ORIGINAL ARTICLES

Nur77 Gene Knockout Alters Dopamine Neuron Biochemical Activity and Dopamine Turnover

François Gilbert, Marc Morissette, Michel St-Hilaire, Brigitte Paquet, Claude Rouillard, Thérèse Di Paolo, and Daniel Lévesque

Background: *Transcription factors of the Nur family (*Nurr1*,* Nur77*, and* Nor-1*) are orphan nuclear receptors closely associated with dopamine neurotransmission in the central nervous system.* Nur77 *expression is strongly modulated by antipsychotic and ant-parkinsonian drugs in dopaminoceptive brain areas. However, the role of* Nur77 *in dopamine neuron activity and turnover remains elusive.*

Methods: *We compared various behavioral and biochemical parameters between* Nur77 *knockout −/− and wild-type +/+ mice in basal and haloperidol-challenged conditions.*

Results: *We report here that* Nur77*-deficient mice display enhanced spontaneous locomotor activity, greater sensitivity to a small dose of the dopamine D_2 receptor agonist quinpirole acting mainly at autoreceptor sites, and higher levels of the dopamine metabolite DOPAC relative to wild-type mice. Dopamine turnover disturbances are also found after acute challenge with haloperidol, a dopamine D_2 receptor antagonist. These alterations are associated with increased tyrosine hydroxylase expression and activity, and reduced catechol-O-methyltransferase expression.*

Conclusion: *Taken together, these results are consistent with the involvement of* Nur77 *in dopamine neuron biochemical activity and dopamine turnover.*

Key Words: NGFI-B, antipsychotic drug, tyrosine hydroxylase (TH), catechol-O-methyltransferase (COMT), dopamine D_2 autoreceptor, dopamine metabolism, *Nurr1*

Transcription factors represent a vast family of genes that encode regulatory factors, which modulate the expression of target genes. They play an important role during central nervous system (CNS) development and actively participate in adaptive responses after changes in the environment of neuronal cells, such as after ischemia, lesion, or denervation and after exposure to drugs that affect neurotransmitter systems in a mature CNS (Evans 2004; Gronemeyer et al 2004; Olson et al 1998). Recent evidence suggests that orphan receptor members of the nuclear receptor family of transcription factors, namely the *Nur* family (*Nurr1*, *Nur77* and *Nor*-1), are closely associated with dopamine (DA) neurotransmission. For example, in the absence of *Nurr1* (NR4A2), DA midbrain precursors adopt normal localization and neuronal phenotype, but fail to differentiate into DA neuron phenotype, as demonstrated by the lack of tyrosine hydroxylase (TH) expression (Zetterström et al 1997). *Nurr1* can activate the transcription of DA biosynthetic enzymes, such as TH and L-aromatic amino-acid decarboxylase (AADC) in cultured cell lines (Hermanson et al 2003; Iwawaki et al 2000; Sakurada et al 1999). In addition, *Nurr1* can modulate the expression of the DA transporter (DAT) and vesicular monoamine transporter (VMAT) (Hermanson et al 2003; Sacchetti et al 2001). Responsive elements sensitive to *Nurr1* present in those gene promoters can also represent putative targets for other *Nur* members (Maira et al 1999; Perlmann and Jansson 1995). However, *Nur77* (NR4A1) and *Nor*-1 (NR4A3) messenger RNA (mRNA) levels are extremely low in the substantia nigra (SN) and ventral tegmental area (VTA) in basal conditions (Zetterström et al 1996). On the other hand, *Nur77* and *Nor*-1 are highly expressed in target areas of DA neurons, such as the striatal complex and prefrontal cortex (Beaudry et al 2000; Werme et al 2000a; Zetterström et al 1996). In these areas, strong modulation of *Nur77* and *Nor*-1 has been observed after manipulation of DA neurotransmission with DA receptor antagonists (neuroleptics) or psychostimulants, or after DA denervation (Beaudry et al 2000; Ethier et al 2004a; St-Hilaire et al 2003a; 2003b; Werme et al 2000a; 2000b). In addition, we have shown that *Nur77*-deficient mice had an altered response after treatment with antipsychotic drugs (Ethier et al 2004a; 2004b). The cataleptic response after conventional antipsychotic drug administration, such as haloperidol, was strongly attenuated, while orofacial dyskinesias (vacuous chewing movements), which developed after prolonged treatment, were exacerbated (Ethier et al 2004a; 2004b). *Nur77* is selectively induced by antipsychotic drug treatment in striatal enkephalin-containing cells (bearing D_2 receptors) (Beaudry et al 2000). Thus, alteration of these behavioral responses after haloperidol suggests an involvement of *Nur77* in intracellular signaling events associated with the blockade of the DA D_2 receptor in a medium spiny (postsynaptic to DA cells) cell population of the striatum (Ethier et al 2004a). Interestingly, we recently observed that *Nur77* could be strongly induced in the SN/VTA complex after acute treatment with numerous antipsychotic drugs (Maheux et al 2005). This observation suggests that *Nur77* may influence DA neuron biochemical activity under specific circumstances. Taken together, these observations indicate that *Nur77* is involved in biochemical and behavioral effects of antipsychotic drugs.

To further investigate the role of *Nur77* in DA neurotransmission, we conducted a series of experiments aimed at exploring the role of *Nur77* in DA turnover and DA neuron biochemical activity. In the present study, we report that genetic ablation of *Nur77* alters multiple components related to DA neuron activity, including DA metabolites, TH and *Nurr1* levels, and TH activity, as well as catechol-O-methyltransferase (COMT) expression and autoreceptor-related locomotor behavior. In addition, basal and haloperidol-induced DA turnover are strongly altered in *Nur77*-deficient mice, suggesting a role of *Nur77* in DA clearance.

From the Neuroscience Unit (FG, MS-H, BP, CR, DL) and the Molecular Endocrinology and Oncology Unit (MM, TDP), CRCHUL and Faculty of Medicine, Laval University, Ste-Foy, Quebec, Canada.

Address all reprint requests to Dr. Daniel Lévesque, Faculty of Pharmacy, University of Montreal, C.P. 6128, succursale Centre-Ville, Montreal, Qc, H3C 3J7 Canada; E-mail: daniel.levesque.1@umontreal.ca.

Received March 1, 2006; revised April 25, 2006; accepted April 26, 2006.

0006-3223/06/$32.00
doi:10.1016/j.biopsych.2006.04.023

© 2006 Society of Biological Psychiatry

Methods and Materials

Animals and Treatments

All procedures, including means to minimize discomfort, were reviewed and approved by the Laval University Animal Care Committee. Male wild-type C57BL/6 mice (*Nur77* +/+) were purchased from Charles River Laboratories, St-Constant, Quebec, Canada. *Nur77* knockout (*Nur77* −/−) mice were developed and graciously provided by Dr. Jeff Milbrandt from the University of Washington in St. Louis, Missouri (Lee et al 1995). These mice were healthy and reproduced normally. They were produced in a mixed background and have been backcrossed into the C57BL/6 strain for at least 10 generations to reduce genetic background heterogeneity (Jeff Milbrandt, personal communication, 2002). We maintain a *Nur77* −/− mouse colony at the animal care facility of our research center. Young adults *Nur77* +/+ and *Nur77* −/− male mice weighing 20–25 g were used for the present experiments. A group of C57BL/6 mice were purchased from Harlan (Teckland, Indianapolis, Indiana) to evaluate the contribution of the difference in the genetic background of the C57BL/6 mouse strain from Charles River, Canada, and the C57BL/6 mouse strain that has been used to backcross *Nur77* −/− mice.

Two series of *Nur77* +/+ and −/− mice were used for locomotor evaluation in basal condition and after saline or quinpirole administration. Two other groups of *Nur77* +/+ and *Nur77* −/− mice were treated with vehicle (saline) or haloperidol (.5 mg/kg intraperitoneal [IP]). One group of both strains was sacrificed 1 hour after haloperidol challenge to assess *Nur77* and *Nurr1* mRNA levels. The other groups were sacrificed 5 hours after haloperidol administration in order to evaluate the levels of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and serotonin (5-HT) and its metabolite 5-hydroxyindolacetic acid (5-HIAA), as well as TH mRNA, catechol-O-methyltransferase (COMT) mRNA and DAT-binding capacity.

For catecholamine measurements, brains were rapidly removed from the skull and placed onto a mouse brain stainless steel matrix (Plastic One Inc., Roanoke, Virginia) and sliced at three levels to isolate the frontal cortex (Bregma 2.70 mm), striatal complex (Bregma −1.20 mm) and substantia nigra/ventral tegmental area (SN/VTA) regions (from Bregma −4.50 to −6.20 mm). Note that the frontal region section mainly includes the prefrontal cortex. Then, regions of interest were dissected out from these brain pieces and immediately homogenized in .1 N perchloric acid ($HClO_4$) and frozen at −80°C until assayed.

For in situ hybridization and autoradiographic techniques, brains were rapidly removed from the skull and immediately frozen in dry ice–cooled isopentane (−40°C) and stored at −80°C until used. For immunohistochemistry, mice were deeply anesthetized with a ketamine/xylazine solution (80 and 10 mg/kg, respectively, IP) and perfused intracardially with 150 mL of saline solution (.9% NaCl), followed by 250 mL of 4% paraformaldhehyde (PFA) in .1 mol/L borax buffer (pH 9.5 at 4°C). After perfusion, the brains were removed and postfixed 90 min in 4% PFA. After postfixation, the brains were cryoprotected by an overnight immersion in 20% sucrose diluted in the fixative solution, and then frozen and stored until further processing. Frozen brains were sectioned at 30-μm with the use of a sliding microtome (Leica Microsystem SM200R, Richmond Hill, Ontario, Canada) in the coronal plane. Sections were collected in cold cryoprotectant solution and stored at −20°C.

Detection of DA and Its Metabolites

The concentration of DA and its metabolites, DOPAC and HVA, were evaluated by high-performance liquid chromatography (HPLC) with electrochemical detection according to previously published procedures with slight modifications (Di Paolo et al 1986; Morissette and Di Paolo 1996). Striatum extracts were homogenized at 0 to 4°C in 250 μL of .1 N $HClO_4$ and centrifuged at 10,000*g* for 10 min to precipitate proteins; SN/VTA and frontal cortex extracts were homogenized in 100 μL of .1 N $HClO_4$. The pellets were dissolved in 100 μL of .1 mol/L NaOH for the determination of protein content (Lowry et al 1951). The supernatant of striatal, SN/VTA, and frontal cortex tissues, as well as standards, were injected into a HPLC system consisting of an autosampler automatic injector (Waters 717+, Waters Ltd, Mississauga, Ontario, Canada), a pump (Waters 515) equipped with a C-18 column (Waters, Nova-Pak C_{18}, 3 μm, 3.9 × 150 mm), an electrochemical detector (LC-4C, BASi, West Lafayette, Indiana), and a glassy carbon electrode. The mobile phase consisted of .025 mol/L citric acid, 1.7 mmol/L 1-heptane-sulfonic acid, and 10% methanol, in filtered distilled water, delivered at a flow rate of .8 mL/min. The final pH of 3.85 was obtained with the addition of NaOH. The electrochemical potential was set at .8 V with respect to an Ag/AgCl reference electrode. All HPLC reagents were of analytical grade.

L-DOPA Measurement

Levels of L-DOPA were measured by HPLC with electrochemical detection. Supernatants of striatal, frontal cortex, or SN/VTA tissue were directly injected into the HPLC system, as described for DA and 5-HT measurements. The mobile phase consisted of 50 mmol/L KH_2PO_4, .2 mmol/L 1-heptane-sulfonic acid, .1 mmol/L EDTA, and 10% methanol, in filtered distilled water, delivered at a flow rate of .8 mL/min. The final pH of 2.70–2.80 was obtained by addition of phosphoric acid. The electrochemical potential was set at .8 V with respect to an Ag/AgCl reference electrode.

In Situ Hybridization Procedures

Cryostat coronal brain sections (12 μm) were mounted onto Snowcoat X-tra slides (Surgipath; Winnipeg, Manitoba, Canada) and stored at −80°C until used. Brain sections were fixed in 4% PFA at 4°C for 20 min. For single in situ hybridization, specific [^{35}S]UTP-radiolabeled complementary RNA (cRNA) probes were used. The production and synthesis of *Nur77, Nurr1,* and TH probes were previously described in detail (Beaudry et al 2000; Cossette et al 2004; Ethier et al 2004a). The cRNA probe for COMT was generated from a 565-bp fragment (nucleotides 155–720) from the mouse sequence (Gogos et al 1998), subcloned into PCRII-TOPO plasmid, and linearized with *Apa*I. In situ hybridization of the riboprobes with tissue sections were done at 55°C –58°C, overnight, in a standard hybridization buffer containing 50% formamide (Beaudry et al 2000; Ethier et al 2004a). Tissue sections were then apposed against BiomaxMR (Kodak; New Haven, Connecticut) radioactive sensitive films for 1–5 days. Quantification of autoradiograms was performed by means of computerized analysis (ImageJ 1.32i software, Wayne Rasband, National Institutes of Health, Bethesda, Maryland). Optical gray densities were transformed into nCi/g or μCi/g of tissue equivalent by using standard curves generated with ^{14}C-microscales (ARC 146A-^{14}C standards; American Radiolabeled Chemicals Inc., St. Louis, Missouri). Average levels of labelling for each area were calculated from four adjacent brain sections of the same animals. Background intensities were subtracted from every measurement.

The double in situ hybridization procedure was performed as

Download English Version:

https://daneshyari.com/en/article/4179972

Download Persian Version:

https://daneshyari.com/article/4179972

Daneshyari.com