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Striatal ablation of GABAergic neurons prevents the in vivo neuroprotective effect of DCG-IV against the MPP^+-induced neurotoxicity on dopaminergic nerve terminals

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ABSTRACT

In previous studies we found that intrastriatal DCG-IV administration, an agonist for group II metabotropic glutamate receptor: (i) protected striatal dopaminergic terminals against MPP^+-induced neurotoxicity (Matarredona et al., 2001); (ii) selectively destroyed striatal GABAergic neurons (Venero et al., 2002) and (iii) induced early robust up-regulation of brain-derived neurotrophic factor (BDNF) in nigral dopaminergic neurons afferents in a target-dependent manner (Rite et al., 2005). Considering that BDNF protein is anterogradely transported to dopaminergic nerve endings, an autocrine role of BDNF could account for the neuroprotective effect of DCG-IV against the MPP^+-induced toxicity of dopaminergic terminals. To test this possibility, we first performed a previous insult with quinolinic acid (QA) to specifically damage the striatal GABAergic neuronal cell bodies in order to remove the nigral BDNF target. Fourteen days later, we explored the potential in vivo neuroprotective action of DCG-IV against MPP^+-induced toxicity on striatal dopaminergic nerve terminals by in vivo microdialysis. Integrity of GABAergic system was evaluated by glutamic acid decarboxylase (GAD) in situ hybridization. We demonstrate that previous striatal target ablation with QA prevented the neuroprotective effect of DCG-IV perfusion against the MPP^+-induced neurotoxicity on dopaminergic terminals. Our results strongly suggest an important autocrine neuroprotective role of BDNF on striatal dopaminergic nerve terminals. In addition, we found an unexpected regulatory response of surviving striatal GABAergic neurons in terms of high levels of GAD mRNA expression.

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1. Introduction

Metabotropic glutamate (mGlu) receptors are G-protein-coupled receptors and are not generally thought to be involved in fast synaptic neurotransmission. Eight different receptor subtypes have been identified, falling into three families according to sequence homology and pharmacological properties: group I (mGlu 1 and 5), group II (mGlu 2 and 3) and group III (mGlu 4, 6, 7 and 8) (for reviews, see Byrnes et al., 2009; Kim et al., 2008; Gerber et al., 2007). Group I are coupled to phosphoinositide hydrolysis as well as to various classes of K^+ channels via G_o or G_q GTP binding protein (Hinoi et al., 2001). Groups II and III are coupled to a G_i protein and are negatively linked to adenylyl cyclase. In addition, native group II or group III mGlu receptors in the CNS are coupled to multiple transduction pathways, including inhibition of voltage-sensitive Ca^{2+} channels, stimulation of cAMP formation inhibition, stimulation of phosphatidylinositol hydrolysis and activation of the mitogen-activated protein kinase pathway (for review, see Pin and Duvoisin, 1995).

Activation of group II and group III mGlu receptors has been shown to be protective against different types of insults. For instance, group II mGlu receptor agonists attenuate hypoxia-induced neuronal degeneration, combined with glucose deprivation (Buisson and Choi, 1995) and protect cultured neurons against neuronal degeneration induced by excitotoxic concentration of NMDA (Battaglia et al., 1998; Bruno et al., 1993; Kingston et al., 1999); of kainate (Bruno et al., 1993; Miyamoto et al., 1997) or of ischaemia (Garcia de Arriba et al., 2006).

In a previous work (Matarredona et al., 2001), we have studied the effect of DCG-IV (2S:2′R:3′R:-2-(2′3′-dicarboxycyclopropyl)-glycine), an agonist for group II mGluRs (mGlu 2), on the MPP^+-induced degeneration of striatal dopaminergic terminals. We found a protective effect of DCG-IV against MPP^+-induced dopaminergic striatal degeneration. This protective effect was dependent on protein synthesis and, in fact, striatal DCG-IV

Abbreviations: BDNF, brain-derived neurotrophic factor; DCG-IV, 2S:2′R:3′R:-2-(2′3′-dicarboxycyclopropyl)glycine; MPP^+, 1-methyl-4-phenylpyridinium ion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; QA, quinolinic acid.

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infusion greatly induced a subset of brain-derived neurotrophic factor (BDNF) mRNA-expressing cells (Matarredona et al., 2001). Based on these precedents, we hypothesized in another work (Venero et al., 2002) that local microglial activation of BDNF was due to an unexpected selective toxicity of DCG-IV on GABAergic cells, a typical feature of axon-sparing neurotoxins (Schwarcz et al., 1983, 1984). We concluded that the selective DCG-IV-induced degeneration of striatal GABAergic neurons and the associated trophic glial response were responsible for protecting dopaminergic terminals against the MPP^+-induced neurotoxicity (Venero et al., 2002). However, in more recent studies, we have demonstrated that nigral BDNF expression is totally dependent on striatal target integrity (Rite et al., 2003, 2005). Striatal DCG-IV injection was shown to strongly induce nigral expression of BDNF mRNA within the first 24 h after injections. Considering that BDNF protein is anterogradely transported to dopaminergic nerve endings (Altar and DiStefano, 1998), an autocrine role of BDNF could account for the neuroprotective effect of DCG-IV against the MPP^+-induced toxicity of dopaminergic terminals.

If our assumption is correct, then preventing BDNF mRNA induction in substantia nigra in response to DCG-IV treatment should overcome the ability of this compound to protect striatal dopaminergic terminals from MPP^+-induced toxicity. To achieve this, we first performed a previous insult with quinolinic acid (QA) to cause specific damage to the striatal GABAergic neuronal cell bodies in order to remove the nigral BDNF target. QA is an agonist of NMDA receptors, causing the death of medium spiny neurons while sparing dopaminergic fibers (Karl et al., 2008) and it reproduces some of the behavioral and biochemical alterations of Huntington's disease (Estrada Sanchez et al., 2008; Schwarcz et al., 2010). Fourteen days later, we explored the potential *in vivo* neuroprotective action of DCG-IV against MPP^+-induced toxicity on striatal dopaminergic nerve terminals. DCG-IV was intrastriatally perfused through a dialysis probe with and without the inclusion of 1 mM MPP^+ in the perfusate and the toxicity was evaluated by microdialysis (Santiago et al., 2001). Integrity of the GABAergic system was evaluated by *in situ* hybridization using specific antisense riboprobe to glutamic acid decarboxylase (GAD67) mRNA. Our results support an autocrine role of BDNF within adult striatal dopaminergic terminals. An unexpected regulatory response of surviving striatal and cortical GABAergic neurons in terms of high levels of GAD67 mRNA expression was also found.

2. Materials and methods

2.1. Animals and drug treatment

Animals were male albino Wistar rats weighing 270–320 g at the time of probe implantation. The rats were kept, three or four per cage, at constant room temperature (22 ± 2 °C) and relative humidity (60%) with a 12-h light–dark cycle and unlimited access to food and water. Experiments were carried out in accordance with the Guidelines of the European Union Council (86/609/EU) and following the Spanish regulations (BOE 67/8509-12, 1988) for the use of laboratory animals, and approved by the Scientific Committee of the University of Sevilla.

The following drugs were used: 1-methyl-4-phenylpyridinium (MPP+ iodide; Research Biochemical Inc., Natick, MA, U.S.A.), DCG-IV (Tocris Cookson, Bristol, UK) and Quinolinic acid (QA, Sigma Chemical Co., St. Louis, MO, U.S.A.).

2.2. QA lesion

QA (120 nmol) was dissolved in a volume of 2 μl of Ringer's solution. The solution was injected at a rate of 0.5 μl/min into the left striatum with a Hamilton syringe positioned at the following coordinates relative to bregma and dura: A/P 0.5, L/M 2.5, and V/D 6.5. The Hamilton syringe was left in place for an additional 5 min after the end of the infusion. Rats were allowed to recover and were housed in groups of 3–4.

2.3. Brain dialysis

Fourteen days after the QA lesion, animals were anesthetized with general chloral hydrate (400 mg/kg, i.p.) and local lidocaine (10%, w/v in water) anesthesia and mounted in a stereotaxic apparatus (David Kopf Instruments) with the nose positioned 3.3 mm below the horizontal bar. Following a midline incision, the skull was exposed and 2 burr holes were drilled, through which 2 probes were implanted in both corpus striata with coordinates from bregma point and dura (A/P +0.6, L/M ±2.5, V/D −6.0) (Paxinos and Watson, 1986). Following surgery, animals were housed individually in plastic cages (35 cm × 35 cm × 40 cm) and allowed to recover overnight, with free access to food and water.

Microdialysis in the corpus striatum was performed with an I-shaped cannula (Santiago and Westerink, 1992). The exposed tip of the dialysis membrane was 4 mm. The dialysis tube (ID: 0.22 mm; OD: 0.31 mm) was prepared from polyacrylonitrile/sodium methalyl sulfonate copolymer (AN 69, Hospal, Barcelona, Spain). The dopamine in vitro recovery of the membrane was 20.3 ± 1.6% ($N = 5$).

The perfusion experiments were carried out 24 h (day 1) and 48 h (day 2) after implantation of the probe (Santiago et al., 2001). Microdialysis and subsequent chemical analysis were performed using an automated on-line sample injection system (Westerink et al., 1987). The corpus striatum was perfused at a flow rate of 3.0 μl/min, using a microperfusion pump (model 22, Harvard Apparatus, South Natick, MA, U.S.A.), with a Ringer solution containing (in mM): NaCl, 140; KCl, 4.0; $CaCl_2$, 1.2; and $MgCl_2$, 1.0. With the help of an electronic timer, the injection valve was held in the load position for 15 min, during which the sample loop (40 μl) was filled with dialysate. The valve then switched automatically to the injection position for 15 s. This procedure was repeated every 15 min—the time needed to record a complete chromatogram. After establishing a steady baseline of levels in four consecutive samples (in fmol/min), drugs were administered and sampling was continued for 2.5 h thereafter. All drugs were dissolved in Ringer's solution. On day 1, 1 mM DCG-IV was perfused for 1 h and/or 1 mM MPP^+ for 15 min in animals without and with QA lesion. On day 2, 1 mM MPP^+ was perfused for 15 min.

2.4. Chemical assays

Dopamine level in dialysates was analysed by HPLC with electrochemical detection. A Merck L-6200A intelligent pump was used in conjunction with a glassy carbon electrode set at 780 mV (ANTEC, The Netherlands). A Merck Lichrocart cartridge (125 mm × 4 mm) column filled with Lichrospher reverse-phase C_{18} 5 μM material was used. The mobile phase consisted of a mixture of 0.05 M of sodium acetate, 0.4 mM of 1-octanesulfonic acid, 0.3 mM of Na2EDTA and 70 ml methanol/l, adjusted to pH 4.1 with acetic acid. All reactive agents and water were HPLC grade. The flow rate was 0.8 ml/min and the detection limit for dopamine was 5 fmol per injection.

2.5. Preparation of riboprobes

A pBluescript SK plasmid containing the cDNA sequence for GAD67 was kindly provided by Dr. A. Tobin (UCLA, Los Angeles, USA). The GAD67 cDNA is about 3.2 kb and was isolated from a 1 gt-11 cDNA library made from poly(A) RNA from adult rat brain (Erlander et al., 1991). Sense and antisense riboprobes used for in situ hybridization were transcribed in the presence of [35S]UTP (1300 Ci/mmol; Amersham International). Single-strand antisense cRNA probes were synthesized with RNA polymerases according to a protocol provided by the RNA polymerase supplier (Bethesda Research Laboratories, Bethesda, MD, USA). Sense and antisense riboprobes used were transcribed in the presence of [35S]UTP (1300 Ci/mmol; Amersham International) for isotopic in situ hybridization and digoxigenin-UPT for nonisotopic in situ hybridization.

2.6. In situ hybridization histochemistry

Animals were decapitated 24 h after day 1 perfusions (day 2), the brains were removed immediately and frozen in isopentane at −15 °C. Thaw-mounted 12 μm sections were postfixed for 30 min in 4% paraformaldehyde, followed by three washes of 10 min each in phosphate-buffered saline (PBS), pH 7.4. Sections were then treated with 0.1 M triethanolamine (pH 8) for 1 min, followed by 10 min in 0.25% acetic anhydride/0.1 M triethanolamine. After a 1-min wash in 2× SSC, sections were dehydrated in graded ethanol and then air-dried. Hybridization was done for 3 h at 50 °C in a buffer containing 50% deionized formamide, 10% dextran sulfate, 1× Denhardt solution, 2× SCC, 0.1% sodium pyrophosphate, 100 μg/ml tRNA, 100 μg/ml denaturated salmon sperm DNA and the [35S]riboprobe. Following incubation, the sections were rinsed in 4× SSC/20 mM dithiothreitol, 4× SSC alone, and treated with RNase for 30 min at 37 °C (20 μg/ml RNase A in 0.5 M NaCl, 0.01 M Tris–HCl, 0.001 M EDTA, pH 8.0). The sections were then washed for 2 h in 2× SSC at 25 °C and in 0.1× SSC at 60 °C for 1 h, dehydrated in a series of ethanol and air-dried.

2.7. Quantification of in situ hybridization

Quantification of GAD67 mRNA expression in brain sections, dry-film autoradiographs were analyzed by densitometry using the computer program analySIS® (Soft Imaging System, Germany). The system identifies objects within a user defined window and measures the optical density based upon calibrated grey scale densities resulting in relative values.

2.8. Expression of results and statistics

Difference between the average dialysate concentrations of the control and drug treatments was compared by Kruskal–Wallis analysis of variance by ranks, and,

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