L-DOPA reverses the MPTP-induced elevation of the arrestin2 and GRK6 expression and enhanced ERK activation in monkey brain

E. Bezard,[a] C.E. Gross,[a] Li Qin,[b] V.V. Gurevich,[c] J.L. Benovic,[d] and E.V. Gurevich[c,*]

[a]Basal Gang, CNRS UMR 5543, Université Victor Segalen-Bordeaux 2, 33076 Bordeaux Cedex, France
[b]Laboratory Animal Research Center, China Agricultural University, Beijing, China
[c]Department of Pharmacology, Vanderbilt University Medical Center, Preston Research Building Room 422,
 2200 Pierce Avenue, Nashville, TN 37232, USA
[d]Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA 19107, USA

Dysregulation of dopamine receptors (DARs) is believed to contribute to Parkinson disease (PD) pathology. G protein-coupled receptors (GPCR) undergo desensitization via activation-dependent phosphorylation by G protein-coupled receptor kinases (GRKs) followed by arrestin binding. Using quantitative Western blotting, we detected profound differences in the expression of arrestin2 and GRKs among four experimental groups of nonhuman primates: (1) normal, (2) parkinsonian, (3) parkinsonian treated with levodopa without or (4) with dyskinesia. Arrestin2 and GRK6 expression was significantly elevated in the MPTP-lesioned group in most brain regions; GRK2 was increased in caudal caudate and internal globus pallidus. Neither levodopa-treated group differed significantly from control. The only dyskinesia-specific change was an elevation of GRK3 in the ventral striatum of the dyskinetic group. Changes in arrestin and GRK expression in the MPTP group were accompanied by enhanced ERK activation and elevated total ERK expression, which were also reversed by L-DOPA. The data suggest the involvement of arrestins and GRKs in Parkinson disease pathology and the effects of levodopa treatment.
© 2004 Elsevier Inc. All rights reserved.

Keywords: Arrestin; G protein-coupled receptor kinase; Dyskinesia; Parkinson disease

Introduction

Parkinson disease (PD) is a neurodegenerative disorder caused by degeneration of dopaminergic neurons that provide dopamine (DA) to the striatum (Ehringer and Hornykiewicz, 1960). DA replacement therapy with levodopa (L-DOPA) often results in motor complications such as wearing off or dyskinesia (Cotzias et al., 1969; Stocchi et al., 1997). Denervation-induced super-sensitivity of DA receptors (DARs), which are members of the G protein-coupled receptor (GPCR) superfamily, is widely believed to be a contributing factor to lesion-induced motor disturbances as well as to dyskinesia, although the exact mechanisms remain to be elucidated. It has been hypothesized that altered subcellular DAR localization accompanying the complex changes in DAR expression (reviewed in Bezard et al., 2001a) plays a role in dyskinesia (Muriel et al., 1999).

Upon persistent stimulation, many GPCRs undergo desensitization via a two-step process: activation-dependent receptor phosphorylation by G protein-coupled receptor kinases (GRKs) followed by the binding of "uncoupling" proteins termed arrestins. Arrestin binding precludes further signaling via G proteins and induces receptor internalization. Internalized receptor can either be recycled back to the plasma membrane or targeted for degradation, which leads to receptor down-regulation. Two of the four arrestins, rod and cone arrestins, are expressed almost exclusively in the retina. The two nonvisual arrestins, arrestin2 and arrestin3,[1] are ubiquitous and appear to participate in the desensitization of various GPCRs. There are seven GRKs, five of which, GRK2, 3, 4, 5, and 6, are expressed in the brain (Arriza et al., 1992; Benovic and Gomez, 1993; Premont et al., 1994). Thus, five GRKs and two arrestins appear to be involved in the desensitization of hundreds of different GPCR subtypes.

Many receptors are phosphorylated equally well in vitro by several GRKs (Ménard et al., 1996; Richardson and Hosey, 1993). Similarly, many receptors in vitro bind both nonvisual arrestins equally well (Gurevich et al., 1995; Krupnick and Benovic, 1998; Richardson and Hosey, 1993). However, studies with mice

---

* Corresponding author. Fax: +1 615 343 6532.
E-mail address: Eugenia.Gurevich@vanderbilt.edu (E.V. Gurevich).
Available online on ScienceDirect (www.sciencedirect.com).

---

[1] Here we use the systematic names of arrestin and GRK proteins adopted by the Alliance for Cellular Signaling (cf. http://cellularsignaling. org). The synonyms of arrestin2 are β-arrestin and β-arrestin1; arrestin3 is also called β-arrestin2. The synonyms of GRK2 and GRK3 are β-adrenoreceptor kinase1 (or βARK1) and β-adrenoreceptor kinase2 (or βARK2), respectively.

overexpressing or lacking various GRKs suggest that in vivo receptors may prefer specific GRKs (Gainetdinov et al., 1999; Iaccarino et al., 1998a,b; Koch et al., 1995; Rockman et al., 1996). In living cells, receptors appear to have preferences for specific arrestins (Kohout et al., 2001; Oakley et al., 2000). Thus, the sensitivity of GPCRs may be differentially regulated depending on the cellular complement of arrestins and GRKs. We hypothesized that changes in DA tone caused by nigrostriatal degeneration and/ or subsequent pulsatile DA receptor stimulation by L-DOPA lead to alterations in arrestin/GRK gene expression that result in dysregulation of DA receptor signaling.

Here we report multiple changes in the concentrations of arrestins and GRKs within the basal ganglia of four nonhuman primate experimental groups: normal, parkinsonian, parkinsonian chronically treated with L-DOPA without exhibiting dyskinesia, and parkinsonian L-DOPA-treated with overt dyskinesia. Arrestin binding to GRK-phosphorylated GPCRs can initiate second round of signaling via MAP kinase cascades. Therefore, we investigated the changes in the expression and phosphorylation status of extracellular signal-regulated kinase (ERK1/2), which may be connected to modifications in arrestin/GRK expression.

Materials and methods

Animals

We used 15 female cynomolgus monkeys (*Macaca fascicularis*, Shared Animal Health, Beijing, PR of China). Animals were housed in individual primate cages under controlled conditions of humidity (50% ± 5%), temperature (24 ± 1°C), and light (12-h light/12-h dark cycle, lights on at 8:00 AM); food and water were available ad libitum, and animal care was supervised by veterinarians skilled in the healthcare and maintenance of nonhuman primates. Experiments were carried out in accordance with the European Communities Council Directive of November 24, 1986, (86/609/EEC) for the care of laboratory animals. All efforts were made to minimize animal suffering and to use the minimum number of animals necessary to perform statistically valid analysis. To maximize data obtained from these animals, brain tissues acquired in the present experiment have been (Bezard et al., 2003a) and will be used for further experiments on the mechanism of dyskinesia.

Experimental protocol

Experiments were conducted according to previously published procedures and methods (Bezard et al., 2001b, 2003a). Four monkeys were kept normal (control group), and eleven were intoxicated with MPTP hydrochloride. Once a bilateral parkinsonian syndrome had stabilized (i.e., unchanged disability score over several weeks), three monkeys were kept without any dopaminergic supplementation (MPTP group) and eight were treated chronically with twice-daily administration of L-DOPA (Modopar®, L-DOPA/carbidopa, ratio 4:1) for 6–8 months at a tailored dose designed to produce a full reversal of the parkinsonian condition (Bezard et al., 2003a). Four monkeys developed severe and reproducible dyskinesia (MPTP-intoxicated, dyskinetic monkey group), while four did not (MPTP-intoxicated, nondyskinetic monkey group). Animals were killed by sodium pentobarbital overdose (150 mg/kg, i.v.) 1 h after the last L-DOPA dose. Brains

were removed quickly after death. Each brain was bisected along the midline, and the two hemispheres were immediately frozen by immersion in isopentane (−45°C) and then stored at −80°C. Tissue was sectioned coronally at 20 μm in a cryostat at −17°C, thaw-mounted onto gelatin-subbed slides, dried on a slide warmer, and stored at −80°C.

Behavioral assessment

Parkinsonian condition (and its reversal) was assessed on a parkinsonian monkey rating scale using videotape recordings of monkeys as previously described (Bezard et al., 2003a). A score of 0 corresponds to a normal animal and a score above 6 to a parkinsonian animal. The severity of dyskinesia was rated using the Dyskinesia Disability Scale: 0, dyskinesia absent; 1, mild, fleeting, and rare dyskinetic postures and movements; 2, moderate, more prominent abnormal movements, but not interfering significantly with normal behavior; 3, marked, frequent and, at times, continuous dyskinesia intruding on the normal repertoire of activity; or, 4, severe, virtually continuous dyskinetic activity replacing normal behavior and disabling to the animal.

Assessment of lesion

Several indices were used to estimate the extent of the lesion in the MPTP-treated groups. First, DA transporter (DAT) binding in the striatum was measured using $[^{125}I]$-(E)-N-(3-iodoprop-2-enyl)-2β-carboxymethyl-3β-(4′-methylphenyl)-nortropane (PE2I; Chelatec, France) as previously described (Bezard et al., 2001b, 2003a). DA fiber density was assessed in the striatum by densitometric analysis of sections using an image analysis system (Biocom, Visioscan v4.12, Les Ulis, France) at three rostrocaudal levels in accordance with the functional organization of the striatum as previously described: the rostral level including the caudate, putamen, and nucleus accumbens (A21.0); the midlevel including the caudate, putamen, and globus pallidus pars externalis (A17.2); and the caudal level including the body of the caudate, the putamen, and both parts of the globus pallidus (i.e., pars externalis and pars internalis) (A14.6). Where appropriate, both caudate and putamen were divided into dorsolateral, dorsomedial, ventrolateral, and ventromedial quadrants for analysis. Three sections per animal per striatal level were analyzed by an examiner blind with regard to the experimental condition. Slides were then stained with hemalun to allow further anatomical identification. Optical densities were averaged for each region in each animal, converted to amount of radioactivity bound by comparison to the standards, and expressed in fmol/mg of tissue equivalent (mean ± SEM).

Second, striatal sections were stained for tyrosine hydroxylase (TH) and analyzed in the same basal ganglia subdivisions as DAT binding (Bezard et al., 2001b). Third, the TH content in the basal ganglia subdivisions was measured by Western blot using anti-TH antibody (Chemicon, Temecula, CA). Finally, the number of surviving dopaminergic (TH-positive) neurons and total number of surviving neurons were measured on sections containing the substantia nigra parc compacta (SNc) processed for TH immunohistochemistry and counterstained with Nissl. The boundaries of the SNc were chosen on three consecutive sections corresponding to a representative median plane of the SNc by examining the size and shape of the different TH-immunoreactive (TH-IR) neuronal groups, cellular relationships to axonal projections and nearby fiber bundles. The number of both TH-IR and Nissl-stained neurons per