The Dietary Polyphenols trans-Resveratrol and Curcumin Selectively Bind Human CB1 Cannabinoid Receptors with Nanomolar Affinities and Function as Antagonists/Inverse Agonists

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ABSTRACT

The dietary polyphenols trans-resveratrol [5-[(1E)-2-(4-hydroxyphenyl)ethyl]-1,3-benzenediol; found in red wine] and curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1E,6E-heptadiene-3,5-dione; (found in curry powders)] are known to exert anti-inflammatory and antioxidant effects via poorly defined mechanisms. It is interesting that these polyphenols, derived from the marijuana plant (Cannabis sativa), produce similar protective effects via poorly defined mechanisms. It is unclear whether the beneficial effects in vitro generally require relatively high concentrations (>1 μM) and are thought to involve antioxidative effects or CB1 activity. COX-2 inhibitors and conventional antioxidants, producing neutral antagonists, whereas competitively antagonizing CB1 receptors at dietary relevant concentrations. Therefore, these polyphenols and their derivatives might be developed as novel, nontoxic CB1 therapeutics for obesity and/or drug dependence.

Dietary polyphenols, such as resveratrol [5-[(1E)-2-(4-hydroxyphenyl)ethyl]-1,3-benzenediol] (found in red wine) and curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1E,6E-heptadiene-3,5-dione] (found in curry powders), have been used safely for centuries as traditional medicines. As a consequence, increasing scientific investigation suggests that they may prove useful as therapeutics for a broad range of conditions (Scalbert et al., 2005), from inflammatory diseases (Rahman et al., 2006) to cancer (Hadi et al., 2007). The protective effects of resveratrol and curcumin seem to be related to their antioxidant (Fraga, 2007) and anti-inflammatory (Surh et al., 2005) properties. Although the specific mechanisms responsible for these beneficial effects remain unclear, the beneficial effects in vitro generally require relatively high concentrations (>1 μM) and are thought to involve antioxidative effects or CB1 activation.

ABBREVIATIONS: ASC-J9, 1,7-bis(3,4-dimethoxyphenyl)-5-hydroxy-1E,4E,6E-heptatriene-3-one; [3H]CP-55,950, (−)-cis-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl) cyclohexanone; [3H]GTP, S, guanosine 5′-O-[3,5-(3H)trimethylphosphoramide]; CHO, Chinese hamster ovary; h, human; WIN-55,212-2, [(R)-(−)]-2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)piperidino[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethylamine mesylate; CP-55,940, (1S,3R)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol; ANOVA, analysis of variance; HU-210, (−)-11-hydroxy-(8)-tetrahydrocannabinol-dimethylheptyl; AM-251, N-(4-piperidinyl-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; m, mouse; O-2050, (SAR,10aR)-3-(1-methanesulfonylamino-4-hexyl-6-yl)-6a,7,10a,14a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran; rimonabant, 5-(p-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-piperidinopyrazole-3-carboxamide hydrochloride; AM1241, (R,S)-3-(2-iodo-5-nitrobenzoyl)-1-(1-methyl-2-piperidinyl)methyl)-1H-indole.
volve multiple receptor- and nonreceptor-mediated processes (Stevenson and Hurst, 2007).

Recently, it has been reported that resveratrol and other polyphenols bind with high affinity to a distinct, yet unidentified, plasma membrane bound receptor that occurs in high density throughout the brain (Han et al., 2006). Cannabinoid receptors seem to share many characteristics with this newly discovered, uncharacterized resveratrol receptor. Originally isolated from the marijuana plant (Cannabis sativa), both synthetic and naturally occurring cannabinoids, such as Δ9-tetrahydrocannabinol, produce their effects by acting at two G-protein-coupled receptors, CB1 (Matsuda et al., 1990) and CB2 (Munro et al., 1993). CB1 receptors are expressed in high abundance throughout the central nervous system, whereas CB2 receptors are expressed predominantly in immune cells and non-neuronal tissues. Cannabinoids acting at both receptors produce antioxidant (Hampson et al., 1998) and anti-inflammatory (Klein, 2005) effects, similar to that reported for resveratrol and curcumin. Therefore, the current studies were conducted to determine whether two important dietary polyphenols, resveratrol and curcumin, and an analog of curcumin (ASC-J9) act as ligands at cannabinoid receptors. It is important that our study identifies the human CB1 cannabinoid receptor as a high-affinity target for all three polyphenols: resveratrol (K_i = 45 nM), curcumin (K_i = 6 nM), and ASC-J9 (K_i = 64 nM, an analog of curcumin). Furthermore, all polyphenols examined seem to act as CB1 antagonists/inverse agonists and share common structural motifs with other known cannabinoid receptors. It is important that these results indicate the CB1 receptor may be one of the highest affinity targets for dietary polyphenols, resveratrol and curcumin and may have significant implications for future development.

Materials
All drugs used were purchased from Sigma-Aldrich (St. Louis, MO). [3H]Adenine ([3H]A) (168 Ci/mmol) and [35S]GTPgS ([35S]GTPgS) (1.100 Ci/mmol) were purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). Rats (B6SJL) were obtained from an in house breeding colony. Whole brains were pooled before beginning homogenization. Pellets of frozen/thawed cells or freshly harvested brain tissue were resuspended in a homogenization buffer containing 50 mM HEPES, pH 7.4, 3 mM MgCl_2, and 1 mM EGTA. Using a 40-ml Dounce glass homogenizer (Wheaton, Philadelphia PA), samples were subjected to 10 complete strokes and centrifuged at 18,000 rpm for 10 min at 4°C. After repeating the homogenization procedure twice more, the samples were resuspended in HEPES buffer (50 mM, pH 7.4) and subjected to 10 strokes utilizing a 7-ml glass homogenizer. Membranes were stored in aliquots of approximately 1 mg/ml at −80°C.

Competition Receptor Binding
Increasing concentrations of WIN-55,212-2 or different polyphenols were incubated with 0.1 nM (mouse brain or CHO-hCB2) or 0.5 nM (CHO-hCB1) [3H]CP-55,940 in a final volume of 1 ml of binding buffer as described previously (Shoemaker et al., 2005). Each binding assay contained 100 (mouse brain or CHO-hCB2) or 150 (CHO-hCB1) μg of membrane protein, and reactions were incubated for 90 min at room temperature with mild agitation. Nonspecific binding was defined as binding observed in the presence of 1 μM nonradioactive CP-55,940. Reactions were terminated by rapid vacuum filtration through Whatman GF/B glass fiber filters (Whatman, Clifton, NJ) followed by two washes with ice-cold binding buffer. Analysis of the binding data were performed using the nonlinear regression (Curve Fit) function of GraphPad Prism version 4.0b (GraphPad Software Inc., San Diego, CA) to determine the concentration of the drug that displaced 50% of [3H]CP-55,940 (IC50). A measure of affinity (K_i) was derived from the IC50 values utilizing the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

Measurement of cAMP Levels in Intact Cells
[3H]Adenine-labeled ATP pools to cAMP was measured as a functional measure of cannabinoid activity (Shoemaker et al., 2005). CHO-hCB1 cells were seeded into 24-well plates and cultured to confluence. Dulbecco’s modified Eagle’s medium containing 0.9% NaCl, 500 μM 3-isobutyl-1-methylxanthine, and 2 μCi/well [3H]adenine was added to the cells for 2 h at 37°C. The labeled adenine mixture was removed, and the cannabinoids were added for 15 min in a Krebs-Ringer-HEPES buffer containing 500 μM 3-isobutyl-1-methylxanthine and 10 μM forskolin. The reaction was terminated with 50 μl of 2.2 N HCl and [3H]cAMP separated by aluminu column chromatography.

Animal Studies
Mice. Animal use protocols employed in this study were approved by the University of Arkansas for Medical Sciences Institutional Animal Care and Use Committee and conducted in accordance with the United States Public Health Service policy on humane care and use of laboratory animals. Male and female B6SJL mice were obtained from an in house breeding colony.

Hypothermia Experiments. Body temperature of age- and weight-matched mice was measured by a digital thermometer (model 17025; Thermo Fisher Scientific) inserted ~1 cm into the rectum. Body temperature was measured 1 h after a subcutaneous injection of CP-55,940, a time interval resulting in maximal hypothermia (data not shown). When testing CB1 antagonism, drugs were given 30 min before CP-55,940 injections by the intraperitoneal route. For all experiments, body temperature was measured before any injection, 30 min after antagonist or vehicle injection and 1 h after injection of CP-55,940. The injection vehicle used for these experiments contained 50% polyethylene glycol and 50% saline.

Body Weight Reduction Experiments. Age- and weight-matched mice were injected intraperitoneally with the indicated
Selectivity of polyphenols for human CB1 and CB2 receptors

Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>( K_i ) mCB1</th>
<th>( K_i ) hCB1</th>
<th>( K_i ) hCB2</th>
<th>( K_i ) hCB2/hCB1</th>
<th>Selectivity</th>
</tr>
</thead>
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<tr>
<td>WIN-55,212-2</td>
<td>3.4 ± 1.6 (5)</td>
<td>7.7 ± 1.3 (5)</td>
<td>5.8 ± 1.2 (4)</td>
<td>0.75</td>
<td>Nonselective</td>
</tr>
<tr>
<td>Curcumin</td>
<td>73.1 ± 23.5 (7)</td>
<td>5.9 ± 2.1 (6)</td>
<td>2,600 ± 900 (4)</td>
<td>446</td>
<td>CB1</td>
</tr>
<tr>
<td>ASC-J9</td>
<td>190 ± 110 (8)</td>
<td>64 ± 17 (3)</td>
<td>13,000 ± 1300 (4)</td>
<td>201</td>
<td>CB1</td>
</tr>
<tr>
<td>trans-Resveratrol</td>
<td>270 ± 160 (6)</td>
<td>45 ± 17 (3)</td>
<td>&gt;100,000 (4)</td>
<td>&gt;2227</td>
<td>CB1</td>
</tr>
</tbody>
</table>

Statistical Analysis

Curve-fitting and statistical analysis was performed using GraphPad Prism version 4.0b. Data obtained from three or more experimental groups were analyzed by a one-way ANOVA, followed by a Dunnett’s post hoc comparison of individual groups. A non-parametric test was employed to statistically compare data obtained from two experimental groups.

Statistical Error

Six animals were included in each experimental group. All experiments were conducted in triplicate. Statistical significance was set at \( p < 0.05 \).
under certain conditions, employing the same nonradioactive compound to define nonspecific binding of the radioactive compound may identify binding erroneously as specific, when in reality it is nonspecific but inhibitable. Therefore, experiments were performed to compare the maximal displacement of [3H]CP-55,940 produced by CP-55,940 (1 μM) and a second high-affinity nonselective cannabinoid agonist HU-210 (1 μM) (data not shown). Results from these experiments revealed that nonradioactive CP-55,940 and HU-210 produce near-identical maximal displacement of [3H]CP-55,940 in membrane homogenates prepared from mouse brain, CHO-hCB1, and CHO-hCB2 cells. This suggests that the residual [3H]CP-55,940 binding observed for all cannabinoid ligands tested was not due to the use of nonradioactive CP-55,940 to define nonspecific binding. Although the exact reason for the observed residual binding is unknown, it is possible that the highly hydrophobic properties of the ligands tested, relative to CP-55,940, might contribute these results.

**trans-Resveratrol, Curcumin, and ASC-J9 Act as Antagonists/Inverse Agonists at Human CB1 Receptors in Membrane Preparations of CHO-hCB1 Cells.** To determine the intrinsic activity concerning G-protein function, the ability of the three polyphenols to modulate [35S]GTPγS binding in CHO-hCB1 membranes was examined (Fig. 2). Characteristic of agonists, the nonselective full CB1/CB2 agonist WIN-55,212-2 produces a concentration-dependent increase of approximately 90% in the binding of [35S]GTPγS to CHO-hCB1 membranes, with an ED50 of 31 ± 6.4 nM, open diamonds). The ED50 values are presented under Results. (Trans)-resveratrol, curcumin, and ASC-J9 act as neutral antagonists, rather than inverse agonists, in intact CHO-CB1 cells. Indicative of competitive antagonism, coinoculation with a fixed concentration of each of the polyphenols with the agonist WIN-55,212-2 resulted in a significant (p < 0.05) 7- to 10-fold parallel shift to the right in the concentration-effect curve of WIN-55,212-2 (+ curcumin, 120 ± 3.5 nM, n = 3; + trans-resveratrol, 130 ± 25 nM, n = 3) (Fig. 2B). It is interesting that coinoculation with ASC-J9 resulted in a much greater, 63-fold reduction in the potency of WIN-55,212-2 to activate G-proteins (Fig. 2B). Both curcumin and trans-resveratrol produced a significant (p < 0.05), 3-fold shift to the right in the concentration-effect curve of WIN-55,212-2 (+ curcumin, 120 ± 3.5 nM, n = 3; + trans-resveratrol, 130 ± 25 nM, n = 3) (Fig. 2B).

Before conducting in vivo studies in mice, in vitro studies were conducted to determine the affinity and [35S]GTPγS binding to CHO-hCB1 membranes. All polyphenols (100 μM) fail to produce any change in [35S]GTPγS binding to membranes prepared from wild-type CHO cells (data not shown). This suggests that the polyphenols act as inverse agonists, suppressing G-protein activation produced by constitutively active hCB1 receptors. However, the potency (e.g., IC50) of the polyphenols required to observe inverse agonism is relatively low (curcumin, 1.3 ± 0.3 μM, n = 3; ASC-J9, 56 ± 22 μM, n = 3; trans-resveratrol, 47 ± 17 μM, n = 3) compared with their high nanomolar affinity for hCB1 receptors (Fig. 1). Consistent with an antagonist/inverse agonist profile, coinoculation with a fixed concentration of each polyphenol that produced minimal reduction of [35S]GTPγS binding alone resulted in a significant reduction in the potency of the agonist WIN-55,212-2 to activate G-proteins (Fig. 3A). Both curcumin and trans-resveratrol produced a significant (p < 0.05), 3-fold shift to the right in the concentration-effect curve of WIN-55,212-2 (+ curcumin, 120 ± 3.5 nM, n = 3; + trans-resveratrol, 130 ± 25 nM, n = 3) (Fig. 2B). It is interesting that coinoculation with ASC-J9 resulted in a much greater, 63-fold reduction in the potency of WIN-55,212-2 to activate G-proteins (Fig. 2B).

**Similar to Human CB1 Receptors, trans-Resveratrol, Curcumin, and ASC-J9 Bind with Nanomolar Affinity to and Act as Antagonists/Inverse Agonists at Mouse CB1 Receptors in Membrane Preparations of Whole-Brain Tissue.** Before conducting in vivo studies in mice, in vitro studies were conducted to determine the affinity and...
intrinsic activity of the polyphenols at mouse CB1 receptors (Fig. 4). Homologous competition receptor binding with [3H]CP-55,940 showed that mouse brain membranes contain a density of mCB1 cannabinoid receptors of 0.59 ± 0.14 pmol/mg protein, to which CP-55,940 binds with an affinity (Kd) of 2.6 ± 0.55 nM (n = 3, data not shown). The affinity for WIN-55,212-2 for brain mCB1 receptors (3.4 ± 0.3 nM, Table 1) is similar to that observed for brain membranes (Fig. 1). Furthermore, the rank order of potency of the polyphenols tested to mCB1 receptors with high intrinsic activity in the same rank order of potency as observed for hCB1 (Table 1). Curcumin binds mCB1 with the highest affinity (73 nM) and ASC-J9 (190 nM) more weakly. In comparison, resveratrol acts as a pure neutral antagonist, whereas curcumin to reduce body weight in mice was examined (Fig. 2A). In Mice, Repeated Administration of trans-Resveratrol, curcumin, and ASC-J9 act as neutral antagonists at human CB1 receptors in intact CHO-hCB1 cells. A, forskolin (10 μM)-stimulated adenylyl cyclase assays were conducted in whole CHO-hCB1 cells. Intracellular cAMP levels were measured in response to increasing concentrations of the CB1 agonist WIN-55,212-2 (filled squares), curcumin (open circles), ASC-J9 (open triangles), or trans-resveratrol (open diamonds) alone. Data are presented as the percentage of cAMP levels measured in the presence of the indicated drug concentrations, compared with that observed in the absence of drugs (i.e., percentage of control). B, WIN-55,212-2 concentration-effect curves for inhibition of forskolin-stimulated adenylyl cyclase activity were determined in the absence (filled squares) or presence of a single, fixed 10 μM concentration of curcumin (open circles), ASC-J9 (open triangles), or trans-resveratrol (open diamonds). The IC50 values determined in A and B are presented under Results. C, curcumin (10 μM), ASC-J9 (10 μM), and trans-resveratrol (10 μM) significantly block inhibition of adenylyl cyclase activity produced by 10 nM WIN-55,212-2. D, curcumin (10 μM), ASC-J9 (50 μM), and trans-resveratrol (10 μM) significantly attenuate stimulation of adenylyl cyclase activity produced by 10 nM inverse agonist AM-251. Values designated with different letters above the error bars are significantly different (one-way ANOVA followed by Dunnett’s post hoc comparison, P < 0.05).

In Mice, Repeated Administration of trans-Resveratrol, Curcumin, and ASC-J9 Antagonizes Hypothermia Produced by a CB1 Agonist. Cannabinoid agonists produce a classic tetrad of effects in mice (hypothermia, analgesia, catalepsy, and reduced locomotor activity), mediated by activation of CB1 receptors (Smith et al., 1994). To determine whether the polyphenols act as antagonists/inverse agonists at mCB1 receptors in vivo (as predicted by in vitro assays), the ability of each compound to antagonize hypothermia produced by the cannabinoid agonist CP-55,940 was examined (Fig. 5, A and B). Administration of a single, fixed dose of CP-55,940 (14 mg/kg) significantly decreased body temperature (P < 0.05). A rank order of potency (IC50) for reversal of CP-55,940-induced hypothermia was determined. In Mice, Acute Administration of trans-Resveratrol, curcumin, and ASC-J9 Antagonizes Hypothermia in Body Weight, Similar to That Produced by the CB1 Antagonist/Inverse Agonist AM-251. CB1 antagonists/inverse agonists produce reductions in food intake and body weight in mice (Pavon et al., 2008). Because in vitro assays suggest that all polyphenols tested act as antagonists/inverse agonists at mCB1, the ability of trans-resveratrol and curcumin to reduce body weight in mice was examined (Fig. 5C). As anticipated, the CB1 antagonist/inverse agonist AM-251 (10 mg/kg) administered twice daily for 3 days results in a significant (P < 0.01) weight loss of 2.8 ± 0.47 g (Fig. 5C, left; n = 6). Likewise, repeated administration of curcumin produces a dose-related weight loss, equivalent to that produced by AM-251 (Fig. 5C, center; n = 5). Although slightly
higher doses are required, trans-resveratrol also results in significant (p < 0.05), dose-dependent weight loss (Fig. 5C, right, n = 5).

**In Silico Comparison of the Structures of trans-Resveratrol and Curcumin with Known Cannabinoids Reveals Common Structural Motifs.** Molecular modeling studies employing CAChe molecular modeling software (Fujitsu America, Inc., Sunnyvale, CA) with structure minimizations performed with a PM5 wave function in water reveals that the favored conformation of trans-resveratrol (Fig. 6A, in red) is similar to that of a series of novel synthetic resorcinol-derived cannabinoids (Wiley et al., 2002), as graphically illustrated by comparison with the resorcinol O-1422 (Fig. 6A, in green). When the resorcinol rings of both molecules are overlaid, the similarities are striking. Although the cyclohexyl group of O-1422 is not present in trans-resveratrol, the dimethylheptyl side chain (also present in many other cannabinoids) of O-1422 is similar in length to the trans-double bond and phenol ring of resveratrol.

In addition, a subsequent overlay of the CB1-selective ligand rimonabant (Fig. 6B, in blue), trans-resveratrol (Fig. 6B, in red), and curcumin (Fig. 6B, in purple) reveals several areas of similarity that closely match a three-dimensional pharmacophore model of CB1-selective ligands recently proposed by Wang et al. (2008). For example, an aromatic region (A) and a hydrophobic region (B), which are located in aromatic rings containing electron-withdrawing groups, are present in all three molecules. Furthermore, the amide car-
bonyl (of rimonabant), the carboxyl of trans-resveratrol, and two oxygens (hydrogen bond donors) that reside in the middle region; hence, region C.

Based on in vitro studies, however, it is interesting that all three polyphenols were shown to contain regions similar to that occurring in the CB1 selective ligand rimonabant (in blue).

**Discussion**

The most significant finding of this study is the identification of human CB1 cannabinoid receptors as a high-affinity target for three distinct polyphenols; trans-resveratrol ($K_i = 45$ nM), curcumin ($K_i = 6$ nM), and ASC-J9 ($K_i = 64$ nM, an analog of curcumin). All polyphenols examined seem to act as CB1 antagonists/inverse agonists, at dietary-relevant concentrations, in both in vitro and in vivo assays. Furthermore, in silico comparison of the structures of trans-resveratrol and curcumin with known cannabinoid receptor ligands reveals common structural motifs. Coupled with their proven safety, these studies indicate that trans-resveratrol, curcumin, and/or their derivatives might be developed as novel, nontoxic CB1 therapeutics for use in obesity, diabetes, drug dependence, and additional disease states in which CB1 antagonists have shown efficacy.

Polyphenols, including trans-resveratrol and curcumin, are known to produce many biological effects by acting on multiple targets (Stevenson and Hurst, 2007). trans-Resveratrol and curcumin are very efficacious antioxidants (Fraga, 2007) and anti-inflammatory (Surh et al., 2005) agents; however, their in vitro effects require relatively high concentrations (>$1$ μM) and are thought to involve multiple receptor- and nonreceptor-mediated processes. Therefore, the specific molecular mechanisms responsible for these effects remain unclear. This study identifies CB1 receptors as one of the highest affinity targets for trans-resveratrol and curcumin reported to date. For example, although trans-resveratrol inhibits the activity of quinone reductase 2, with a dissociation constant of 35 to 50 nM (Buryanovskyy et al., 2004), much higher concentrations are required to stimulate adenylyl cyclase (800 nM) (El-Mowafy and Alkhalaf, 2003) or inhibit the activity of IkB kinase (1 μM) (Kundu et al., 2006) and lipoxigenase (3.7 μM) (Jang et al., 1997). Likewise, curcumin inhibits the activity of adenyl cyclase with an IC50 of 63 nM (Yang et al., 2005); however, significantly greater concentrations (500 nM) are required to reduce the aggregation of α-synuclein (Yang et al., 2005) or inhibit the activity of glycogen synthase kinase-3B (GSK-3B) (Hayashi et al., 2002). Therefore, these polyphenols clearly play different roles in action for effectively lower, subphysiologically attainable concentrations to produce near-full efficacy.

It is additionally important because the CB1 targets are high-affinity, high-affinity, receptor-mediated antagonists/inverse agonists that probably contribute to many of the reported physiological effects of these and other structurally related polyphenols in a variety of disease states. For example, both CB1 antagonists/inverse agonists and polyphenols (including trans-resveratrol and curcumin) are efficacious anti-inflammatory agents (Rahman et al., 2006; Muccioli, 2007) and seem to be promising therapeutics for use in cardiovascular disease, cancer, stroke, and diabetes (Scalbert et al., 2005). In addition, curcumin has been used for centuries in the traditional Indian Ayurveda system of medicine to reduce the hallucinatory effects of many psychotropic drugs, including hashish, a potent form of cannabis (Tilak et al., 2004). However, the most direct evidence supporting our observations that certain polyphenols may produce actions through CB1 receptors is provided by the recent report that trans-resveratrol and several other polyphenols bind to a specific, yet unidentified, binding site in rat brain (Han et al., 2006). Similar to CB1 receptors, these binding sites are localized to plasma membranes, expressed in high density, and widely distributed throughout the brain. It is most interesting that [3H]trans-resveratrol binds to these unidentified sites, with an affinity ($K_a$) of 220 nM, very similar to its affinity ($K_i$) for mCB1 receptors of 220 nM reported in this study. It is certainly possible that [3H]trans-resveratrol might also bind to the orphan receptor GPR55 or to other noncannabinoid G-protein-coupled receptors, such as dopamine receptors, to which cannabinoid receptor ligands also bind.

It is interesting that all three polyphenols were shown to possess both neutral antagonist and inverse agonist proper-
ties, depending on the assay or tissue/cell homogenate examined. These data suggest that the polyphenols tested might act as protean agonists at CB1 receptors, similar to that recently described for the CB2 ligand AM-1241 (Yao et al., 2006). A protean agonist is a compound that changes its apparent intrinsic activity to exhibit agonist, antagonist, or inverse agonist activity at the same receptor, depending on the specific assay systems employed for detection. Alternatively, a more simple explanation for the current observations might be due to differences between assay conditions used for the GTPγS binding assay (employing membrane homogenates and relatively high concentrations of guanine nucleotides), relative to that employed for the cAMP assay (employing whole cells).

trans-Resveratrol and curcumin, like most polyphenols, are extensively and rapidly metabolized by glucuronidation and sulfation in the liver and other tissues (Singh et al., 2008). This predicts that relatively poor bioavailability, particularly in the central nervous system, might preclude observation of significant antagonism of effects mediated by central CB1 receptors in mice as reported here. However, even with such unfavorable pharmacokinetic properties, peak serum concentrations in mice of approximately 1 to 2 μM parent drug after a single, acute intraperitoneal injection of moderate doses (~20–100 mg/kg) of either trans-resveratrol (Asensi et al., 2002) or curcumin (Pan et al., 1999) have been reported. In addition, curcumin can accumulate to concentrations as high as 1 to 2 μM in the brains of mice and rats after oral administration of a single 7.5 mg/kg daily dose over a period of 3 to 4 months (Begum et al., 2008). Very low doses of resveratrol (~1 mg/kg) can produce peak serum concentrations in mice of approximately 1 to 2 μM even with such unfavorable pharmacokinetic properties, as reported here, if such micromolar (or even high nanomolar concentrations of trans-resveratrol, curcumin, or ASC-J9 are attained in the brain, near-full receptor occupancy would be predicted. Alternatively, it is also certainly possible that a metabolite of trans-resveratrol and/or curcumin might bind with high (or superior) affinity to CB1 receptors to mediate the in vivo effects reported here. In any case, because of the potential therapeutic promise of these drugs in a number of disease states, several methods to improve their systemic bioavailability, including the development of liposomal and nanoparticle preparations, are actively being pursued (Anand et al., 2007). Based on the present findings, future development of polyphenol-based CB1 ligands should include similar studies to improve systemic bioavailability.

Activation of peripheral CB1 receptors is effective at suppressing inflammation that leads to chronic pain states (Gutierrez et al., 2007). However, the potential use of current CB1 agonists for this application is severely limited by concurrent stimulation of central CB1 receptors, resulting in unacceptable psychotropic side effects. Furthermore, the CB1 antagonist/inverse agonist rimonabant is very effective for management of obesity (Pavon et al., 2008). However, several adverse effects, presumed to be mediated via blockade of central CB1 receptors, resulted in the recent discontinuance of all ongoing clinical trials of rimonabant in Europe (Jones, 2008), thus virtually assuring a lack of future Food and Drug Administration approval for use in the United States. Several studies indicate that the metabolic benefits of CB1 antagonists/inverse agonists in obese animals is due to action at peripheral, but not central, CB1 receptors (Pavon et al., 2008). Results from the present study demonstrating that repeated administration of curcumin or trans-resveratrol produces a dose-dependent reduction in body weight provide additional evidence for this observation. It is interesting that, although not attributed to action at CB1 receptors, others also report that trans-resveratrol reduces body weight in Zucker obese rats (Lekli et al., 2008). Therefore, polyphenol-derived, peripherally restricted CB1 antagonists or antagonists might be developed as a class of nontoxic cannabinoids. The observation that high doses of either trans-resveratrol (Esposito et al., 2006) or curcumin (Chainani-Wu, 2003) seem to reduce body weight suggests a very limited number of doses might provide further evidence for this hypothesis.

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