Loss of striatal type 1 cannabinoid receptors is a key pathogenic factor in Huntington’s disease

Cristina Blázquez,1,2 Anna Chiarlone,1,2 Onintza Sagredo,1,3 Tania Aguado,1,2 M. Ruth Pazos,1,3 Eva Resel,1,2 Javier Palazuelos,1,2 Boris Julien,1,2 Maria Salazar,1,2 Christine Börner,4 Cristina Benito,1,5 Carolina Carrasco,1,2 María Diez-Zaera,1,6 Paola Paoletti,1,7 Miguel Díaz-Hernández,1,8 Carolina Ruiz,1,9 Michael Sendtner,10 José J. Lucas,1,8 Justo G. de Yébenes,1,9 Giovanni Marsicano,11 Krisztina Monory,12 Beat Lutz,12 Julián Romero,1,5 Jordi Alberch,1,7 Silvia Gineás,1,7 Jürgen Kraus,4 Javier Fernández-Ruiz,1,3 Ismael Galve-Roperh1,2 and Manuel Guzmán1,2

1 Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Huntington’s Disease and Ataxias Collaborative Project, 28040 Madrid, Spain
2 Department of Biochemistry and Molecular Biology I, School of Biology, Complutense University, 28040 Madrid, Spain
3 Department of Biochemistry and Molecular Biology III, School of Medicine, Complutense University, 28040 Madrid, Spain
4 Department of Pharmacology and Toxicology, University of Magdeburg, 39120 Magdeburg, Germany
5 Hospital Universitario Fundación Alcorcón, Research Unit, 28922 Madrid, Spain
6 Department of Biochemistry and Molecular Biology IV, School of Veterinary, Complutense University, 28040 Madrid, Spain
7 Cell Biology and Pathological Anatomy Department, School of Medicine, Institut d’Investigacions Biomèdiques August Pi i Sunyer, Barcelona University, 08036 Barcelona, Spain
8 Centro de Biología Molecular ‘Severo Ochoa’, Consejo Superior de Investigaciones Científicas/Universidad Autónoma, 28049 Madrid, Spain
9 Department of Neurobiology, Ramón y Cajal Hospital, 28034 Madrid, Spain
10 Institute of Clinical Neurobiology, University of Würzburg, 97078 Würzburg, Germany
11 U862 INSERM, Bordeaux University 2, 33077 Bordeaux, France
12 Department of Physiological Chemistry, Johannes Gutenberg University Mainz, 55099 Mainz, Germany

Correspondence to: Manuel Guzmán, Department of Biochemistry and Molecular Biology I, School of Biology, Complutense University, C/ José Antonio Novais 2, 28040 Madrid, Spain
E-mail: mgp@bbm1.ucm.es

Correspondence may also be addressed to: Ismael Galve-Roperh. E-mail: igr@quim.ucm.es

Endocannabinoids act as neuromodulatory and neuroprotective cues by engaging type 1 cannabinoid receptors. These receptors are highly abundant in the basal ganglia and play a pivotal role in the control of motor behaviour. An early downregulation of type 1 cannabinoid receptors has been documented in the basal ganglia of patients with Huntington’s disease and animal models. However, the pathophysiological impact of this loss of receptors in Huntington’s disease is as yet unknown. Here, we generated a double-mutant mouse model that expresses human mutant huntingtin exon 1 in a type 1 cannabinoid receptor-null background, and found that receptor deletion aggravates the symptoms, neuropathology and molecular pathology of the disease. Moreover, pharmacological administration of the cannabinoid Δ9-tetrahydrocannabinol to mice expressing human mutant huntingtin exon 1 exerted a therapeutic effect and ameliorated those parameters. Experiments conducted in striatal...
cells show that the mutant huntingtin-dependent downregulation of the receptors involves the control of the type 1 cannabinoid receptor gene promoter by repressor element 1 silencing transcription factor and sensitizes cells to excitotoxic damage. We also provide in vitro and in vivo evidence that supports type 1 cannabinoid receptor control of striatal brain-derived neurotrophic factor expression and the decrease in brain-derived neurotrophic factor levels concomitant with type 1 cannabinoid receptor loss, which may contribute significantly to striatal damage in Huntington’s disease. Altogether, these results support the notion that downregulation of type 1 cannabinoid receptors is a key pathogenic event in Huntington’s disease, and suggest that activation of these receptors in patients with Huntington’s disease may attenuate disease progression.

Keywords: cannabinoid; receptor; Huntington’s disease; neuroprotection; experimental therapeutics

Abbreviations: BDNF = brain-derived neurotrophic factor; CAT = chloramphenicol acetyltransferase; CB1 = type 1 cannabinoid; FAAH = fatty acid amide hydrolase; GABA = gamma-aminobutyric acid; GAD67 = glutamic acid decarboxylase 67 KDa isofrom; GFP = green fluorescent protein; NMDA = N-methyl-D-aspartate; PSD95 = post-synaptic density protein 95; RE1 = repressor element 1; REST = repressor element 1 silencing transcription factor; THC = Δ9-tetrahydrocannabinol

**Introduction**

Endocannabinoids are a family of neural retrograde messengers that act by engaging type 1 cannabinoid (CB1) receptors, the same receptors targeted by Δ9-tetrahydrocannabinol (THC), the major active component of marijuana (Gaoni and Mechoulam, 1964; Piomelli, 2003). Endocannabinoid generation occurs by on-demand synthesis and cleavage of plasma membrane lipid precursors and is tightly controlled by neuronal activity. Endocannabinoid signalling serves as a major feedback mechanism to prevent excessive presynaptic activity, and thus tunes the functionality and plasticity of many synapses (Piomelli, 2003; Katona and Freund, 2008). In concert with this well-established neuromodulatory function, studies in various animal models support that CB1 receptor activation promotes neuron survival upon acute brain injury and neuroinflammatory insults (Nagayama et al., 1999; Panikashvili et al., 2001; Parmentier-Batteur et al., 2002; Marsicano et al., 2003; Pryce et al., 2003). This neuroprotective action of endocannabinoid signalling relies on the inhibition of excitotoxic glutamatergic neurotransmission as well as on other mechanisms, and is supported by the observation that the brain overproduces endocannabinoids upon damage (Mechoulam et al., 2002; Marsicano et al., 2003; Galve-Roperh et al., 2008).

CB1 is the most abundant G protein-coupled receptor in the brain and, specifically, is very highly expressed in the neocortex, hippocampus, cerebellum and basal ganglia (Katona and Freund, 2008). In the latter, CB1 receptors are mostly localized at synapses established by neurons containing gamma-aminobutyric acid (GABA; e.g. striatal projection neurons and some striatal interneuron subpopulations) and glutamate (e.g. corticostriatal and subthalamonic neurons) as transmitters, and play a pivotal role in the inhibitory control of motor behaviour (Katona and Freund, 2008; Pazos et al., 2008). Of possible clinical importance, alterations in CB1 receptor expression have been reported in various pathologies affecting the basal ganglia (Maccarrone et al., 2007; Pazos et al., 2008). Specifically, a significant downregulation of CB1 receptor binding and messenger RNA levels has been documented in the basal ganglia of patients (Glass et al., 2000) and animal models (Denovan-Wright and Robertson, 2000; Lastres-Becker et al., 2002; McCaw et al., 2004) of Huntington’s disease, a devastating neurodegenerative disorder that is primarily caused by a degeneration of medium-sized spiny striato-epiferent GABAergic neurons and that is clinically characterized by a variety of movement disturbances, including chorea, dystonia and Parkinson’s disease-like symptoms, as well as by cognitive and behavioural impairment (Walker, 2007). Of interest, CB1 receptors are abundant in the great majority of medium-sized spiny neurons of the striatum (Marsicano and Lutz, 1999; Hohmann and Herkenham, 2000; Hermann et al., 2002), but their loss in mutant huntingtin transgenic mice is brain region-specific, as it occurs in the lateral striatum and, to a lesser extent, in the medial striatum, but not in the cortex (Denovan-Wright and Robertson, 2000; McCaw et al., 2004). Moreover, the downregulation of CB1 receptor expression observed in patients with Huntington’s disease and animal models seems to occur at early stages of the disease and prior to the appearance of overt clinical symptoms, neurodegeneration and changes in other neurochemical parameters (Maccarrone et al., 2007; Pazos et al., 2008).

Although Huntington’s disease has long been known to be caused by a single-gene mutation, specifically a CAG repeat expansion in exon 1 of the huntingtin gene that translates into an expanded polyglutamine tract in the N-terminal domain of the huntingtin protein (The Huntington’s Disease Collaborative Research Consortium, 1993), the mechanisms by which mutant huntingtin produces the progressive degeneration of striatal neurons are extremely complex and as yet incompletely understood (Walker, 2007; Imarisio et al., 2008). Hence, this work was undertaken to evaluate the potential contribution of the loss of CB1 receptors to Huntington’s disease pathogenesis and the molecular mechanism underlying this event.

**Materials and methods**

**Animals**

Hemizygous male mice transgenic for exon 1 of the human huntingtin gene with a greatly expanded CAG repeat (R6/2 mice) (Mangiarini et al., 1996) and wild-type littermates were purchased from The Jackson Laboratory [Bar Harbor, ME; code...
CB1 receptors in Huntington’s disease

Brain 2011: 134; 119–136 | 121

B6CBA-Tg(HDexon1)62Gpb(1J); 155–175 CAG repeats) or kindly provided by Gill Bates (King’s College London School of Medicine, London, UK). The colony was maintained by back-crossing R6/2 males with (CBA x C57BL/6J) F1 females. Animals were housed and maintained in groups of mixed genotypes (Hockly et al., 2003) with free access to food and water and on a 12-h light/dark cycle. From Week 10 of age, animals were provided with extra in-cage food and water. Due to welfare considerations based on the 3Rs (replacement, reduction and refinement) principle, animals were not allowed to die naturally (Olsson et al., 2008). Instead, they were routinely sacrificed for brain samples for biochemical and histological analyses. Some experiments were conducted on hemizygous male R6/1 mice, which were maintained and handled as described (Canals et al., 2004). Animal handling procedures were approved by Complutense University Animal Research Committee in accordance with Directive 86/609/EU of the European Commission.

To obtain double-mutant mice that express human mutant huntingtin exon 1 and are deficient in CB1 cannabinoid receptors, we first cross-mated wild-type CBA female mice with CB1–/– (C57BL/6J) male mice (Marsicano et al., 2002). The CB1+/– (CBA x C57BL/6J) F1 females were crossed with R6/2 males (Mangiarini et al., 1996), and the resulting R6/2:C57,+/– (C57BL/6J) F2 males were back-crossed with the aforementioned CB1–/– F1 females to generate the CB1–/–, CB1+/–, R6/2:C57,+/– and R6/2:C57,–/– (CBA x C57BL/6J) animals. All experiments were performed with male littermates from this population to avoid strain and sex differences. These animals were not treated with any vehicle or drug. The uniformity of the CBA x C57BL/6J background in our mouse colony was routinely assessed by Illumina Bead Scanner-based profiling of 256 representative single-nucleotide polymorphisms of the CBA and the C57BL/6J backgrounds. Analyses were conducted at Centro Nacional de Genotipado (Centro Nacional de Investigaciones Oncológicas, Madrid, Spain).

Cannabinoid administration to animals

On the basis of their basal RotaRod performance and body weight, wild-type and R6/2 mice were matched into the different treatment groups and injected daily (intraperitoneally) with vehicle [1% (v/v) dimethyl sulfoxide in 100 µl Tween/saline (1:18, v/v)] or THC (The Health Concept, Richelbach, Germany) at 2 mg/kg body weight per day. Behaviour tests were conducted prior to injections.

Behaviour analyses

Motor coordination (RotaRod) analysis was conducted with acceleration from 4 to 40 r.p.m. over a period of 570 s in an LE8200 device (Harvard Apparatus, Barcelona, Spain). Any mice remaining on the apparatus after 600 s were removed and their time scored as 600 s. For basal RotaRod performance, mice were tested on four consecutive days, for three trials per day with a rest period of ~30 min between trials. At each successive age analysed, mice were tested on three consecutive days, for three trials per day with a rest period of ~30 min between trials. Data from the three trials per day were averaged for each animal, and the mean value of each day averaged for each animal. Data from the first day (or the first 2 days in the basal test) were not used in statistical analyses.

Motor activity and exploration analyses were conducted in an automated actimeter (ActiTrack; Panlab, Barcelona, Spain). This consisted of a 22.5 × 22.5-cm area with 16 surrounding infrared beams coupled to a computerized control unit. Activity was recorded for a period of 10 min, and total distance travelled, resting time and movements >5 cm/s were recorded for each animal.

Limb-clasping analysis was conducted in animals that were tail-suspended and video-recorded for 45 s. We evaluated total clasping time (in at least one limb) of each animal.

Magnetic resonance imaging

Striatal volume was calculated by magnetic resonance imaging. Experiments were performed at the Nuclear Magnetic Resonance Centre of Complutense University (Madrid, Spain) using a BIOSPEC BMT 47/40 (Bruker, Ettingen, Germany) operating at 4.7 T, equipped with a 12 cm, actively shielded gradient system. Mice were anaesthetized with oxygen/isofluorane and subsequently placed in prone position inside a cradle. The animal’s head was immobilized and placed underneath a 4 cm surface coil. A respiration sensor was used to control the animals. First global shimer was assessed, and then three gradient-echo scout images in axial, sagittal and coronal directions were acquired (time to repetition/echo time = 100/3.2 ms, matrix = 128 × 128). A 3D fast-spin-echo experiment with axial slice orientation was subsequently performed using the following acquisition parameters: time to repetition= 3000 ms, effective echo time = 86.5 ms, number of averages = 2, field of view = 2.56 × 2.56 × 1.28 cm3, matrix size = 256 × 256 × 32. The reconstructed matrix size was 256 × 256 × 32. The total time of the acquisition experiment was 27 min.

Real-time quantitative polymerase chain reaction

RNA was isolated using Trizol Reagent or RNeasy (Invitrogen, Carlsbad, CA). Complementary DNA was obtained with Transcriptor (Roche, Basel, Switzerland). Real-time quantitative polymerase chain reaction assays were performed using the FastStart Master Mix with Rox (Roche) and probes were obtained from the Universal Probe Library Set (Roche). Amplifications were run in a 7900 HT-Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). Each value was adjusted to β-actin levels as reference. Relative gene expression data were determined by the 2^{-ΔΔCT} method. The 18S RNA levels were routinely used as an additional control to further validate the data. Probes and primers used are shown in Supplementary Table 1.

Microscopy analyses

Cells were cultured on coverslips and fixed in 4% paraformaldehyde. Coronal free-floating sections were obtained from paraformaldehyde-perfused mouse brains (Aguado et al., 2006). Sections were incubated with anti-CB1 receptor [raised against a glutathione S-transferase fusion protein containing the first 77 residues of the CB1 receptor (Twitchell et al., 1997); 1:500, kindly provided by Ken Mackie, Indiana University, Bloomington, IN, USA], anti-brain-derived neurotrophic factor (BDNF; 1:500; generated at Michael Sendtner’s laboratory, University of Würzburg, Germany), anti-glutamic acid decarboxylase 67 KDa isoform (GAD67; 1:250; Chemicon, Temecula, CA; cat. no. AB5188), anti-synaptophysin (1:250; Synaptic Systems; cat. no. 100 002), anti-post-synaptic density protein 95 (PSD95; 1:1000, Abcam, Cambridge, UK; cat. no. ab27223) or anti-NeuN (1:400; Chemicon; cat. no. MAB377) antibodies, followed by staining with the corresponding highly cross-adsorbed Alexa Fluor 488, 594 or 647 antibodies (1:500; Molecular Probes, Leyden, The Netherlands).
After washing, samples were incubated with Hoescht 33342 (1:2000; Invitrogen) to stain cell nuclei and subsequently mounted in Mowiol solution. Immunofluorescence images of cells were obtained with an Axioplan 2 microscope (Carl-Zeiss, Oberkochen, Germany). Confocal fluorescence images were acquired using TCS-SP2 software and a SP2 AOBS microscope (Leica, Wetzlar, Germany). Pixel quantification and co-localization were analysed with Metamorph-Offline software (Universal Imaging, Downingtown, PA).

For quantification of huntingtin aggregates, 30 μm coronal sections were pre-treated with 1% bovine serum albumin, 5% foetal bovine serum and 0.2% Triton X-100, and then incubated with anti-human huntingtin antibody (1:1000; Chemicon; cat. no. MA85374). Samples were subsequently incubated in avidin–biotin complex using the mouse Elite Vectastain kit (Vector Laboratories) and chromogen reactions were performed with 0.05% diaminobenzidine (Sigma-Aldrich, St. Louis, MO) and 0.01% H2O2. Sections were mounted with Mowiol, analysed in an Olympus BX-41 microscope (Barcelona, Spain) with a CCD ColorView IIIu camera and quantified employing Metamorph-Offline software. Specifically, counting of huntingtin inclusions was conducted in the caudate-putamen area of both hemispheres in a 1-in-10 series per animal, ranging from bregma +1.18 mm to −0.46 mm coronal coordinates. Sections were analysed at a magnification of ×40 and spots sized 5–2000 pixels were recorded. Data are presented as number of huntingtin aggregates relative to the control animal group.

Western blot
Western blot analysis was conducted with antibodies against CB1 receptor (see characteristics of the antibody above; 1:1000), fatty acid amid hydrolase (FAAH; 1:1000; Chemicon; cat. no. AB5644P) or α-tubulin (1:4000; Sigma-Aldrich; cat. no. T9026) following standard procedures. Specifically, samples were lysed in a buffer containing 50 mM Tris, 0.1% Triton X-100, 1 mM ethylenediaminetetraacetic acid, 1 mM ethylene glycol tetraacetic acid, 50 mM NaF, 10 mM sodium glycerophosphate, 5 mM sodium pyrophosphate and 1 mM sodium orthovanadate (pH 7.5) supplemented with a protease inhibitor cocktail (Roche; cat. no. 11697498001), 0.1% phenylmethylsulphonylfluoride, 0.1% β-mercaptoethanol and 1 mM microcystin. The running buffer consisted of 200 mM glycine, 25 mM Tris and 1% sodium dodecyl sulphate (pH 8.3), and the transfer buffer contained 200 mM glycine, 25 mM Tris and 20% methanol (pH 8.3). Blots were incubated with Tris-buffered saline (20 mM Tris and 0.5 mM NaCl, pH 7.5)/Tween-20 (0.1%) supplemented with 1% bovine serum albumin. Densitometric analysis was performed with Quantity One software (Bio-Rad, Hercules, CA).

Cell and slice culture
Conditionally immortalized striatal neuroblasts obtained from wild-type mice (STHdh+/Y cells) or knock-in cells expressing one copy (STHdh1/2+Y cells) or two copies (STHdh1/1+1 cells) of a mutant huntingtin allele, thus expressing endogenous levels of full-length huntingtin with only seven glutamines, 7 and 111 glutamines or only 111 glutamines in the protein N-terminal domain, respectively, were used (Trettel et al., 2000). Cell infection with a defective retrovirus transducing the temperature-sensitive A58/U19 large T antigen, selection of geneticin-resistant colonies at the permissive temperature of 33°C and analysis of colonies by immunostaining has been previously described (Trettel et al., 2000; Paoletti et al., 2008). Cells were grown at 33°C in Dulbecco’s modified eagle’s medium supplemented with 10% foetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine and 400 μg/ml geneticin (Paoletti et al., 2008).

Adult striatal slices were obtained from wild-type and R6/2 mice. Brains were dissected and cut coronally with a vibratome. Slices (300 μm thick) were cultivated for 20 h in semidry conditions in wells containing Neurobasal medium supplemented with B27, N2 and 2.5 mM L-glutamine.

Cell viability
Cells were transfected to serum-free Dulbecco’s modified eagle’s medium for 24 h and incubated for a further 5 h in Locke’s solution (154 mM NaCl, 5.6 mM KCl, 2.3 mM CaCl2, 3.6 mM NaHCO3, 5 mM Hepes, 20 mM glucose and 10 mM glycine) supplemented or not with N-methyl-D-aspartate (NMDA) and cannabinoid receptor agonists (THC, HU-210, WIN-55,212-2), the CB1 cannabinoid receptor antagonist SR141716 (kindly provided by Sanofi-Aventis, Montpellier, France) or the respective vehicle (dimethyl sulphoxide, 0.1–0.2% (v/v) final concentration). The medium was subsequently replaced by NMDA/serum-free Dulbecco’s modified eagle’s medium and cell viability was determined after 24 h by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide test.

Cell transfection
Cells were transfected transiently with constructs expressing human wild-type huntingtin exon 1 with 17 glutamines fused to green fluorescent protein (GFP) (17Q-GFP), human mutant huntingtin exon 1 with 72 glutamines fused to GFP (72Q-GFP) (kindly provided by Montserrat Arrasate and Steven Finkbeiner, The Gladstone Institute of Neurological Disease, San Francisco, CA, USA), human full-length wild-type huntingtin with 17 glutamtes (17Q-FL), human full-length mutant huntingtin with 79 glutamines (79Q-FL) (kindly provided by Frédéric Saudou, Institut Curie, Orsay, France), mouse pcDNA3-CB1 cannabinoid receptor complementary DNA (generated at Beat Lutz’s laboratory, Johannes Gutenberg University Mainz, Germany) or with their respective empty vectors, using Lipofectamine 2000 (Invitrogen). In other experiments, cells were transfected with small interfering RNA duplexes corresponding to mouse huntingtin (5′-GAAAGGACCACCAG UUGAAA-3′) or a non-targeted control (5′-UGUUUACUAGUG GACUA-3′) using the DharmaFECT 1 transfection reagent (Dharmacon, Lafayette, CO), and/or with double-stranded repressor element 1 (RE1) decoy oligonucleotides (5′-GCCCGAGGGCAG ACAGGT-3′) or a non-targeted control (5′-CTCCGAGCTGTCACG TACAAT-3′) using Lipofectamine 2000.

CB1 cannabinoid receptor gene promoter activity
Cells were transfected transiently with the aforementioned huntingtin-expressing plasmids together with a construct encoding the –3016 to +142 sequence (referring to the first nucleotide of exon 1) of the human CB1 receptor gene promoter fused to the chloramphenicol acetyltransferase (CAT) reporter gene (pHB-1-3016-CAT) (Borner et al., 2008). All reporter gene constructs were based on the pBLCAT2/pBLCAT3 system, in which the thymidine kinase minimal promoter was replaced for the human CB1 receptor promoter upstream of CAT. The 5′ deletion constructs of this plasmid were generated either by site-specific restriction enzyme deletion (–2420: AfeI; –1880: SpeI; –1583: EagI; –648: Sphi) or by a deletion strategy using the sequence-unspecific enzyme Bal31 (–9099; –989; –559; –223).
The reporter plasmids phCB₁-962/-934-tk-CAT and pRE1-tk-CAT were constructed by ligation of double-stranded oligonucleotides (Metabion, Martinsried, Germany) encoding the −962/−934 fragment of the human CB₁ receptor promoter or a consensus RE1 site, respectively, into the BamHI site of pBLCAT2 upstream of the herpes simplex thymidine kinase promoter. The sequences (sense strands) used were 5′-GATCCGCCCCGAGGGCGAGCTGG GCCGC-3′ for the −962/−934 plasmid, and 5′-GATCCTTCAG CGCCAGGCAAGCAGGC-3′ for the RE1 plasmid. The correct insertion of the sequences and the deletions of all the plasmids were verified by DNA sequencing.

Human samples

Human caudate-putamen samples were obtained from patients with Huntington’s disease or without neurological disease (controls) according to the standardized procedures of the Banco de Tejidos para Investigación Neurologica (Madrid, Spain). Briefly, both the patients with Huntington’s disease and the control subjects, according to the Declaration of Helsinki, had signed during their life a donation protocol that was in custody of their relatives and the brain bank. After death, the corpses were immediately stored at 4 °C until autopsy, which was performed within a time interval ranging from 2 to 12 h post mortem. After removal of the brain, the quality of the samples was checked by their pH. The brain was split in two parts by a sagittal section through the midline: the right hemibrain was used for histo-pathological studies and the left hemibrain for Western blot and other biochemical analyses. Both hemibrains were dissected in coronal sections (~1 cm thick) to evaluate the presence of additional lesions, such as cerebral infarctions. The right hemibrain was immersed in formalin, and the slices of the left hemibrain were frozen in a metal plate cooled at −80 °C. The frozen samples were stored at −80 °C in freezers with continuous recording of temperature and a double temperature control (liquid CO₂-backup connection and alarm telephone). All protocols were approved by the institutional ethics committee.

Statistical analyses

Data are presented as mean ± SEM. Statistical comparisons were made by ANOVA with post hoc Student–Neuman–Keuls test or by unpaired Student’s t-test, as appropriate.

Results

Genetic deletion of CB₁ cannabinoid receptors aggravates Huntington’s disease-like symptomatology, neuropathology and molecular pathology in R6/2 mice

To evaluate the pathophysiological relevance of CB₁ receptor loss in Huntington’s disease, we first generated double-mutant mice expressing human mutant huntingtin exon 1 [R6/2 mice, which recapitulate the Huntington’s disease-associated decrease of striatal CB₁ receptors (Denovan-Wright and Robertson, 2000; McCaw et al., 2004)] in a CB₁ receptor-null background. These R6/2:CB₁⁻/⁻ mice showed a significant motor-coordination impairment phenotype—as assessed by RotaRod performance—at Week 4, an age at which R6/2:CB₁⁺/+ animals are overtly normal (Fig. 1A). Moreover, the subsequent decline in motor coordination evidenced by R6/2:CB₁⁻/⁻ mice was exacerbated in R6/2:CB₁⁻/⁻ littermates (Fig. 1A). CB₁ receptor genetic ablation in R6/2 mice induced the appearance of other phenotypic alterations such as impairment of general motor and exploratory behaviour (decreased ambulation, activity and speed; Fig. 1B) and limb clasp- ing (Fig. 1C). Moreover, striatal atrophy, as determined by MRI (Fig. 1D), and accumulation of huntingtin aggregates (Fig. 1E), two hallmarks of Huntington’s disease neuropathology, were exacerbated upon CB₁ receptor deletion in R6/2 mice. Body weight from Week 4 to Week 10 was not significantly different in wild-type, CB₁−/−, R6/2:CB₁⁺/+ and R6/2:CB₁⁻/⁻ mice (n = 20–30 animals per group; data not shown), indicating that CB₁ receptor ablation does not affect the general health status of the animals.

We next evaluated the expression of various molecular markers of neuronal integrity in the double-mutant mice. A remarkable decrease in striatal messenger RNA levels and immunoreactivity of the GABAergic neuron marker GAD67 was evident in R6/2:CB₁⁻/⁻ mice (Fig. 2A). Likewise, the expression of the pre-synaptic marker synaptophysin (Fig. 2B) and the post-synaptic marker PSD95 (Fig. 2C) was reduced in the striata of R6/2:CB₁⁻/⁻ mice when compared with R6/2:CB₁⁺/+ littermates.

Pharmacological activation of CB₁ cannabinoid receptors ameliorates Huntington’s disease-like symptomatology, neuropathology and molecular pathology in R6/2 mice

The worsening of the Huntington’s disease-like phenotype shown by R6/2 mice upon genetic loss of CB₁ receptors suggests that pharmacological activation of CB₁ receptors could have a thera-peutic impact on disease progression. To address this issue we treated R6/2 mice and wild-type littermates with vehicle or THC starting at Week 4 of life and found that cannabinoid treatment attenuated the motor coordination deficits of R6/2 mice, as evaluated in the RotaRod test (Fig. 3A). THC administration also ameliorated the impairment of motor and exploratory behaviour (Fig. 3B) and the limb clasp- ing (Fig. 3C) that appeared in R6/2 mice at later stages of the disease—Weeks 8–10. Striatal atrophy (Fig. 3D) and huntingtin aggregate accumulation (Fig. 3E) were also attenuated by THC delivery to R6/2 mice. THC treatment did not significantly affect body weight from Week 4 to Week 10 in wild-type or R6/2 mice (n = 20–30 animals per group; data not shown).

As CB₁ receptor deficiency downregulated the expression of molecular markers of neuronal integrity in R6/2 mice, we reasoned that pharmacological receptor activation would have the opposite effect, thereby improving the molecular pathology profile of the animals. Thus, THC administration was able to normalize the decline of GAD67 (Fig. 4A), synaptophysin (Fig. 4B) and PSD95 (Fig. 4C) expression observed in vehicle-treated R6/2 mice.
CB₁ cannabinoid receptors protect striatal cells from excitotoxic damage

We next conducted a series of experiments aimed at unravelling the mechanism and consequences of the mutant huntingtin-evoked loss of CB₁ receptors in striatal cells. To address this question we first made use of striatal neuroblasts obtained from wild-type mice (STHdh<sup>Q7/Q7</sup>) and their mutant huntingtin knock-in counterparts (STHdh<sup>Q111/Q111</sup>) cells, which express endogenous levels of huntingtin with 7 and 111 glutamines in the protein N-terminal domain, respectively. We exposed these cells to the ionotropic glutamate receptor agonist NMDA and...
found that THC rescued STHdh^Q7/Q7 cells from death. In contrast, STHdh^Q111/Q111 cells, which express significantly lower levels of CB1 receptors than do STHdh^Q7/Q7 cells (see below), showed an enhanced basal sensitivity to death and an impaired THC-mediated protective response (Fig. 5A). A pivotal role for CB1 receptors in promoting cell survival was supported by the observation that THC-induced protection of STHdh^Q7/Q7 cells was mimicked by the synthetic cannabinoid agonists HU-210 and WIN-55,212-2 (data not shown) and was prevented by the CB1 receptor-selective antagonist SR141716 (Fig. 5A). Moreover, ectopic expression of CB1 receptors in STHdh^Q111/Q111 cells decreased their basal sensitivity to NMDA-induced death and

Figure 2 Genetic deletion of CB1 cannabinoid receptors aggravates Huntington's disease-like molecular pathology in R6/2 mice. (A) Striatal GAD67 mRNA levels and immunoreactivity [given as relative values of GAD67^+ area (in green)/total cell number (nuclei in blue)]. (B) Striatal synaptophysin immunoreactivity [given as relative values of synaptophysin^+ intensity (in red)/NeuN^+ area (in green)]. (Striatal synaptophysin messenger RNA levels were not significantly different in wild-type, CB1^+/+ (WT), R6/2:CB1^+/+ and R6/2:CB1^−/− (R6/) mice; data not shown.) (C) Striatal PSD95 messenger RNA levels and immunoreactivity [given as relative values of PSD95^+ area (in green)/total cell number (nuclei in blue)]. In all panels samples were taken at Week 8 of life (n = 6–8 animals per group; *P < 0.05, **P < 0.01 from the corresponding wild-type group; #P < 0.05, ##P < 0.01 from the corresponding CB1^+/+ group). Representative confocal microscopy images are shown. Scale bar 50 μm.
Huntingtin aggregates in R6/2 mice (% of vehicle)

<table>
<thead>
<tr>
<th>0</th>
<th>80</th>
<th>120</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>THC</td>
<td>Vehicle</td>
<td>THC</td>
</tr>
</tbody>
</table>

Figure 3 Pharmacological activation of CB₁ cannabinoid receptors ameliorates Huntington’s disease-like symptomatology and neuropathology in R6/2 mice. R6/2 mice and wild-type (WT) littermates were treated daily with vehicle (white bars) or THC (2 mg/kg body weight per day; black bars) from Week 4. (A) RotaRod performance at the indicated ages (n = 10–14 animals per group). (B) Motor activity at Weeks 8 and 10 as determined by total distance, resting time and fast movements (n = 14–18 animals per group). (C) Clasping of R6/2 mice at Weeks 8 and 10 (n = 16–20 animals per group). (Clasping was not observed in wild-type littermates at those ages.) (D) Striatal volume relative to total brain volume at Week 8 (n = 10–12 animals per group). (E) Huntingtin aggregates in the striatum at Week 8 (n = 8–10 animals per group). (Aggregates were not detected in wild-type littermates at that age.) In all panels, *P < 0.05, **P < 0.01 from the corresponding wild-type group; #P < 0.05, ##P < 0.01 from the corresponding vehicle-treated group. Representative images are shown in panels D and E (scale bar 50 μm).
Figure 4 Pharmacological activation of CB₁ cannabinoid receptors ameliorates Huntington’s disease-like molecular pathology in R6/2 mice. R6/2 mice and wild-type (WT) littermates were treated daily with vehicle (white bars) or THC (2 mg/kg body weight per day; black bars) from Week 4 of life. (A) Striatal GAD67 immunoreactivity [given as relative values of GAD67⁺ area (in green)/total cell number (nuclei in blue)]. (B) Striatal synaptophysin immunoreactivity [given as relative values of synaptophysin⁺ intensity (in red)/NeuN⁺ area (in green)]. (C) Striatal PSD95 immunoreactivity [given as relative values of PSD95⁺ intensity (in green)/total cell number (nuclei in blue)]. (Striatal GAD67, synaptophysin and PSD95 messenger RNA levels were not significantly different in wild-type or R6/2 mice treated with vehicle or THC; data not shown.) In all panels samples were taken at Week 8 of life (n = 6–8 animals per group; *P < 0.05, **P < 0.01 from the corresponding wild-type group; #P < 0.05, ##P < 0.01 from the corresponding vehicle-treated group). Representative confocal microscopy images are shown. Scale bar 50 μm.
rendered them as responsive as STHdh\textsuperscript{Q7/Q7} cells to cannabinoid-mediated protection (Fig. 5B).

**Transient regulation of huntingtin expression controls CB\textsubscript{1} cannabinoid receptor gene promoter activity via repressor element 1 silencing transcription factor**

To study how mutant huntingtin affects CB\textsubscript{1} receptor expression, we transfected wild-type mouse striatal neuroblasts (STHdh\textsuperscript{Q7/Q7} cells) with constructs expressing human wild-type huntingtin exon 1 with 17 glutamines fused to GFP or human mutant huntingtin exon 1 with 72 glutamines fused to GFP, or with constructs expressing human full-length wild-type huntingtin with 17 glutamines or human full-length mutant huntingtin with 75 glutamines, together with a construct that encodes a 3 kb human CB\textsubscript{1} receptor promoter fused to the CAT reporter gene. Promoter activity was enhanced by wild-type huntingtin exon 1 (Fig. 6A, left panel) and full-length wild-type huntingtin (Fig. 6B, left panel), but was not affected by their respective mutant huntingtin counterparts. This indicates that, although mutant huntingtin usually dysregulates gene transcription by gain-of-function mechanisms (Walker, 2007; Imarisio et al., 2008), the huntingtin mutation in our system is—at least in part—associated with a loss-of-function process. To further support this notion, we knocked-down endogenous huntingtin with a huntingtin-directed small interfering RNA (which diminished huntingtin messenger RNA levels to 30 ± 3% of control small interfering RNA-transfected cells; \( n = 4 \) experiments, \( P < 0.01 \)) and found that CB\textsubscript{1} receptor promoter activity decreased to 68 ± 9% of control small interfering RNA-transfected cells (Fig. 6C, left panel).

We next aimed to characterize promoter regions involved in the control of CB\textsubscript{1} receptor gene transcription. Cells were thus transfected with reporter constructs containing sequential 5′ deletions of the receptor promoter (Fig. 6D). An increase in reporter activity
was observed upon deletion of the promoter sequence from nucleotide −1099 to nucleotide −898, indicating that it contains negative regulatory elements. On the other hand, the sequence comprising nucleotide −898 to nucleotide −648 may contain enhancer elements as its deletion decreased reporter activity. Of interest, the −898 promoter was insensitive to wild-type or mutant huntingtin ectopic expression (Fig. 6A and B, right panel) as well as to huntingtin downregulation (Fig. 6C, right panel), supporting the involvement of negative regulatory elements at the 5′ side of the −898 position but not of positive regulatory elements at the 3′ side of that position in the huntingtin-mediated control of CB1 receptor gene expression.

To date, the best-established factor that participates in the huntingtin-mediated control of neuronal gene expression and whose transcriptional activity changes upon loss of wild-type huntingtin function is repressor element 1 silencing transcription factor (REST) (Zuccato et al., 2003; Cattaneo et al., 2005). We therefore considered whether REST is involved in CB1 receptor promoter regulation. Computer-aided analysis of the CB1 receptor promoter allowed us to identify three potential REST-binding RE1 sites.

Figure 6 Transient regulation of huntingtin expression controls CB1 cannabinoid receptor gene promoter activity via repressor element 1 silencing transcription factor. (A and B) CB1 receptor promoter activity in STHdhQ7/Q7 cells transfected with GFP, 17Q-GFP or 72Q-GFP (A), or with empty vector, 17Q-FL or 75Q-FL (B) and CAT reporter constructs encoding a 3016- or an 898-bp human CB1 receptor promoter (n = 4 experiments; **P < 0.01 from empty construct; #P < 0.01 from the corresponding −3016 construct). (C) CB1 receptor promoter activity in STHdhQ7/Q7 cells transfected with control small interfering RNA (siC) or huntingtin-directed small interfering RNA (siHtt) and the aforementioned reporter constructs (n = 4 experiments; *P < 0.05 from siC; #P < 0.01 from the corresponding −3016 construct). (D) CB1 receptor promoter activity in STHdhQ7/Q7 cells transfected with reporter constructs encoding sequential 5′-promoter deletions (n = 4 experiments; **P < 0.01 from −3016 construct). (E) Promoter activity in STHdhQ7/Q7 cells transfected with GFP, 17Q-GFP or 72Q-GFP, and reporter constructs encoding the CB1 receptor promoter −962/−934 sequence or a RE1 consensus sequence (n = 4 experiments; **P < 0.01 from the corresponding empty construct). (F) Promoter activity in STHdhQ7/Q7 cells transfected with siC or siHtt, control (C) or REST-directed decoy oligonucleotides, and reporter constructs encoding a 3016-bp CB1 receptor promoter, the CB1 receptor promoter −962/−934 sequence or a RE1 consensus sequence (n = 4 experiments; *P < 0.05, **P < 0.01 from siC; #P < 0.05 from the corresponding decoy C). a.u. = arbitrary units.
Endogenous huntingtin controls CB₁ cannabinoid receptor gene promoter activity via repressor element 1 silencing transcription factor

To evaluate the huntingtin-mediated control of the CB₁ receptor promoter in a huntingtin constitutive expression setting and to search for possible dose-dependent effects of huntingtin on CB₁ receptor expression, we used striatal neuroblasts from wild-type mice (STHdh<sup>Q7/Q7</sup> cells) and from knock-in mice expressing one copy (STHdh<sup>Q7/Q111</sup> cells) or two copies (STHdh<sup>Q111/Q111</sup> cells) of a mutant huntingtin allele. We first observed that CB₁ receptor expression, as determined by real-time quantitative PCR (Fig. 7A, left panel), western blot (Fig. 7B, middle panel) and immunofluorescence (Fig. 7C, right panel), followed the relative order STHdh<sup>Q7/Q7</sup> cells > STHdh<sup>Q7/Q111</sup> cells > STHdh<sup>Q111/Q111</sup> cells. We then transfected those cells with the CB₁ receptor promoter construct and found that reporter activity displayed the same sequential order as receptor expression (Fig. 7B, left panel). Likewise, the reporter activity of the CB₁ receptor promoter −962/−934 sequence (Fig. 7B, middle panel), as well as that of a RE1 consensus sequence (Fig. 7B, right panel), was higher under wild-type huntingtin expression conditions, pointing again to an important role of the RE1 site in the huntingtin-mediated control of the CB₁ receptor promoter. Further support for this notion was provided by the observation that delivery of RE1-targeted decoy oligonucleotides to mutant huntingtin-expressing cells recovered CB₁ receptor promoter activity to values close to those found in STHdh<sup>Q7/Q7</sup> cells (Fig. 7C).

CB₁ cannabinoid receptors control striatal brain-derived neurotrophic factor expression

CB₁ receptors can confer neuroprotection by cross-talking to neurotrophic-factor signalling systems (Galve-Roperh et al., 2008). Specifically, CB₁ receptors have been reported to upregulate BDNF expression, which may play a key mechanistic role in cannabinoid-evoked neuroprotection from excitotoxic damage (Marsicano et al., 2003; Khaspekov et al., 2004). Of interest, the downregulation of this particular neurotrophin is critically involved in Huntington’s disease neurodegeneration (Canals et al., 2004; Cattaneo et al., 2005; Zuccato and Cattaneo, 2007). We therefore evaluated how modulation of CB₁ receptor function affects BDNF expression in R6/2 mice. The messenger RNA levels and immunoreactivity of striatal BDNF were lower in R6/2:CB₁<sup>−/−</sup> mice than in their R6/2:CB₁<sup>+/+</sup> littermates (Fig. 8A). Moreover, THC administration was able to prevent the decline of striatal BDNF expression observed in vehicle-treated R6/2 mice (Fig. 8B). The messenger RNA levels of the BDNF receptor TrkB were not significantly different in the striata of 8-week-old wild-type, CB₁<sup>−/−</sup>, R6/2:CB₁<sup>−/−</sup> and R6/2:CB₁<sup>+/+</sup> mice, or of 8-week-old wild-type or R6/2 mice treated with vehicle or THC (data not shown).

To provide further support for the direct involvement of huntingtin/CB₁ receptors in the control of striatal BDNF expression, we exposed striatal cells to THC. We found that cannabinoid challenge upregulated BDNF expression in STHdh<sup>Q7/Q7</sup> cells, an effect that was prevented by CB₁ receptor blockade (Fig. 8C). In contrast, STHdh<sup>Q111/Q111</sup> cells showed a reduced basal expression of BDNF (data not shown). Of interest, CB₁ receptor antagonism or agonism (Fig. 8C). Next, we conducted experiments in striatal organotypic cultures obtained from wild-type and R6/2 mice. THC increased BDNF expression in slices from wild-type mice of 6 and 10-weeks of age, as well as in slices from 6-week-old R6/2 mice (Fig. 8C). However, BDNF expression in 10-week-old R6/2 mouse slices—in which CB₁ receptors are severely downregulated—was low and refractory to cannabinoid challenge (Fig. 8C).

Striatal fatty acid amide hydrolase expression increases in R6 mice and patients with Huntington’s disease

The experimental evidence described above strongly supports that CB₁ receptor downregulation plays a pivotal role in Huntington’s disease-like pathology in R6/2 mice. Nonetheless, the possible participation of other endocannabinoid system elements in progression of the disease may also be considered. Specifically, the levels of anandamide and other endocannabinoids have been shown to decline in the striatum of symptomatic (10-week-old) R6/2 mice (Bisogno et al., 2008). Therefore, our next question was whether the expression of the endocannabinoid-deactivating enzyme FAAH is altered in the disease. CB₁ receptor expression was always monitored in parallel as a functionally related, well-established control. We found that striatal FAAH messenger RNA levels were higher in symptomatic (8- to 12-week-old) R6/2 mice than in their wild-type littermates (Fig. 9A). Striatal FAAH upregulation was also evident at late stages of Huntington’s disease-like progression in the R6/1 mouse line, a slow-course transgenic model of Huntington’s disease (Fig. 9B). Likewise, western blot analysis of post-mortem samples showed an increase of FAAH expression in the caudate-putamen of patients with Huntington’s disease compared to control subjects (Fig. 9C). In contrast to FAAH, the expression of monoaaclylglycerol lipase, the major enzyme involved in the breakdown of the endocannabinoid...
A loss of striatal CB1 cannabinoid receptors that occurs in an disease pathogenesis remains unclear. Here we show that the huntingtin-evoked gene expression changes on Huntington's disease. However, the functional impact of most of these mutant huntingtin-associated impairment of CB1 receptor gene expression, as determined by real-time quantitative PCR (left), western blot (middle; quantification of optical density values relative to those of loading controls (α-tubulin) as well as a representative blot with the Mr of the selected protein bands are shown) and immunofluorescence (right; given as relative values of CB1+ area (in green)/total cell number (nuclei in blue), n = 4 experiments; *P < 0.05, **P < 0.01 from STHdhQ7/Q7 cells; †P < 0.05, ‡P < 0.01 from STHdhQ7/Q111 cells). (B) Promoter activity in STHdh cells transfected with CAT reporter constructs encoding a 3016-bp human CB1 receptor promoter, the CB1 receptor promoter −962/−934 sequence or a RE1 consensus sequence (n = 6 experiments; *P < 0.05, **P < 0.01 from STHdhQ7/Q7 cells; †P < 0.05, ‡P < 0.01 from STHdhQ7/Q111 cells). (C) Promoter activity in STHdh cells transfected with control (C) or REST-directed decoy oligonucleotides and a reporter construct encoding a 3016-bp CB1 receptor promoter (n = 4 experiments; *P < 0.05, **P < 0.01 from the corresponding STHdhQ7/Q7 cells; †P < 0.05 from the corresponding STHdhQ7/Q111 cells). a.u = arbitrary units.

2-arachidonoylglycerol, remained unchanged in the striata of R6/2 or R6/1 mice along disease progression as determined by real-time quantitative PCR (data not shown).

Discussion

One of the most widely reported effects of mutant huntingtin is the alteration of gene expression, and thus transcriptional dysregulation has emerged as a central pathogenic feature of Huntington’s disease (Cha, 2007; Imarisio et al., 2008). However, the functional impact of most of these mutant huntingtin-evoked gene expression changes on Huntington’s disease pathogenesis remains unclear. Here we show that the loss of striatal CB1 cannabinoid receptors that occurs in an animal model of Huntington’s disease is caused by a mutant huntingtin-associated impairment of CB1 receptor gene expression, and that this event may constitute a key pathogenic factor of the disease. Thus, CB1 receptor genetic ablation in mice aggravates Huntington’s disease symptoms and pathology, while CB1 receptor pharmacological activation attenuates them. Likewise, CB1 receptor downregulation sensitizes striatal cells to excitotoxic damage, while enforced CB1 receptor expression renders striatal cells more resistant to excitotoxic damage. Besides this pivotal role of CB1 receptors, the participation of other endocannabinoid system elements in Huntington’s disease pathology might also be considered. Specifically, the striatal expression of the anandamide-degrading enzyme FAAH is upregulated in symptomatic Huntington’s disease-like mice as well as in patients with Huntington’s disease, most likely reflecting—like in other neuro-pathologies—a process of astroglial activation (Benito et al., 2003, 2007). Accordingly, the levels of anandamide and palmitoylethanolamide (another FAAH substrate) have been shown to decline in the striata of symptomatic—but not pre-symptomatic—R6/2 mice (Bisogno et al., 2008). This decrease in endocannabinoid and endocannabinoid-like messengers might contribute to the aggravation of Huntington’s disease symptomatology at late stages of the disease. In contrast to these findings in striatal specimens, FAAH activity has been reported to decrease—and endocannabinoid levels to increase—in peripheral lymphocytes.
from patients with Huntington’s disease compared to healthy subjects (Battista et al., 2007). As shown in the present study, the expression of monoacylglycerol lipase, the major enzyme involved in the breakdown of the endocannabinoid 2-arachidonoylglycerol, remains however unchanged in the striata of R6/2 or R6/1 mice along disease progression. On the other hand, microglial CB2 cannabinoid receptors are induced upon various neuroinflammatory conditions, in which they are believed to inhibit the production...
of pro-inflammatory cytokines and reactive oxygen species (Fernandez-Ruiz et al., 2007). Thus, the recently described upregulation of CB2 receptors in striatal microglia of Huntington’s disease patient samples and transgenic and neurotoxin-induced Huntington’s disease animal models (Palazuelos et al., 2009, Sagredo et al., 2009) might constitute a defensive response aimed at attenuating microglial overactivation in late stages of Huntington’s disease. We cannot rule out that activation of CB2 receptors participates in the beneficial effects of THC reported here. However, the implication of microglial overactivation selectively in advanced stages of the disease, the strong impact of CB1 receptor genetic ablation at early stages of the disease and the indispensable involvement of CB1 receptors in cannabinoid-induced neuroprotection and BDNF upregulation found in our striatal cell/tissue culture experiments strongly support that CB1 receptors make a major contribution to the observed effects of THC as administered—as in the present study FAAH to 4- to 10-week-old animals. On the other hand, the finding that the modulation of CB1 receptor (the present work) or CB2 receptor (Palazuelos et al., 2009) activity in R6/2 mice affects the immunoreactivity of the pre-synaptic terminal marker synaptophysin—besides that of the post-synaptic marker PSD95 and the GABAergic neuron marker GAD67—supports the possibility that the endocannabinoid system confers protection not only to striatal medium-sized spiny neurons, the cells that degenerate primarily in Huntington’s disease, but also to other types of neurons that are targeted by the disease such as those projecting the striatum (e.g. corticostriatal neurons and nigrostriatal neurons) and striatal interneurons.

Huntington’s disease is usually envisaged as a gain-of-function disease (Walker, 2007; Imarisio et al., 2008). However, although the cellular functions of wild-type huntingtin are still not completely clear, it has been proposed that loss of wild-type huntingtin function also contributes to Huntington’s disease (Cattaneo et al., 2005). Our data support that the impact of CB1 receptor downregulation on Huntington’s disease pathology is associated,
at least in part, to a loss of wild-type huntingtin function process, and that the huntingtin-mediated control of CB1 receptor gene expression relies on REST, a transcriptional repressor that regulates the expression of a large network of neuronal proteins (Johnson and Buckley, 2009). It was previously shown that wild-type huntingtin sequesters REST in the cytoplasm, thereby preventing its gene-silencing action (Zuccato et al., 2003). A subsequent report supported that this interaction is not direct, so that huntingtin binds to REST through two intermediate proteins, dynactin p150glue and REST/neuron restrictive silencer factor-interacting LIM domain protein (Shimojo, 2008). The latter study further suggested that mutant huntingtin binds to that multi-protein complex and alters its conformation, thus permitting REST to translocate to the nucleus and repress gene expression. Our data fit well with this current model of huntingtin/REST action. Nonetheless, it cannot be ruled out that the huntingtin-mediated control of CB1 receptor expression is a more complex issue, as, for example, mutant huntingtin is well known to impact gene/protein expression by a plethora of different transcriptional and post-transcriptional mechanisms (Benn et al., 2008; Imai et al., 2008; Johnson and Buckley, 2009).

Of note, REST also participates in the huntingtin-mediated transcriptional control of BDNF, a particular neurotrophin that is critically involved in Huntington’s disease pathophysiology (Cattaneo et al., 2005; Zuccato and Cattaneo, 2007). In addition, several reports support that CB1 receptors confer neuroprotection by enhancing BDNF expression, although the molecular basis of this connection remains unknown (Galve-Roperh et al., 2008). It is thus conceivable that the decrease of BDNF levels concomitant with CB1 receptor loss contributes significantly to striatal damage in Huntington’s disease, for which our findings support that BDNF is a bona fide marker of Huntington’s disease neurodegeneration (Zuccato and Cattaneo, 2007) and CB1 receptor-evoked neuroprotection (Galve-Roperh et al., 2008). Striatal BDNF can be produced in situ (Timmusk et al., 1995; Canals et al., 1998; Aid et al., 2007; Hasbi et al., 2009). Additionally, striatal GABAergic projections receive BDNF from the cortex (Altar et al., 1997; Mufson et al., 1999), indicating that impaired anterograde BDNF transport in corticostriatal neurons may contribute to the decreased BDNF protein expression found in the striata of Huntington’s disease mice (Cattaneo et al., 2005). Nonetheless, mutant huntingtin has been shown to affect axonal transport of BDNF in striatal neurons but not in cortical neurons (Her and Goldstein, 2008), and CB1 receptor loss or gain of function does not affect cortical BDNF expression in R6/2 mice (Supplementary Fig. 2).

Potential clinical implications

Previous studies on the potential role of CB1 receptors in Huntington’s disease have been undertaken on simpler experimental systems and have provided contradictory data. Thus, screening of a large library of compounds for their ability to protect cultured PC12 pheochromocytoma cells from mutant huntingtin-induced toxicity unveiled THC and other plant-derived cannabinoids as very efficient agents (Aiken et al., 2004). However, this was not replicated in a similar study (Wang et al., 2005). Likewise, administration of THC and other cannabinoid receptor agonists reduced (Lastres-Becker et al., 2004; Pintor et al., 2006) or increased (Lastres-Becker et al., 2003) neuronal loss in rat models of neurotoxin-induced acute striatal damage. Here we used a well-established genetic model of Huntington’s disease, the R6/2 mouse, which recapitulates many of the features of human Huntington’s disease, including motor and cognitive impairments, weight loss, striatal atrophy, mutant-protein aggregates, neurochemical alterations, gene expression dysregulation, metabolic and neuroendocrine changes and premature death (Mangiarini et al., 1996; Hockly et al., 2003; Gil and Rego, 2009). Although this model displays potential limitations such as an accelerated phenotype—which may mimic juvenile-onset Huntington’s disease rather than adult-onset Huntington’s disease—and the expression of a truncated form of mutant huntingtin, a recent study that has compared different transgenic and knock-in models of Huntington’s disease using standardized conditions has confirmed the relevance of the R6/2 line for the study of the disease (Menalled et al., 2009). Our experiments of CB1 receptor pharmacological activation in R6/2 mice, as well as the phenotypic analyses of R6/2:CB1−/− mice, to the best of our knowledge the first double-mutant animals generated so far in which CB1 receptors are ablated in a neuropathology genetic-model background, provide strong evidence for the protective role of CB1 receptors, and may open possibilities for similar studies on other neuropathologies (such as Alzheimer’s disease) in which CB1 receptor levels fall (Benito et al., 2003, Ramirez et al., 2005).

Pharmacological activation of CB1 receptors in patients with early-stage Huntington’s disease might thus be beneficial in attenuating disease progression in these subjects. A first controlled trial conducted with a cannabis component (cannabidiol) reported no effect on chorea severity in 15 patients with Huntington’s disease (Consroe et al., 1991). However, cannabidiol, although structurally similar to THC, is not a cannabinoid receptor agonist. Two subsequent uncontrolled, single-patient studies using nabilone, a synthetic 9-keto derivative of THC that activates CB1 receptors, reported contradictory outcomes on Huntington’s disease-associated chorea—either worsening (Muller-Vahl et al., 1999) or improvement (Curtis and Rickards, 2006). The only double-blind, placebo-controlled, cross-over study of a CB1 receptor agonist (specifically nabilone) in Huntington’s disease has been recently reported (Curtis et al., 2009). This 44-patient trial has shown improvements in total motor score, chorea, cognition, behaviour and neuropsychiatric inventory upon cannabinoid treatment, which was safe and well tolerated. Although it is clear that there is a need for further and more exhaustive trials to establish the use of cannabinoids in Huntington’s disease, this clinical study—for which our work provides strong preclinical support—opens a new therapeutic avenue for the management of this devastating disease. In this respect, THC and other cannabinoids have a favourable drug-safety profile and are already used in clinical practice as anti-emetic, appetite-stimulating and analgesic compounds (Pertwee, 2009). Additionally, approaches aimed at preventing CB1 receptor loss (e.g. by environmental stimulation; Glass et al., 2004) might be also envisaged. Finally, our results
support the potential use of CB₁ receptors as biomarkers for monitoring the onset and progression of Huntington’s disease.

Acknowledgements

The authors are grateful to Elena García-Taboada, José A. Rodríguez-Navarro and Juan PerUCHO for their expert technical assistance, and Michael Patterson and Guillermo Velasco for their valuable comments on the manuscript.

Funding

Ministerio de Ciencia e Innovación (grant numbers SAF2009-08403 to M.G., SAF2009-11847 to J.F.R., SAF2007-61565 to J.R.); Comunidad de Madrid-Universidad Complutense de Madrid (grant numbers SAL2006/261 to M.G., I.G.R., J.F.R. and J.R., 950344 to M.G. and J.F.R.); and German Bundesministerium für Bildung und Forschung (Förderkennzeichen 01ZZ0407 to C.B.). M.R.P., J.P. and M.D.Z. are supported by Ministerio de Ciencia e Innovación. M. Salazar is supported by Comunidad de Madrid.

Supplementary material

Supplementary material is available at Brain online.

References


