Neuroscience 162 (2009) 1072–1080

LONG TERM EXPOSURE TO THE CHEMOKINE CCL2 ACTIVATES THE NIGROSTRIATAL DOPAMINE SYSTEM: A NOVEL MECHANISM FOR THE CONTROL OF DOPAMINE RELEASE

A. GUYON,[b2*] D. SKRZYDELSKI,[a2] I. DE GIRY,[b] C. ROVÈRE,[b] G. CONDUCTIER,[b] J. M. TROCELLO,[a] V. DAUGÉ,[c] P. KITABGI,[a] W. ROSTÈNE,[a] J. L. NAHON[b3] AND S. MÉLIK PARSADANIANTZ[a1,3]

[a]*Institut National de la Santé et de la Recherche Médicale U 732, Université Pierre et Marie Curie-Paris 06, Hôpital Saint-Antoine, 184 Rue du Faubourg Saint-Antoine, 75571 Paris Cedex 12, France*

[b]*Institut de Pharmacologie Moléculaire et Cellulaire, CNRS UNSA, UMR, 6097 CNRS, 660 Route des Lucioles, Sophia Antipolis, 06560, Valbonne, France*

[c]*Institut National de la Santé et de la Recherche Médicale U 513, Université Pierre et Marie Curie-Paris 06, 9 Quai Saint Bernard, 75005 Paris, France*

Abstract—Accumulating evidence show that chemokines can modulate the activity of neurons through various mechanisms. Recently, we demonstrated that CCR2, the main receptor for the chemokine CCL2, is constitutively expressed in dopamine neurons in the rat substantia nigra. Here we show that unilateral intranigral injections of CCL2 (50 ng) in freely moving rats increase extracellular concentrations of dopamine and its metabolites and decrease dopamine content in the ipsilateral dorsal striatum. Furthermore, these CCL2 injections are responsible for an increase in locomotor activity resulting in contralateral circling behavior. Using patch-clamp recordings of dopaminergic neurons in slices of the rat substantia nigra, we observed that a prolonged exposure (>8 min) to 10 nM CCL2 significantly increases the membrane resistance of dopaminergic neurons by closure of background channels mainly selective to potassium ions. This leads to an enhancement of dopaminergic neuron discharge in pacemaker or burst mode necessary for dopamine release. We provide here the first evidence that application of CCL2 on dopaminergic neurons increases their excitability, dopamine release and related locomotor activity.

Key words: CCL2, substantia nigra, dopamine neuron, microdialysis, patch clamp, locomotor activity.

Chemokines constitute a family of small protein ligands signaling through G-protein-coupled receptors which regulate diverse functions in the immune system where they are mainly involved in leukocyte trafficking and activation. In addition, chemokines and their receptors are expressed by all major cell types in the CNS, i.e. neurons, astrocytes, oligodendrocytes, microglia (Banisadr et al., 2005c; Cartier et al., 2005; Biber et al., 2006; Minami et al., 2006) and play important roles in brain development, in several neuropathologies and in neuromodulation (Callewaere et al., 2007; Rostene et al., 2007).

Indeed, there is accumulating evidence that several chemokines (including SDF1/CXCL12, Rantes/CCR5, Fractalkine/CX3CL1) can modulate the electrical activity of different populations of neurons such as hippocampal, cortical, cerebellar, dorsal root ganglion neurons, hypothalamic and dopaminergic (DA) neurons of the substantia nigra (SN). Chemokines operate through various mechanisms like an increase in calcium transients or modulations of presynaptic release of neurotransmitter, of voltage-dependent channels, and of ionotropic and metabotropic receptors (Limatola et al., 2000; Bajetto et al., 2002; Oh et al., 2003; Gosselin et al., 2005; Guyon and Nahon, 2007; Rostene et al., 2007; Kolodziej et al., 2008). Furthermore, chemokines can modulate neuropeptide or neurotransmitter release from neurons as exemplified by recent *in vivo* and *in vitro* studies showing that CXCL12, via its receptor CXCR4, could inhibit vasopressin release from magnocellular hypothalamic neurons and stimulate DA release from nigral neurons (Callewaere et al., 2006; Skrzydelski et al., 2007). In the latter case, evidence was provided that CXCL12 could modulate the electrical activity of rat SN DA neurons both presynaptically through the release of glutamate and GABA onto DA neurons (Guyon et al., 2006) and directly through an increase in the excitability of DA neurons by potentiating high voltage activated calcium (HVA-Ca) currents (Guyon et al., 2008).

We previously reported that another chemokine, CCL2 and its receptor CCR2 are also both constitutively expressed in DA neurons in the SN (Banisadr et al., 2002, 2005a,b,c), suggesting that this ligand/receptor pair might influence the activity of DA neurons.

In the present study, we show that CCL2 injected into the rat SN induces a robust extracellular DA release in the ipsilateral striatum and that this activation of the nigrostriatal pathway leads to an increase in locomotor activity. Using *in vitro* patch clamp experiments, we show that brief CCL2 applications (<3 min) are not sufficient to alter DA neuron activity, while prolonged CCL2 applications (>8

[1] Present address: Centre de recherche de l'Institut Cerveau Moelle (Cr ICM), UMR S 975 INSERM-UMR 7225 CNRS-UPMC, Équipe Douleurs, Faculté de Médecine Pitié Salpêtrière, 91 Bvd Hôpital, 75013 Paris.

[2] These authors contributed equally to the work.

[3] Co-senior authors.

*Corresponding author. Tel: +33-49-39-57-741; fax: +33-04-93-95-77-08.
E-mail address: alice.guyon@ipmc.cnrs.fr (A. Guyon).

Abbreviations: aCSF, artificial cerebrospinal fluid; APV, D-2-amino-5-phosphonovalerate; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DA, dopamine, dopaminergic; DOPAC, 3,4-dihydroxyphenylacetic acid; EDTA, ethylenediamine-tetraacetic acid; HPLC, high pressure liquid chromatography; HVA, homovanillic acid; HVA-Ca, high voltage activated Ca; I_A, inactivating A type potassium current; I_h, hyperpolarization-activated cation current; I_T, low threshold Ca current; MPTP, 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine; PBBS, phosphate bicarbonate buffer saline; PD, Parkinson disease; Rm, input resistance; SN, substantia nigra; TTX, tetrodotoxin.

0306-4522/09 $ - see front matter
doi:10.1016/j.neuroscience.2009.05.048

min) are able to raise DA neuron excitability by increasing their membrane resistance. Our data lend further support to the notion that chemokines represent a new class of neuromodulators, in particular of nigral DA neurons and might represent new targets for the treatment of Parkinson's disease.

EXPERIMENTAL PROCEDURES

Animals

Wistar male rats were bred in the local animal facilities and maintained on a 12-h dark/light cycle (7 AM/7 PM) with food and water *ad libitum*. All the protocols were carried out in accordance with French standard ethical guidelines for laboratory animals (Agreement No. 75-178, 05/16/2000). All efforts were made to minimize the number of animal used and their suffering.

Cannula guide implantation

Wistar male rats (60 days old) were anesthetized by an i.p. injection of chloral hydrate (400 mg/kg) and implanted with stainless steel cannula guide (ref C313, Plastics One, Roanoke, VA, USA) 1 mm above the SN as previously described (Skrzydelski et al., 2007). The stereotaxic coordinates were anteriority −5.6 mm, laterality +2.5 mm from bregma, depth −6 mm from the skull surface (Paxinos and Watson, 1998). Then the guide cannula was fixed to the skull with dental cement and stainless steel screw. Ten days after implantation of the guide cannula, unilateral intranigral injections were made using stainless steel needles (ref C313 I, Plastics One) connected to a 10 μl microsyringe (Hamilton, Bonaduz, Switzerland) by polyethylene tubes. Animals were placed in a cage and were able to move freely during the injection.

Drug administration

CCL2 (50 ng) or vehicle (artificial cerebrospinal fluid, aCSF, Harvard Apparatus, Holliston, MA, USA) were administered as previously described (Skrzydelski et al., 2007) in a volume of 2.5 μl by an infusion pump (Harvard Apparatus) at a constant flow rate (1 μl/min) for 2.5 min. The needle was then left for 1 min after the end of the injection to allow the diffusion of the liquid.

DA and its metabolite tissue contents

One hour after the intranigral infusion of aCSF or CCL2, rats were killed by decapitation and their brains rapidly removed. For each rat, ipsi- and contralateral striata were separately dissected out. The tissues were homogenized in 200 μl of perchloric acid 0.1 mol/l and centrifuged at 10,000×*g* for 30 min at 4 °C to precipitate proteins. The supernatants were collected and stored at −80 °C until analyzed by high pressure liquid chromatography (HPLC) for DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) contents (see below for the neurochemical analysis). The pellets were used for the protein determination with the Bio-Rad D_C Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA).

Microdialysis experiments

Surgery. Wistar adult male rats were anesthetized as previously described (Skrzydelski et al., 2007) and placed in stereotaxic frame. Two guide cannulas were unilaterally implanted: one for the microdialysis probe (ref CMA/10CMA/microdialysis, North Chelmsford, MA, USA) in the dorsal striatum and one for the mesencephalic injection directly above SN. The following stereotaxic coordinates (Paxinos and Watson, 1998) were used for the dorsal striatum: anteriority +0.5 mm, laterality +3 mm from bregma, depth −5 mm from the skull surface, and for the SN: anteriority −5.6 mm, laterality +2.5 mm from bregma, depth −6 mm. The microdialysis experiment was conducted 8–10 days after guide cannula implantation.

Experimental procedure

The evening before the experiment, the animals were transferred to the experimental cage and allowed to habituate. The next morning, the microdialysis probe (ref CMA/11CMA/microdialysis) was inserted through the guide cannula into the striatum. From this time, the freely moving rat was continuously perfused with a dialysis buffer containing (in mmol/l) NaCl 140, KCl 4, $CaCl_2$ 1.2, $MgCl_2$ 1, NaH_2PO_4 0.1, and Na_2HPO_4 1.9, pH 7.4, at a rate of 2.5 μl/min by means of a syringe pump (Harvard Apparatus). Two hours after the probe implantation, baseline dialysate was collected at 20-min intervals for 120 min. After baseline stabilization, aCSF or CCL2 (50 ng) was injected unilaterally in the SN and sample collection continued for the remaining 2 h. Dialysate samples were collected in vials containing 6 μl of perchloric acid (0.1 mol/l) and L-cysteine (0.33 mol/l) solution. Immediately after the collection, fractions were stored at −80 °C until they were analyzed by HPLC.

Neurochemical analysis

The levels of DA and its metabolites (DOPAC and HVA) were determined by HPLC coupled with coulometric detection as previously described in Skrzydelski (2007). Briefly, the HPLC system consisted in a Waters model 515 pump, a refrigerated automatic injector (Waters 717 plus autosampler; Guyancourt, France), and a reverse phase column (C_{18}, 150×4.6 mm internal diameter, particle size 5 μm) (Merck, Lyon, France), controlled at 30 °C. The mobile phase was constituted by sodium acetate buffer (90 mmol/l) containing 130 μmol/l EDTA, 230 μmol/l 1-octanesulfonic acid, 6% methanol, pH 3, delivered at a constant flow of 1.2 ml/min. A coulometric detector (Coulochem II, ESA, Chelmsford, MA, USA) with a 5014 B high performance analytical cell was used. A model 5020 guard cell (ESA) was positioned before the column to oxidize at +450 mV. The first electrode reduced at −300 mV and the second electrode oxidized at +300 mV to quantify DA and DOPAC. For HVA, the potentials of reduction and oxidation were 175 and +175 mV, respectively. Levels of DA, DOPAC, and HVA were calculated from the quantitative comparisons made with external standards of DA, DOPAC, and HVA that were run each day. The detection limits were 1.5 pg for DA and DOPAC and 50 pg for HVA.

Circling behavioral test

To minimize the possible circadian changes in rat behavior, circling motor activity was monitored between 09:00 h and 12:00 h and conducted in a special room dimly illuminated. Tested rats were isolated from the rest of the animals and from outer noises. To analyze circling behavior, the animals were placed in a cylindrical plastic container (27 cm diameter) during 10 min and the direction and number of 360° revolutions were recorded by a video camera apparatus (Videotrack Apparatus, Viewpoint S.A., Champagne au Mont d'Or, France). Before each test, litter was changed to obviate possible biasing effects because of odor clues left by previous rats. Before the intranigral injection, rats were previously habituated to the novel environment during 5 min. Immediately after this habituation period, rat received unilaterally an intranigral injection of aCSF or CCL2 (50 ng) (see above, for intranigral injection procedure). Immediately after the injection, the number of turns was automatically analyzed for 10 min.

Whole-cell patch-clamp recordings

Whole-cell patch-clamp recording were carried out as previously described (Guyon et al., 2006). Briefly, rat SN slices were placed