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The mGluR5 positive allosteric modulator, CDPPB, ameliorates pathology and phenotypic signs of a mouse model of Huntington's disease

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ABSTRACT

Huntington's disease (HD) is an autosomal-dominant neurodegenerative disorder caused by a polyglutamine expansion in the amino-terminal region of the huntingtin protein (htt), leading to motor dysfunction, cognitive decline, psychiatric alterations, and death. The metabotropic glutamate receptor 5 (mGluR5) has been implicated in HD and we have recently demonstrated that mGluR5 positive allosteric modulators (PAMs) are neuroprotective in vitro. In the present study we demonstrate that the mGluR5 PAM, CDPPB, is a potent neuroprotective drug, in vitro and in vivo, capable of delaying HD-related symptoms. The HD mouse model, BACHD, exhibits many HD features, including neuronal cell loss, htt aggregates, motor incoordination and memory impairment. However, chronic treatment of BACHD mice with CDPPB 1.5 mg/kg s.c. for 18 weeks increased the activation of cell signaling pathways important for neuronal survival, including increased AKT and ERK1/2 phosphorylation and augmented the BDNF mRNA expression. CDPPB chronic treatment was also able to prevent the neuronal cell loss that takes place in the striatum of BACHD mice and decrease htt aggregate formation. Moreover, CDPPB chronic treatment was efficient to partially ameliorate motor incoordination and to rescue the memory deficit exhibited by BACHD mice. Importantly, no toxic effects or stereotypical behavior were observed upon CDPPB chronic treatment. Thus, CDPPB is a potential drug to treat HD, preventing neuronal cell loss and htt aggregate formation and delaying HD symptoms.

Introduction

Huntington's disease (HD) is a neurodegenerative disorder caused by an unstable CAG repeat expansion in the amino-terminal region of the huntingtin protein (htt) (Group, 1993). Clinical manifestations of HD usually initiate between 35 and 50 years of age as involuntary body movement, loss of cognitive function, and psychiatric disturbance, inevitably leading to death (Li and Li, 2004; Vonsattel and DiFiglia, 1998; Young, 2003). Neuropathological analysis reveals selective and progressive neuronal loss in the striatum (DiFiglia, 1990; Vonsattel et al., 1985).

Glutamate-mediated neurotoxicity has been postulated to play an important role in the pathogenesis of HD (Anborgh et al., 2005; Calabresi et al., 1999; DiFiglia, 1990; Ribeiro et al., 2010). Stimulation of metabotropic glutamate receptor 5 (mGluR5) leads to the formation of inositol 1,4,5-trisphosphate (InsP3) and release of intracellular Ca^{2+} and mutant htt can enhance this cell signaling pathway, leading to toxic levels of intracellular Ca^{2+} (Ribeiro et al., 2010; Tang et al., 2005; Tang et al., 2003). However, mGluR5 stimulation can also promote activation of neuroprotective cell signaling pathways, involving ERK1/2 and AKT (Doria et al., 2013; Ribeiro et al., 2010). We have recently demonstrated that mGluR5 positive allosteric modulators (PAMs) can promote neuronal survival by activating AKT without triggering Ca^{2+} release (Doria et al., 2013). Moreover, it has been shown that mGluR5 PAMs have a positive effect on memory, facilitating neurotransmission and improving spatial learning (Ayala et al., 2009) and rescuing pharmacologically induced object recognition memory impairment (Reichel et al., 2011; Uslaner et al., 2009). In contrast, both mGluR5 receptor antagonism and genetic deletion have been shown to cause deleterious effects on learning and memory (Simonyi et al., 2010). Thus, mGluR5 PAMs are potential drugs to treat HD.

In the present study we demonstrate that the mGluR5 PAM, 3-Cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide (CDPPB), can ameliorate pathology and phenotypic signs exhibited by a mouse model of HD, BACHD. We performed a concentration–response curve using CDPPB and we found that this mGluR5 PAM is a very potent

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neuroprotective drug as it was capable of protecting neurons against glutamate insult even at low concentrations. We also investigated the effect of CDPPB chronic administration and we found that BACHD mice treated with 1.5 mg/kg CDPPB s.c. for 18 weeks exhibited higher levels of AKT and ERK1/2 activation, as well as enhanced *brain-derived neurotrophic factor* (BDNF) mRNA expression. Moreover, treatment with CDPPB was capable of reducing the neurodegeneration and htt aggregate formation that take place in BACHD brain. Electron microscopy analyses showed that there was a decrease in the number of vesicles at the pre-synaptic active zone of BACHD mice and that CDPPB chronic treatment normalized this deficit. Finally, our behavioral tests demonstrated that CDPPB treatment partially improved motor coordination and normalized memory deficit in BACHD mice. Thus, our results indicate that CDPPB chronic treatment has the potential to prevent the neuronal loss and ameliorate the motor and cognitive symptoms observed in a HD mouse model.

Materials and methods

Materials

Neurobasal medium, N2 and B27 supplements, GlutaMAX (50.0 mg/ml penicillin and 50.0 mg/ml streptomycin), Live/Dead viability assay, TRIzol, Nuclease-Free Water, and Power SYBR® Green PCR Master Mix were purchased from Life Technologies (Foster City, CA, USA). Mouse anti-Huntingtin EM48 (Cat# MAB5374, RRID: AB_177645) and mouse anti-NeuN (Cat# MAB377, RRID: AB_2298772) monoclonal antibodies were purchased from Millipore (Billerica, MA, USA). 3-Cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide (CDPPB) was purchased from Tocris Cookson Inc. (Ellisville, MO, USA). Horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (Cat# 170-6515, http://www.bio-rad.com/pt-br/sku/170-6515-goat-anti-rabbit-igg-h-l-hrp-conjugate) was from BioRad (Hercules, CA, USA). ECL Western blotting detection reagents were from GE Healthcare (Buckinghamshire, UK). Anti-phospho AKT (Cat# DB 127, http://www.dbbiotech.com/products/antibodies/wb/anti-akt1-(pser-473).html), anti-phospho ERK1/2 (Cat# DB 013, http://www.dbbiotech.com/products/antibodies/wb/anti-phospho-erk-1,2.html), anti-AKT (Cat# DB 126, http://www.dbbiotech.com/products/antibodies/wb/anti-akt1.html) and anti-ERK1/2 (Cat# DB 012, http://www.dbbiotech.com/products/antibodies/wb/anti-erk-1,2.html) rabbit monospecific clonal antibodies were from DB Biotech (Kosice, Slovakia). Vectastain Elite ABC Kit (Mouse IgG) and Vector SG Peroxidase Substrate Kit were purchased from Vector Laboratories (Burlingame, CA, USA). All other biochemical reagents were purchased from Sigma-Aldrich (St Louis, MO, USA).

Mouse model

FVB/NJ (wild-type, RRID: IMSR_JAX:001800) and FVB/N-Tg(HTT*97Q) IXwy/°J (BACHD) transgenic mice (RRID: IMSR_JAX:008197, http://jaxmice.jax.org/strain/008197.html) (Gray et al., 2008) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed in an animal care facility at 23 °C on a 12 h light/12 h dark cycle with food and water provided ad libitum. Animal care was in accordance with the Universidade Federal de Minas Gerais Ethics Committee on Animal Experimentation, CETEA.

Neuronal primary culture preparation

Neuronal cultures were prepared from the striatal region of E15 mouse embryo brains. Animal procedures were approved by the Ethics Committee on Animal Experimentation, CETEA/UFMG. After dissection, striatal tissue was submitted to trypsin digestion followed by cell dissociation using a fire-polished Pasteur pipette. Cells were plated on poly-L-ornithine coated dishes in Neurobasal medium supplemented with N2 and B27 supplements, 2.0 mM GlutaMAX, 50.0 μg/ml penicillin, and 50.0 μg/ml streptomycin. Cells were incubated at 37 °C and 5% CO_2 in a humidified incubator and cultured for 10 to 12 days in vitro (DIV) with medium replenishment every 4 days.

Drug administration

CDPPB was dissolved in dimethyl-sulfoxide (DMSO) and was continuously delivered by subcutaneously implanted Alzet® micro-osmotic pumps (200 μl total volume, model 2006, Charles River Laboratories, Sulzfeld, Germany). Filling and preparation of micro-osmotic pumps for implantation were done as suggested by the manufacturer. Pump rate was 0.15 μl/h and pumping duration was 6 weeks. Either vehicle (DMSO) or CDPPB 1.5 mg/kg s.c. per day was continuously delivered to animals during a total of 18 weeks.

EM48 and NeuN immunohistochemistry

Mice were anesthetized with ketamine/xylazine (100/10 mg/kg) i.p. and transcardially perfused with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS). The brains were then dissected out and stored in 4% PFA in PBS for 24 h. Prior to sectioning, brains were put into 30% sucrose in PBS overnight at 4 °C. Brains were coronally sectioned in cryostat and 40 μm slices were stored in cryoprotect solution. Immunohistochemistry was performed on 40 μm free-floating sections using a peroxidase based immunostaining protocol. In brief, endogenous peroxidase activity was quenched using 0.3% hydrogen peroxide, washed 2 × 5 min with 1 × PBS, after which the membranes were permeabilized using 1% Triton X-100 for 10 min. Non-specific binding was blocked using 1.5% horse serum from Vector Elite Kit for 30 min, followed by incubation in either mouse anti-htt EM48 (1:100) or mouse anti-NeuN (1:100) primary antibodies, with 2% normal horse serum (from Vector Elite Kit) and 3% bovine serum albumin (BSA) in PBS overnight at 4 °C. Sections were washed in PBS and then incubated in secondary antibody (biotinylated horse anti-mouse, 1:400, Vector Elite ABC kit mouse) for 90 min at 4 °C. Finally, sections were incubated in avidin–biotin enzyme reagent complex (from Vector Elite Kit) for 90 min at 4 °C, according to the manufacturer's instructions. Immunostaining was visualized using a chromogen (Vector SG substrate). Sections were mounted on slides and visualized using an Axio Imager A2-Carl Zeiss Microscope with a Zeiss 20× lens, representative 710 μm × 532 μm areas of striatum were imaged for analysis. The number of NeuN and EM48-positive puncta per image was counted using the cell counter tool from ImageJ (NIH, USA, RRID:nif-0000-30467).

Immunoblotting

Cortex, striatum and hippocampus were dissected and lysed in Triton buffer (1% Triton X-100, 0.15 M NaCl, 0.05 M Tris–HCl, pH 7.2) containing protease inhibitors (1.0 mM AEBSF and 10.0 μg/ml of both leupeptin and aprotinin). 100.0 μg of total cellular protein for each sample was subjected to SDS-PAGE, followed by electroblotting onto nitrocellulose membranes. Membranes were blocked with 5% BSA in wash buffer (150.0 mM NaCl, 10.0 mM Tris–HCl, pH 7.0, and 0.05% Tween 20) for 1 h and then incubated with either rabbit anti-phospho AKT (S473) (1:1000) or rabbit anti-phospho ERK1/2 (Thr202/Thr204) (1:1000) antibodies in wash buffer containing 3% BSA for 2 h at room temperature. Membranes were rinsed three times with wash buffer and then incubated with secondary peroxidase-conjugated anti-rabbit IgG antibody diluted 1:5000 in wash buffer containing 3% skim milk for 1 h. Membranes were rinsed three times with wash buffer and incubated with ECL Western blotting detection reagents. Antibodies were then stripped and membranes were incubated with either rabbit anti-AKT (1:1000) or rabbit anti-ERK1/2 (1:1000) antibodies for 2 h and probed with secondary antibody anti-rabbit IgG diluted 1:5000 to determine total AKT and ERK1/2 expression. Non-saturated,