49; K_i ratio = 0.74				No difference between CB₁ and CB₂, *P*=0.34; K_i ratio = 0.63			

Abbreviations: AEA, anandamide; CB₁, cannabinoid receptor subtype 1; CB₂, cannabinoid receptor subtype 2; CBD, cannabidiol; cannabinol; *Hs, Homo sapiens*; *Rn, Rattus norvegicus*; 2-AG, *sn*-2 arachidonoyl glycerol. The results in the table are expressed as $K_i \pm s.e.$ (nM) and the number of studies extracted for determination of pooled means. Means in bold typeface had their numerical validity confirmed by sensitivity analyses. Differences between validated means were tested for statistical significance with Mann–Whitney *U*-test (Systat; Evanston, IL, USA). ND = no K_i data in Supplementary Table 1.

CB$_1$ receptor distribution

A total of 119 CB$_1$ receptor distribution studies met the inclusion criteria (87 studies reported only CB$_1$ distribution data, the remaining 32 studies also contained affinity data), cited and extracted in Supplementary Table 4. A subset of publications compared CB$_1$ density in $\geqslant 2$ brain regions, enabling us to transform relative densities into rank orders, arrayed from the highest density to the lowest density. Aggregate rank orders derived from this subset of publications are presented in Supplementary Table 5. Heterogeneity tests could not be calculated for receptor distribution data, because the results were qualitative (rank orders). Subgroup analysis was based on the methodology presented in Supplementary Table 5. Autoradiography, immunohistochemistry and *in situ* hybridization methods varied in their sensitivity and level of resolution. Results of autoradiographic studies broadly agreed with each other, irrespective of the radioligand used. Autoradiographic study results generally agreed with studies that used regional dissection and studies that used immunohistochemical methods, allowing for the high degree of variability in brain tissue and the low sample sizes. Many immunohistochemical studies reported subcellular or ultrastructural findings, but did not provide regional comparisons for our meta-analysis. Rank orders from *in situ* hybridization studies differed from rank orders reported in autoradiographic and immunohistochemical studies (see Discussion). Therefore, *in situ* hybridization studies were excluded and the remaining studies (autoradiography, regional dissection and immunocytochemistry) were assembled in the following rank orders:

Hs*CB$_1$*. Substantia nigra > globus pallidus internus > globus pallidus externus > dentate gyrus > hippocampus > cerebral cortex > putamen > caudate > cerebellum > amygdala > thalamus = hypothalamus.

Rn*CB$_1$*. Substantia nigra > globus pallidus externus > entopeduncular nucleus (homologue of GPi) > cerebellum > hippocampus = caudate-putamen (striatum) > dentate gyrus > cerebral cortex > amygdala > hypothalamus > thalamus.

Narrative comparisons were employed by some researchers to describe aspects of CB$_1$ distribution that were not quantified: Glass *et al.* (1997) reported that CB$_1$ expression in human forebrain and thalamus showed greater complexities than CB$_1$ expression patterns seen in rat brain. The human cortex in Brodmann's area 22 (the planum temporale, part of Wernicke's area, the temporal lobe) showed greater CB$_1$ density in the left hemisphere than the right hemisphere (Figure 3), whereas rat studies showed no lateralization. Harkany *et al.* (2005) reported significant area-specific differences in expression of CB$_1$ in primate (lemur, *Microcebus murinus*) compared with a largely homogenous distribution of CB$_1$ afferents in the rat. Eggan and Lewis (2006) reported similar CB$_1$ distribution within the neocortex of the human and the long-tailed macaque (*Macaca fascicularis*).

Results of sensitivity testing

Additional methodological covariates

For affinity studies, *post hoc* observations identified eight additional methodological covariates:

(1) Ligand affinity at CB$_1$ may vary among specific brain regions, although regional differences have not been significant in studies reporting small sample sizes (Rodriguez de Fonseca *et al.*, 1994; Breivogel *et al.*, 1997).

(2) In native brain tissues, ligand affinity may vary depending on the usage of synaptosome fractions (primarily presynaptic nerve terminals) versus whole-membrane homogenates, which contain pre- and postsynaptic nerve terminals (Steffens *et al.*, 2004).

(3) Rodent studies used a variety of inbred lines; ligand affinity may vary among mouse strains (Hungund and Basavarajappa, 2000) and rat strains (Arnold *et al.*, 2001; Hoffman *et al.*, 2005).

(4) Affinities for AEA and WIN55212-2 may have been affected by additional binding targets in mouse brain (Breivogel *et al.*, 2001) and presumably human brain.

(5) Affinity may vary among transfected cell types. CB$_1$ receptors are expressed at the cell surface in some recombinant systems, such as *At*T-20 cells, HEK 293 cells and *Xenopus* oocytes, yet are not well expressed in CHO and COS-7 cells (Andersson *et al.*, 2003). However, Felder *et al.* (1995) reported non-significant differences between *At*T-20 and CHO cells.

(6) Studies were included whether or not they used methylarachidonoyl fluorophosphonate, which may
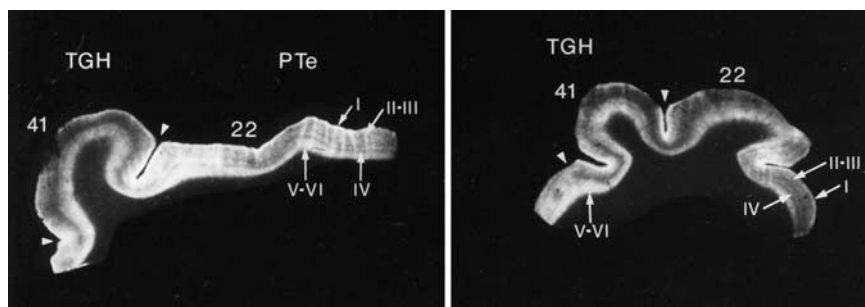

Figure 3 Autoradiograms of human cortex with [³H]CP55,940, showing greater CB$_1$ density (brightness) in the left hemisphere (left panel) compared to the right hemisphere (right panel). In both panels, Brodmann's area 22, labelled '22,' lies to the right of the transverse gyrus of Heschl (TGH), marked by arrow heads. Reproduced from Glass (1995), with permission. CB$_1$, cannabinoid receptor subtype 1; TGH, transverse gyrus of Heschl.

be $\approx 70\,000$-fold more potent than PMSF at preventing 2-AG degradation (Savinainen et al., 2003).

(7) Various autoradiographic studies used different ligand concentrations. Lower concentrations of tritiated ligand (that is, closer to ligand K_d) may be sensitive to the coupling state of the receptor and therefore be measuring differences in coupling rather than receptor B_{max}.

(8) Studies used a variety of vehicles (for example, ethanol, dimethylsulphoxide, bovine serum albumin or none), detergents (for example, 3-[(3-cholamidopropyl)dimethyl-ammonio]-1-propanesulfonate (CHAPS)), buffer concentrations (particularly Na^+ and Mg^{2+} with agonists) and other reagents, which may have affected affinity data. Studies rarely described methods that prevented photo-oxidation of cannabinoids, which are sensitive to light.

Testing the numerical validity of pooled means
Numerical validity was tested in three ways. First, the pooled means in Tables 2 and 3 were scalar-transposed into rank orders, ranked from the highest affinity (HU210) to the lowest affinity (CBD). These rank orders were compared to rank orders derived from studies that directly examined $\geqslant 2$ ligands per receptor. Studies that examined $\geqslant 2$ ligands per receptor are presented in Supplementary Table 6. These studies were aggregated using a bubble-sort algorithm, and the aggregate rank orders are presented in Supplementary Table 7. Rank orders from pooled means generally agreed with rank orders from the bubble-sort at $HsCB_1$, $RnCB_1$ and $HsCB_2$, but not at $RnCB_2$ receptors (see comparisons in Supplementary Table 7).

Second, we compared pooled means in Tables 2 and 3 to studies that made direct comparisons between receptors, which were listed in Supplementary Table 1, column seven. Means derived from direct comparisons were presented in Supplementary Table 8. For example, the pooled affinity of THC at $HsCB_1$ versus $HsCB_2$ (Table 3), when expressed as a ratio, was $(25.1/35.2\,nM =)\ 0.71$. This was consistent with eight studies that directly compared THC at $HsCB_1$ versus $HsCB2$; the ratios in the eight studies ranged from 0.1 to 1.6, with a mean ratio of 0.75 (Supplementary Table 8). Affinity ratios from pooled means generally agreed with affinity ratios derived from studies that made direct comparisons at $HsCB1$, $RnCB1$ and $HsCB_2$, excepting a dearth of data for CBD, CBN and 2-AG. Lack of data at $RnCB_2$ again prevented the validation of pooled means at those receptors, except for CP55,940 and AEA. Direct comparisons of WIN55212-2 diverged widely in the literature; $HsCB_1$ versus $HsCB_2$ ratios varied from 1.3 to 28.5, and $HsCB_1$ versus $RnCB_1$ ratios varied from 0.45 to 70.5 (Supplementary Table 1, column seven).

Third, we tested the precision of pooled means at $RnCB_1$ and $RnCB_2$ by comparing them to $MmCB_1$ and $MmCB_2$ affinity data (Supplementary Table 2). Pooled means of $MmCB_1$ and $MmCB_2$ data are presented in Supplementary Table 9 and compared to $RnCB_1$ and $RnCB_2$ data. This sensitivity analysis agreed with the previous two sensitivity analyses, except for two surprises: the pooled mean for THC at $RnCB_2$ (13.0 nM; Table 3) was supported by a similar pooled mean at $MmCB_2$ (13.4 nM; Supplementary Table 9). The pooled mean for 2-AG at $RnCB_1$ (1180.5 nM; Table 3) was

supported by a similar pooled mean at $MmCB_2$ (1626.6 nM; Supplementary Table 9).

Testing the validity of CB_1 distribution rank orders
The CB_1 distribution rank order for $RnCB_1$ was broadly consistent with the $MmCB_1$ rank order (Supplementary Table 5). The most prominent exception was seen in cerebellum, which ranked fourth in rat and second in mouse. The CB_1 distribution rank order for $HsCB_1$ deviated somewhat from that seen in the rhesus macaque, especially regarding the relative rank orders of cerebellum and cerebral cortex (Supplementary Table 5). This sensitivity analysis was hampered, however, by a low sample size in the other primates ($n = 3$), and possible $MfCB_1$ staining artefacts (K Mackie, personal communication).

Discussion

Critics may refute meta-analyses as mixtures of apples and oranges. We feel, however, that it can be a powerful tool to deal with questions not addressed by individual studies. We have collected individual studies and compared them, as a group, to a null hypothesis: orthologues of cannabinoid receptors do not differ between species. Data heterogeneity was obviated by subgroup meta-regression and by putting limits on data selection. Meta-analysis of individual studies provided an interpretive context not available in any single study, and enabled the adjudication of inconsistencies between studies. Meta-analysis also uncovered data lacunae. For example, the affinity of CBD at $HsCB_1$ receptors has never been reported in the peer-reviewed literature. This is a notable oversight, given the recent evaluation of CBD in several human clinical trials (Guy et al., 2004).

Meta-analysis is particularly appropriate when combining studies that have small sample sizes; the median sample size for affinity studies was $n = 3$. Meta-analyses in the medical literature commonly 'weight' larger studies that have less variance. Our weighted means lacked validity because many affinity studies did not provide the information required for weighting: sample size and sample variance. For this meta-analysis, weighting may not have been particularly advantageous, because 80% of affinity studies used identical sample sizes ($n = 3$) and studies that employed larger samples also reported larger variances.

Receptor–ligand affinity
Despite strict exclusion criteria, our search strategy identified 211 receptor–ligand affinity studies for meta-analysis. In comparison, recent literature reviews have cited an average of 28 studies (Diaz-Laviada and Ruiz-Llorente, 2005; Pertwee, 2005; Demuth and Molleman, 2006). The seeming wealth of information in 211 studies, however, was winnowed by subgroup analysis, then partitioned among nine cannabinoid ligands, and further shared by four receptors ($HsCB_1$, $HsCB_2$, $RnCB_1$ and $RnCB_2$).

Meta-regression identified several methodological covariates that generated heterogeneity in pooled means (Table 1). Studies that used brain sections differed significantly from

the majority of studies that used brain homogenates. Use of PMSF significantly lowered the K_i of AEA in brain tissues, but not in transfected cells. Use of PMSF upon spleen tissues could not be tested, because of a paucity of data. For that same reason, we did not test the effects of PMSF upon 2-AG affinity. Use of centrifugation versus filtration did not generate significantly different K_i values in any ligands except AEA in native brain tissues. In that assay, centrifugation produced K_i values that did not vary in the presence or absence of PMSF (originally demonstrated by Sheskin et al., 1997). Mean K_d (saturation binding) values differed from mean K_i (competition binding) values and the difference was primarily due to K_i studies that used [3H]SR141716A (Table 1), as originally demonstrated by Thomas et al. (1998). When unlabelled agonists were assayed with [3H]SR141716A, they produced biphasic competition curves (Houston and Howlett, 1998). Kearn et al. (1999) noted that [3H]SR141716A labelled two populations of receptors. In our meta-regression, [3H]WIN55212-2 produced mean K_i values that differed from mean K_i values of [3H]CP55,940, [3H]HU243 and [3H]BAY38-7271, but this trend fell short of statistical significance. Petitet et al. (1996) first noted this phenomenon; Thomas et al. (2005) attributed differences to WIN55212-2's divergent binding domain.

Meta-regression of native tissues versus heterologous expression systems produced controversial results. At RnCB$_1$, CP55,940 exhibited greater affinity in native versus transfected receptors, although the difference fell short of significance (Table 1). This is surprising, considering the differences in receptor stoichiometry among heterologous expression systems. For example, CB$_1$ receptors are expressed at the cell surface in AtT20 cells, HEK 293 cells and $Xenopus$ oocytes, but not well expressed at the cell surface in CHO cells and COS-7 cells (Andersson et al., 2003). Six studies in the literature made direct comparisons between native versus transfected: five reported slightly greater affinity (but not statistically different) in native tissues (Felder et al., 1992, 1995; Mauler et al., 2002; Thomas et al., 2005; Paugh et al., 2006), whereas Rhee et al. (1997) reported greater affinity in transfected cells (see data in Supplementary Table 1). If native tissue does indeed produce higher affinities than transfected cells, we may be underreporting interspecies differences for ligands that demonstrate greater affinity for Hs than Rn (for example, THC), because RnCB$_1$ studies are primarily assayed in native tissues, whereas HsCB$_1$ studies primarily use transfected cells. Contrarily, ligands with greater affinity at Rn than Hs (for example, WIN55212, CP55,940 and SR141716A) may actually exert less interspecies differences than estimated by our meta-analysis. The interspecies differences reported in our meta-analysis should be confirmed by the gold standard: a large, adequately powered, $in vitro$ comparative study (LeLorier et al., 1997). Interestingly, the K_i of THC at HsCB$_1$ places it around the median affinity for current small-molecule drugs, which is 20 nM (Overington et al., 2006).

Receptor distribution

Autoradiography studies that used different radioligands were broadly consistent with each other. This agreed with

Breivogel et al. (1997), who made a direct comparison between [3H]WIN55212-2 and [3H]SR141716A and reported a good correlation ($r = 0.89$) in brain region B_{max} values. Studies that used regional dissection showed greater variability in their results, perhaps due to the difficulty of obtaining clean regional dissections. For example, dissected thalamus, a region with low B_{max}, may be contaminated by fragments from the entopeduncular nucleus, a region with high B_{max} (Breivogel et al., 1997). Immunohistochemistry studies used a variety of tagged antibodies raised against N-terminal or C-terminal regions, but their results were broadly consistent (Supplementary Table 5). In agreement, Harkany et al. (2005) directly compared a variety of N-terminus and C-terminus epitopes and reported nearly identical staining patterns, after correcting for methodological considerations that produced staining artefacts. Conversely, Egertova and Elphick (2000) suggested that antibodies directed against the C-terminus were rendered non-immunoreactive by phosphorylation of amino-acid residues in the C-terminus, and it remains untested whether the transient binding of interacting proteins such as GASP1 (Martini et al., 2007) can alter antibody recognition. Furthermore, antibodies directed against the N-terminus cannot label either splice variant of the CB$_1$ receptor. Thus immunohistochemical approaches risk labelling only a subset of total receptors.

Immunohistochemistry studies broadly correlated with radioligand results, given that antibodies specifically label CB$_1$ receptors, whereas [3H]CP55,940 binds to CB$_1$ as well as CB$_2$ receptors and CB$_2$ receptors may be present in the brain (Van Sickle et al., 2005; Gong et al., 2006). Furthermore, cannabinoid ligands may bind to targets other than CB$_1$ receptors (Breivogel et al., 2001; Pertwee et al., 2005; Baker et al., 2006), although the extent of the contribution of these 'new' receptors in tissue preparations has yet to be elucidated (Petitet et al., 2006). $In situ$ hybridization studies yielded different rank orders than immunohistochemistry and radioligand studies, due to the fact that labelled oligonucleotides: (1) identified mRNA in brain regions that may not have translated into the corresponding protein product; (2) identified mRNA in cell bodies (perikarya), whereas the corresponding protein product (CB$_1$) translocated to distal sites before inserting into cell membranes. For example, many neurons with cell bodies in the striatum project axons to the globus pallidus and substantia nigra. Thus, high levels of CB$_1$ mRNA were expressed in the striatum and negligible levels were found in the globus pallidus and substantia nigra. And the reverse was true regarding the corresponding protein product (CB$_1$), with high densities in the globus pallidus, entopeduncular nucleus and substantia nigra, but only moderate densities in the striatum. $In situ$ hybridization studies used a variety of probes, but their results were broadly consistent with each other (Supplementary Table 5).

Interspecies differences could be discerned in CB$_1$ receptor distribution rank orders: HsCB$_1$ densities were relatively greater in brain regions associated with cognitive function (frontal cortex) and memory (hippocampus), whereas RnCB$_1$ densities were relatively greater in movement-associated areas (cerebellum) and the caudate-putamen. These differences were first described by Herkenham et al. (1990). They noted that human brain, compared with rat (and rhesus,

surprisingly), expressed greater CB_1 densities in the cortex and amygdala, and lesser densities in the cerebellum. Romero *et al.* (1998) were the first to show that mouse brain, compared to rat brain, exhibited even greater densities in the cerebellum compared to cortex.

Narrative comparisons in the literature described more subtle interspecies differences. According to Glass *et al.* (1997), the human cortex showed CB_1 enrichment in the left (not the right) planum temporale, which is part of Wernicke's area in the temporal lobe. This region is associated with verbal language development, human communication and musical talent. CB_1 in the human forebrain expressed a greater density and complexity than in rat forebrain; this difference likely mediates a THC 'high' in humans that may be absent in rats. Similarly, CB_1 distribution is more elaborate in the human dorsomedial nucleus, an area that mediates emotional tones from the amygdaloid complex and an area that contributes to the formation of human personality. According to Harkany *et al.* (2005), CB_1 architecture was more complex in primate than rodent, in the cingulate and frontal cortex (regions associated with motor control), hippocampus (associated with memory and learning), amygdala (emotional behaviours) and parietotemporal areas (visual and auditory information processing). In contrast, only minor differences between the primate and rodent were seen in basal forebrain territories (associated with olfaction). Eggan and Lewis (2006) compared the human neocortex with that of the long-tailed macaque (*Macaca fascicularis*). Human CB_1 cytoarchitecture was slightly more complex in prefrontal area 46 (associated with the ability to retain and manipulate visual spatial information) and occipital area 17 (the primary visual cortex).

Conclusion

Sensitivity analyses provided confidence in meta-analytic estimates of K_d (CP55,940, WIN55,212-2, SR141716A) and K_i (THC, AEA, HU210) distilled from studies on $HsCB_1$, $HsCB_2$ and $RnCB_1$ receptors. We consider those pooled K_d and K_i values to be the most valid estimates in the literature, able to be generalized to studies that employed tissue homogenates (not tissue sections) and tritiated agonists (not tritiated inverse agonists), applied PMSF in AEA assays and used either filtration or centrifugation of radioligands. Meta-regression observed a trend towards greater affinity at native tissues compared to heterologous expression systems, but fell short of significance. In other words, heterogeneity arising between studies that used native tissues versus transfected cells is likely due to pipetting inaccuracies and other experimental errors, rather than due to the methodological dichotomy. We lack confidence in our results at $RnCB_2$ receptors, as well as the pooled K_i values of 2-AG, CBD and CBN, due to small sample sizes and lack of validation in sensitivity analyses.

Only $0.05 \, \text{mg kg}^{-1}$ of THC affects a human (Isbell *et al.*, 1967; Ohlsson *et al.*, 1980), whereas it may take $3 \, \text{mg kg}^{-1}$ of THC to affect a rat (Jarbe *et al.*, 1976; Browne and Weissman, 1981; Wiley *et al.*, 1995). Comparing doses across *in vivo* animal models is controversial, because drug discrimination

in the rat and the human perception of 'high' may not involve the same populations of receptors; furthermore only limited pharmacokinetic data exists for cannabinoids across species. Nevertheless this 60-fold disparity suggests phenotypic differences exist between species. Our meta-analysis confirmed differences in ligand binding between human and rat orthologues of CB_1 and CB_2 receptors The fact that synthetic cannabinoids (for example, WIN55,212-2) discriminated between human and rat orthologues did not surprise us. After all, ligands have been synthesized for that very purpose (Astolfi *et al.*, 1997), and WIN55,212-2 induces behavioural changes that are strikingly different between species (Haller *et al.*, 2007). The *R*-enantiomer of AM1241 acts as an agonist at $HsCB_2$, but an inverse agonist at $RnCB_2$, and $MmCB_2$ (Bingham *et al.*, 2007). Species differences in ligand affinity are the norm at vanilloid receptors (Szallasi, 1994; Huang *et al.*, 2002) and melanocortin receptors (Schioth *et al.*, 2005). Phytocannabinoids that discriminate between human and rat orthologues are more interesting from an evolutionary viewpoint, although the concept is not without precedents; Russo *et al.* (2005) showed that CBD discriminated between human and rat orthologues of the $5-HT_{1A}$ receptor.

This study did not evaluate functional (efficacy) assays, such as cannabinoid inhibition of cAMP accumulation by forskolin-stimulated adenylyl cyclase, or cannabinoid stimulation of radiolabelled non-hydrolysable guanosine 5'-*O*-(3-thio)-triphosphate ([^{35}S]GTPγS). Given the disparate affinities for THC and AEA at human versus rat receptors, we hypothesize that efficacies also vary between species. We plan to explore this possibility by conducting our next meta-analysis with efficacy data, to better assess how cannabinoid ligands interact with cannabinoid receptors to evoke responses.

Acknowledgements

Conflict of interest

The authors state no conflict of interest.

References

Andersson H, D'Antona AM, Kendall DA, Von Heijne G, Chin CN (2003). Membrane assembly of the cannabinoid receptor 1: impact of a long N-terminal tail. *Mol Pharmacol* **64**: 570–577.

Arnold JC, Topple AN, Mallet PE, Hunt GE, McGregor IS (2001). The distribution of cannabinoid-induced Fos expression in rat brain: differences between the Lewis and Wistar strain. *Brain Res* **921**: 240–255.

Astolfi M, Patacchini R, Maggi M, Manzini S (1997). Improved discriminatory properties between human and murine tachykinin

NK1 receptors of MEN 10930: a new potent and competitive antagonist. *Neuropeptides* 31: 373–379.

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

Bingham B, Jones PG, Uveges AJ, Kotnis S, Lu P, Smith VA *et al.* (2007). Species-specific *in vitro* pharmacological effects of the cannabinoid receptor 2 (CB2) selective ligand AM1241 and its resolved enantiomers. *Br J Pharmacol*: 2007 Jun 4 [E-pub ahead of print].

Boyce MS, Irwin LL, Barker R (2005). Demographic meta-analysis: synthesizing vital rates for spotted owls. *J Appl Ecol* 42: 38–49.

Breivogel CS, Griffin G, Di Marzo V, Martin BR (2001). Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol Pharmacol* 60: 155–163.

Breivogel CS, Sim LJ, Childers SR (1997). Regional differences in cannabinoid receptor/G-protein coupling in rat brain. *J Pharmacol Exp Ther* 282: 1632–1642.

Browne RG, Weissman A (1981). Discriminative stimulus properties of delta 9-tetrahydrocannabinol: mechanistic studies. *J Clin Pharmacol* 21: 227S–234S.

Cochran W (1954). The combination of estimates from different experiments. *Biometrics* 10: 101–124.

Demuth DG, Molleman A (2006). Cannabinoid signalling. *Life Sci* 78: 549–563.

Diaz-Laviada I, Ruiz-Llorente L (2005). Signal transduction activated by cannabinoid receptors. *Mini Rev Med Chem* 5: 619–630.

Egertova M, Elphick MR (2000). Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB. *J Comp Neurol* 422: 159–171.

Eggan SM, Lewis DA (2006). Immunocytochemical distribution of the cannabinoid CB1 receptor in the primate neocortex: a regional and laminar analysis. *Cereb Cortex* 17: 175–191.

Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O *et al.* (1995). Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. *Mol Pharmacol* 48: 443–450.

Felder CC, Veluz JS, Williams HL, Briley EM, Matsuda LA (1992). Cannabinoid agonists stimulate both receptor- and non-receptor-mediated signal transduction pathways in cells transfected with and expressing cannabinoid receptor clones. *Mol Pharmacol* 42: 838–845.

Glass GV, McGaw B, Smith ML (1981). *Meta-analysis in Social Research*. SAGE Publications: Beverly Hills, CA.

Glass M (1995). *Doctorate Dissertation, Department of Pharmacology and Clinical Pharmacology*. University of Auckland: Auckland, pp. 220.

Glass M, Dragunow M, Faull RL (1997). Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* 77: 299–318.

Gong JP, Onaivi ES, Ishiguro H, Liu QR, Tagliaferro PA, Brusco A *et al.* (2006). Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Res* 1071: 10–23.

Guy G, Robson R, Strong K, Whittle B (2004). *The Medicinal Use of Cannabis*. Royal Society of Pharmacists: London.

Haller J, Matyas F, Soproni K, Varga B, Barsy B, Nemeth B *et al.* (2007). Correlated species differences in the effects of cannabinoid ligands on anxiety and on GABAergic and glutamatergic synaptic transmission. *Eur J Neurosci* 25: 2445–2456.

Harkany T, Dobszay MB, Cayetanot F, Hartig W, Siegemund T, Aujard F *et al.* (2005). Redistribution of CB1 cannabinoid receptors during evolution of cholinergic basal forebrain territories and their cortical projection areas: a comparison between the gray mouse lemur (*Microcebus murinus,* primates) and rat. *Neuroscience* 135: 595–609.

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR *et al.* (1990). Cannabinoid receptor localization in brain. *Proc Natl Acad Sci USA* 87: 1932–1936.

Higgins JPT, Green S (2005). Cochrane Handbook for Systematic Reviews of Interventions 4.2.5. [updated May 2005]. In: Higgins JPT, Green S (eds) *The Cochrane Library*. John Wiley & Sons, Ltd.: Chichester, UK.

Hoffman AF, Macgill AM, Smith D, Oz M, Lupica CR (2005). Species and strain differences in the expression of a novel glutamate-

modulating cannabinoid receptor in the rodent hippocampus. *Eur J Neurosci* 22: 2387–2391.

Houston DB, Howlett AC (1998). Differential receptor-G-protein coupling evoked by dissimilar cannabinoid receptor agonists. *Cell Signal* 10: 667–674.

Howlett AC (2002). The cannabinoid receptors. *Prostaglandins Other Lipid Mediat* 68–69: 619–631.

Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F *et al.* (2002). An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc Natl Acad Sci USA* 99: 8400–8405.

Hungund BL, Basavarajappa BS (2000). Distinct differences in the cannabinoid receptor binding in the brain of C57BL/6 and DBA/2 mice, selected for their differences in voluntary ethanol consumption. *J Neurosci Res* 60: 122–128.

Isbell H, Gorodetzsky CW, Jasinski D, Claussen U, Von Spulak F, Korte F (1967). Effects of (−)delta-9-trans-tetrahydrocannabinol in man. *Psychopharmacologia* 11: 184–188.

Jarbe TU, Johansson JO, Henriksson BG (1976). Characteristics of tetrahydrocannabinol (THC)-produced discrimination in rats. *Psychopharmacology (Berlin)* 48: 181–187.

Kearn CS, Greenberg MJ, DiCamelli R, Kurzawa K, Hillard CJ (1999). Relationships between ligand affinities for the cerebellar cannabinoid receptor CB1 and the induction of GDP/GTP exchange. *J Neurochem* 72: 2379–2387.

LeLorier J, Gregoire G, Benhaddad A, Lapierre J, Derderian F (1997). Discrepancies between meta-analyses and subsequent large randomized, controlled trials. *N Engl J Med* 337: 536–542.

Martini L, Waldhoer M, Pusch M, Kharazia V, Fong J, Lee JH *et al.* (2007). Ligand-induced down-regulation of the cannabinoid 1 receptor is mediated by the G-protein-coupled receptor-associated sorting protein GASP1. *FASEB J* 21: 802–811.

Mauler F, Mittendorf J, Horvath E, De Vry J (2002). Characterization of the diarylether sulfonylester (−)-(R)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate (BAY 38-7271) as a potent cannabinoid receptor agonist with neuroprotective properties. *J Pharmacol Exp Ther* 302: 359–368.

McPartland JM, Matias I, Di Marzo V, Glass M (2006). Evolutionary origins of the endocannabinoid system. *Gene* 370: 64–74.

Ohlsson A, Lindgren JE, Wahlen A, Agurell S, Hollister LE, Gillespie HK (1980). Plasma delta-9 tetrahydrocannabinol concentrations and clinical effects after oral and intravenous administration and smoking. *Clin Pharmacol Ther* 28: 409–416.

Overington JP, Al-Lazikani B, Hopkins AL (2006). How many drug targets are there? *Nat Rev Drug Discov* 5: 993–996.

Paugh SW, Cassidy MP, He H, Milstien S, Sim-Selley LJ, Spiegel S *et al.* (2006). Sphingosine and its analog, the immunosuppressant 2-amino-2-(2-[4-octylphenyl]ethyl)-1,3-propanediol, interact with the CB1 cannabinoid receptor. *Mol Pharmacol* 70: 41–50.

Pertwee RG (2005). Pharmacological actions of cannabinoids. *Handb Exp Pharmacol* 168: 1–51.

Pertwee RG, Thomas A, Stevenson LA, Maor Y, Mechoulam R (2005). Evidence that (−)-7-hydroxy-4′-dimethylheptyl-cannabidiol activates a non-CB(1), non-CB(2), non-TRPV1 target in the mouse vas deferens. *Neuropharmacology* 48: 1139–1146.

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.

Petitet F, Marin L, Doble A (1996). Biochemical and pharmacological characterization of cannabinoid binding sites using [^{3}H]SR141716A. *Neuroreport* 7: 789–792.

Reed GF, Lynn F, Meade BD (2002). Use of coefficient of variation in assessing variability of quantitative assays. *Clin Diagn Lab Immunol* 9: 1235–1239.

Rhee MH, Vogel Z, Barg J, Bayewitch M, Levy R, Hanus L *et al.* (1997). Cannabinol derivatives: binding to cannabinoid receptors and inhibition of adenylylcyclase. *J Med Chem* 40: 3228–3233.

Rodriguez de Fonseca F, Gorriti MA, Fernandez-Ruiz JJ, Palomo T, Ramos JA (1994). Downregulation of rat brain cannabinoid binding sites after chronic delta 9-tetrahydrocannabinol treatment. *Pharmacol Biochem Behav* 47: 33–40.

Romero J, Fernandez-Ruiz JJ, Vela G, Ruiz-Gayo M, Fuentes JA, Ramos JA (1998). Autoradiographic analysis of cannabinoid receptor binding and cannabinoid agonist-stimulated [35S]GTP gamma S

binding in morphine-dependent mice. *Drug Alcohol Depend* **50**: 241–249.

Russo EB, Burnett A, Hall B, Parker KK (2005). Agonistic properties of cannabidiol at 5-HT1a receptors. *Neurochem Res* **30**: 1037–1043.

Savinainen JR, Saario SM, Niemi R, Jarvinen T, Laitinen JT (2003). An optimized approach to study endocannabinoid signaling: evidence against constitutive activity of rat brain adenosine A1 and cannabinoid CB1 receptors. *Br J Pharmacol* **140**: 1451–1459.

Schioth HB, Haitina T, Ling MK, Ringholm A, Fredriksson R, Cerda-Reverter JM *et al.* (2005). Evolutionary conservation of the structural, pharmacological, and genomic characteristics of the melanocortin receptor subtypes. *Peptides* **26**: 1886–1900.

Sese J, Morishita S (2001). Rank aggregation method for biological databases. *Genome Inform* **12**: 506–507.

Sheskin T, Hanus L, Slager J, Vogel Z, Mechoulam R (1997). Structural requirements for binding of anandamide-type compounds to the brain cannabinoid receptor. *J Med Chem* **40**: 659–667.

Steffens M, Engler C, Zentner J, Feuerstein TJ (2004). Cannabinoid CB1 receptor-mediated modulation of evoked dopamine release and of adenylyl cyclase activity in the human neocortex. *Br J Pharmacol* **141**: 1193–1203.

Szallasi A (1994). The vanilloid (capsaicin) receptor: receptor types and species differences. *Gen Pharmacol* **25**: 223–243.

Thomas BF, Francisco ME, Seltzman HH, Thomas JB, Fix SE, Schulz AK *et al.* (2005). Synthesis of long-chain amide analogs of the cannabinoid CB1 receptor antagonist *N*-(piperidinyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyra zole-3-carboxamide (SR141716) with unique binding selectivities and pharmacological activities. *Bioorg Med Chem* **13**: 5463–5474.

Thomas BF, Gilliam AF, Burch DF, Roche MJ, Seltzman HH (1998). Comparative receptor binding analyses of cannabinoid agonists and antagonists. *J Pharmacol Exp Ther* **285**: 285–292.

Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K *et al.* (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* **310**: 329–332.

Wiley JL, Lowe JA, Balster RL, Martin BR (1995). Antagonism of the discriminative stimulus effects of delta 9-tetrahydrocannabinol in rats and rhesus monkeys. *J Pharmacol Exp Ther* **275**: 1–6.

Supplementary Information accompanies the paper on British Journal of Pharmacology website (http://www.nature.com/bjp)