Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Letters 375 (2005) 107–111
Neuroscience Letters
www.elsevier.com/locate/neulet

Tumor necrosis factor-alpha receptor ablation in a chronic MPTP mouse model of Parkinson's disease

Andreas Leng, Anna Mura, Joram Feldon, Boris Ferger*

Behavioural Neurobiology Laboratory, Swiss Federal Institute of Technology Zurich, CH-8603 Schwerzenbach, Switzerland

Received 2 July 2004; received in revised form 7 August 2004; accepted 27 October 2004

Abstract

Recently, we demonstrated that mice deficient of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-alpha) were partly protected against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity. Here we extended the study and investigated TNF-alpha receptor 1 (−/−) (TNFR1) and TNF-alpha receptor 2 (−/−) (TNFR2) mice using a chronic MPTP dosing regimen (15 mg/kg MPTP on 8 consecutive days). One week after the last MPTP treatment, HPLC determination of striatal dopamine (DA) and immunostaining for the dopamine transporter (DAT) in the substantia nigra pars compacta (SNpc) was performed.

MPTP treatment reduced striatal DA levels significantly; nigral DAT immunoreactivity was reduced to a lower extent. However, there was no difference in DA levels and the number of DAT positive neurons between TNFR1 (−/−), TNFR2 (−/−) and wild type mice after MPTP treatment.

In contrast to TNF-alpha deficiency neither TNFR1 nor TNFR2 gene ablation showed protection against MPTP neurotoxicity, which argues for a protective mechanism of TNF-alpha not mediated by TNFR1 and TNFR2 signaling.

Keywords: TNF-alpha; MPTP; Inflammation; Parkinson's disease; Cytokine; Tumor necrosis factor

Parkinson's disease (PD) is a slowly progressing movement disorder characterized by a loss of dopaminergic cells in the substantia nigra pars compacta (SNpc) and a massive reduction in striatal dopamine (DA). The impact of inflammatory processes in PD [9] is supported by the observation that the number of activated microglia [14] and level of pro-inflammatory cytokines are elevated in PD patients [2,15,16]. These findings are also supported by animal models of PD. Activated microglia and elevated cytokine levels are present in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model [13,12] and in the 6-hydroxydopamine model of PD [17]. Among the pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-alpha) is thought to play a prominent role in the pathological process of PD. TNF-alpha can induce microglia activation [19]. Neurons in the SNpc express both TNF-alpha receptors 1 and 2 [2]. Elevated levels of TNF-alpha receptors were found in PD patients [16], and TNF-alpha polymorphism has been observed in patients with sporadic PD [11,18]. Under pathological conditions TNF-alpha is mainly expressed by astroglial and microglial cells [3]. Interestingly, TNF-alpha is involved in both neurodegenerative and neuroprotective pathways [8]. The two subtypes of TNF-alpha receptors (TNFR), TNF-alpha receptor 1 (TNFR1) and TNF-alpha receptor 2 (TNFR2), and their distinct signaling pathways might be responsible for this ambivalent function [23]. The TNFR1 includes an intracellular death domain and is involved in apoptotic cell death [7]. The TNFR2 has no death domain and is considered to activate anti-apoptotic pathways [23]. However, TNFR2 can also enhance the apoptotic action of TNFR1, which can be involved in anti-apoptotic pathways (for review see [7,8,19]). In a previous study, we

* Corresponding author. Department CNS Research, Boehringer Ingelheim Pharma GmbH and Co. KG, Birkendorfer Str. 65, 88397 Biberach an der Riss, Germany. Tel.: +49 7351 54 94 820; fax: +49 7351 54 92 451.
E-mail address: boris.ferger@bc.boehringer-ingelheim.com (B. Ferger).

0304-3940/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2004.10.077

demonstrated that TNF-alpha (−/−) mice and the TNF-alpha synthesis inhibitor thalidomide attenuated MPTP toxicity [4]. Additionally, others [20,21] and we [4] found elevated TNF-alpha mRNA levels after MPTP treatment. Consequently, we were interested whether TNFR1 or TNFR2 signaling is involved in the protective effect observed in TNF-alpha (−/−) mice against MPTP toxicity. Meanwhile two other groups performed MPTP studies using TNFR1 or TNFR2 mice [20,21] showing a protective [21] or no protective effect [20] against MPTP toxicity by genetic ablation of TNFR1 and TNFR2. Both studies used acute MPTP treatment schedules whereas in the present study a chronic MPTP dosing schedule was applied. The chronic MPTP model was selected for the present study, because a protective effect indicated by striatal DA and DAT measurements was obtained in a previous experiment using TNF-alpha (−/−) mice only after chronic MPTP treatment but not after acute MPTP administration [4].

The present study was conducted in male C57bl/6 mice and in homozygous mice deficient in the TNFR1 or TNFR2 gene. All mice were about 10–12 weeks old at the beginning of the experiment. TNFR1 (−/−) and TNFR2 (−/−) mice (Jackson Laboratory, Bar Harbor, ME) were bred at the Research Unit Schwerzenbach, Switzerland, and maintained under standard conditions (temperature, 21 ± 1.0 °C; humidity, 55 ± 5%) on a 12 h light/12 h dark cycle (lights on at 7 a.m.) with free access to standard food (Nafag 9431, Nafag Ecossan, Gossau, Switzerland) and water ad libitum. All animal studies were carried out in accordance with the European Convention for Animal Care and Use of Laboratory Animals and were approved by the appropriate institutional governmental agency (Kantonales Veterinäramt Zürich, Switzerland).

TNFR1 (−/−) and TNFR2 (−/−) mice, which have been backcrossed over 10 generations to a C57bl/6 background, were genotyped using polymerase chain reaction (PCR) amplification from genomic DNA obtained from a tail biopsy. The presence of TNFRs was tested with the following oligonucleotide primer: for TNFR1: 5′-TGT GAA AAG GGC ACC TTT ACG GC-3′ and 5′-GGC TGC AGT CCA CGC ACT GG-3′, and for TNFR2: 5′-CCT CTC ATG CTG TCC CGG AAT-3′ and 5′-AGC TCC AGG CAC AAG GGC GGG-3′ (Microsynth, Balgach, Switzerland). The PCR mix consisted of 0.2 mM dNTP, 1 mM $MgCl_2$, 1 μM primer and 0.5 units Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany) in PCR buffer. One microliter of DNA extract (approximately 50 ng DNA) was used for the following PCR reaction carried out in a thermocycler (Perkin-Elmer PE 9600, Perkin-Elmer Switzerland AG, Hünenberg, Switzerland): After an initial denaturating step at 95 °C for 5 min, the cycle consisted of denaturating for 30 s at 95 °C, annealing for 30 s at 55 °C, and extending for 30 s at 72 °C. The cycle was repeated 35 times. The final extension step was done at 72 °C for 5 min. PCR products (470 bp fragment for the wild type TNF-alpha R1 allele and 200 bp fragment for the TNF-alpha R2 allele) were separated on ethidium bromide-stained 2% agarose gel using TAE buffer (40 mM Tris–acetate; 1 mM EDTA; pH 8.0) at 80 V and visualized by ultraviolet light.

MPTP (15 mg/kg, i.p., calculated as free base) was injected daily on 8 consecutive days as a chronic treatment. Seven days after the last MPTP or saline administration, the mice were sacrificed by cervical dislocation. The brains were rapidly removed and placed on an ice-cooled plate for dissection of the striatum. Immediately after dissection, the striata were weighed and placed in 1.5 ml plastic tubes containing ice-cooled perchloric acid (500 μl, 0.4 M), homogenized for 10 s using ultrasound and centrifuged for 20 min at 15000 × *g* and 4 °C. The supernatant was passed through a 0.2 μm filter and kept at 4 °C until HPLC analysis. DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were analyzed using reversed-phase ion-pair chromatography combined with electrochemical detection under isocratic conditions [22]. The detector potential was set at +750 mV using a glassy carbon electrode and an Ag/AgCl reference electrode. The mobile phase (0.6 mM 1-octanesulfonic acid, 0.27 mM Na_2EDTA, 0.043 M triethylamine and 50 ml acetonitrile/l, adjusted to pH 2.95 with H_3PO_4) was delivered at a flow rate of 0.5 ml/min at 22 °C onto the reversed phase column (125 mm × 3 mm with pre-column 5 mm × 3 mm, filled with Nucleosil 120-3 C18, Knauer, Berlin, Germany). Ten microliters of aliquots were injected by an autosampler with a cooling module set at 4 °C. Data were calculated by an external standard calibration.

The midbrain including the substantia nigra pars compacta (SNpc) was post-fixed for 2–3 days in cold fixative with 4% paraformaldehyde in 0.01 M phosphate-buffered saline (PBS, pH 7.4), then transferred to a 30% sucrose solution and kept at 4 °C until it sunk. Subsequently, the tissue was cut with a freezing microtome, and coronal sections (25 μm thick) were collected throughout the rostro-caudal extent of the SNpc and stored in a cryoprotectant solution. As a specific marker for dopaminergic cells the dopamine transporter (DAT) was used to estimate the extension of the MPTP lesions in the SNpc. Sections were processed using the standard peroxidase-antiperoxidase method. After 3 × 5 min rinses in PBS, the free floating sections were blocked for 1 h in PBS containing 5% normal goat serum plus 0.3% Triton X-100. The sections were then incubated in a solution of PBS and 2% normal goat serum plus 0.15% Triton X-100 containing the primary antibody rat anti-DAT (1:2000, Chemicon, Lucerne, Switzerland) for 2 days at 4 °C. Following this, the sections were rinsed and incubated for 1 h in biotinylated secondary antibodies (goat anti-rat, 1:300, Jackson Immuno Research, West Grove, USA) in a solution of 2% goat serum plus 0.15% Triton X-100 at room temperature. Subsequently, the sections were treated with avidin-biotin-horseradish peroxidase complex (Vectastain Elite, Vector Laboratories, Burlingame, CA) for 1 h at room temperature, followed by 3 min × 5 min rinses in 0.1 M Tris buffer (TB, pH 7.4). Immunoreactivity was visualized with 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma, Buchs, Switzerland) and 0.004% H_2O_2 in TB for 3–5 min. Nickel chloride (0.08%) was added