

Research report

Increased extracellular DA and normal evoked DA release in the rat striatum after a partial lesion of the substantia nigra

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Abstract

After injection of 6-hydroxydopamine into the lateral part of the rat substantia nigra, tissue dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were reduced in the corresponding lateral part of the ipsilateral caudate/putamen (CP) complex (13, 40 and 56% of controls, respectively). In this region, tyrosine hydroxylase (TH, the rate limiting enzyme of the DA synthesis) immunautoradiography decreased by more than 80% as was the case for the binding of tritiated GBR12935 (a specific marker of the DA-carrier protein). In the medial region of the CP, only very moderate reductions of DA, DOPAC and HVA (77, 76 and 84% of controls, respectively) were observed. In this region, TH immunautoradiography and GBR12935 binding were only reduced by about 20% reflecting weak DA denervation. However, using *in vivo* voltammetry, extracellular basal DA levels were found to be particularly high in the medial region of CP complex when compared to unoperated animals (up to 235%). In the medial region, TH activity was also significantly increased (161%) but the electrical stimulation of DA fibers produced the same DA overflow in control and lesioned animals. From these results, it may be concluded that elevated basal DA levels in this region cannot be attributed to the reduced DA uptake and/or to an increased ability of DA neurons to release DA in response to impulse flow. © 2001 Elsevier Science B.V. All rights reserved.

Theme: Disorders of the nervous system

Topic: Degenerative disease: Parkinson's

Keywords: Dopamine uptake; Dopamine release; Substantia nigra lesion; Parkinson's disease

1. Introduction

Using animal models of striatal dopaminergic denervation, several authors have reported that even if as little as

about 10–20% dopamine (DA) neurons are preserved in the substantia nigra pars compacta (SNc), then the dopaminergic function remains almost unmodified [9,33,53]. Various compensatory mechanisms have been evoked to explain the apparent conservation of DA function, including modified activity patterns in the spared dopaminergic neurons [25], increased DA release in response to impulse flow [43,44] or reduced DA uptake in the caudate/putamen (CP) complex [15,18,24,32,54]. These mechanisms are generally considered to be a direct consequence of the loss of cell-bodies and/or of end-terminals, and to be induced by lateral disinhibition [55]. The rationale of the present experiments lays in a possible enhancement in efficacy of the spared DA neurons to releasing DA. To consider such an hypothesis, an experimentally-induced lesion model was chosen which allows to compare, in the same animal, a largely denervated area with a weakly affected region of the CP. After a 6-hydroxydopamine (6-OHDA)-induced

Abbreviations: 6-OHDA, 6-hydroxydopamine; BSA, bovine serum albumin; CP, caudate/putamen; DA, dopamine; DAT_{bind}, dopamine-transporter binding; DNPV, differential normal pulse voltammetry; DOPA, dihydroxy-phenylalanine; DOPAC, dihydroxy-phenylacetic-acid; DPA, differential pulse amperometry; HVA, homovanillic-acid; LCED, liquid chromatography with electrochemical detection; MAO-A, mono-aminooxidase, A type; MFB, medial forebrain bundle; PBS, saline phosphate buffer; PBS-G-T, saline phosphate buffer with triton and gelatin; SNc, substantia nigra, pars compacta; TH_{im}, tyrosine-hydroxylase immunautoradiography; TH, tyrosine hydroxylase

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lesion restricted to the lateral part of the substantia nigra pars compacta (SNc) only the lateral part of the ipsilateral CP is greatly affected, the remaining medial region being only slightly altered [7,8,13]. Four weeks after the toxin injection, a precise study of the lesion was performed by counting tyrosine hydroxylase (TH) immunopositive cells in the SNc, and by evaluating the surface area and extent of the lesion in CP using both quantitative immunohistochemical detection of TH (TH_{im}) and quantitative autoradiographic detection of DA uptake sites (DAT_{bind}). DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and the activity of TH enzyme were measured in both regions (lateral and medial) of the CP. To further characterize dynamic alterations of DA neuron function, basal and stimulation-evoked DA overflow were measured in the two regions using *in vivo* voltammetry.

2. Materials and methods

All chemicals (DA, DOPAC, DOPA, HVA, 6-OHDA, pargyline, epinine, α -methyl-DOPA, GBR-12909, NSD-1015) were obtained from Sigma–Aldrich (France) and were of the highest purity available.

2.1. Animals

The experiments conducted were in accordance with the rules of the French Animal Ethical Committee and the European Communities Council Directive (86/609/EEC). Male Sprague–Dawley rats (Iffa-Credo, France) weighing 220–250 g were housed under standard conditions (24°C and 50% humidity) with a 12-h light, 12-h dark cycle and with free access to food and water.

2.2. Experimental groups

Two groups of rats were used, controls and lesioned. Each group was then divided into five subgroups: the first for TH immunohistochemical of the SNc (four controls, five lesioned); the second for quantitative autoradiographic studies (TH_{im} , DAT_{bind}); the third for measurement of extracellular DA concentration (eight controls, 13 lesioned); the fourth for measurement of tissue DA, DOPAC and HVA measurements (nine controls, seven lesioned); the fifth for TH activity measurement (seven controls, nine lesioned). Measurements were performed 1 month after the lesion, a delay which corresponds to the maximal extent of the lesion [12,13].

2.3. Lesion procedure

Rats were anaesthetized with chloral hydrate (400 mg/kg) and placed in a David Kopf stereotaxic device. The SNc was unilaterally injected with 1 μ l of an isotonic saline 6-OHDA hydrochloride solution (3 μ g free base/ μ l)

in 0.2% ascorbic acid (pH 4). The following coordinates were used (mm): A: 3.0, L: 2.4 and V: –2.0, according to Ref. [28].

2.4. Control of the lesions in the CP and the SNc

Rats were sacrificed, the brains were removed, frozen in isopentane at –30°C and stored at –80°C. Two consecutive 10- μ m sections were cryostat-cut and collected every 100 μ m from the caudal to the rostral extremities of the CP and SNc. The first set of sections was used for GBR autoradiography and the second for TH-immunohistochemistry.

2.5. GBR-autoradiography

DA uptake sites were labelled with [3 H]GBR 12935 (New England Nuclear, UK) as previously described [36]. Briefly, tissue sections were incubated at 4°C for 20 h in 50 mM Tris–HCl, pH 7.5, containing 450 mM NaCl, 0.02% bovine serum albumin (BSA), 1 μ M *cis*-flupentixol and 2 nM of [3 H]GBR 12935 (30 Ci/mmol). Consecutive sections were incubated with [3 H]GBR 12935 in the presence of 50 μ M mazindol in order to determine non-specific binding. Sections were then rapidly dipped in ice-cold distilled water and quickly dried under a stream of cold air. Autoradiograms were obtained by apposing the labelled tissue sections on tritium-sensitive film ([3 H]Hyperfilm, Amersham) at 4°C for 3 weeks. Calibrated tritiated microscales (Amersham) were simultaneously exposed with the tissue sections.

2.6. TH-immunohistochemistry

Adjacent sections to those used for DAT_{bind} were processed for TH_{im} as previously described [6,7]. Briefly, after saturation of non-specific antibody binding sites by incubating the tissue sections for 30 min in phosphate-buffered saline (PBS) solution (50 mM, pH 7.4) containing 1% BSA, the sections were incubated at room temperature in the presence of the primary TH antibody (dilution 1:1000; Boehringer Mannheim, France) for 2 h in the same buffer. Tissue sections were then washed three times with PBS/BSA for 10 min and transferred to a solution of PBS containing 35 S-labeled anti-mouse immunoglobulin (dilution 1:100, Amersham, UK, specific activity 310 Ci/mmol) for 1 h. Finally, the sections were rinsed in PBS three times for 10 min, dried rapidly under a stream of air, and put on hyperfilm β max (Amersham) at room temperature for 1 week.

Optical densities of autoradiograms were quantified using an automatic image analyzer (SAMBA 2005; Alcatel T.I.T.N, France) and the topology was reconstructed using the previously described method [5]. For the CP denervation as well as the SNc, only the three sections on which

the surface area of the lesion was maximum were considered.

2.7. Further control of the lesion in the SNc

Animals were anesthetized with fluothane (3%) and perfused transcardially with 15 ml of PBS (0.1 M; pH 7.4), followed by 650 ml of paraformaldehyde (4%) prepared in PBS (0.1 M; pH 7.4). The brains were removed, immersed in the same fixative for 2 h, transferred to 15% sucrose (in 0.1 M PBS) overnight at 4°C, frozen by immersion in isopentane at -30°C and stored at -80°C. Cryostat-cut coronal sections (20 µm) were collected throughout the caudal and rostral extremities of the SNc [39]. For each brain, two consecutive sections were collected every 220 µm. The first section was used for TH immunohistochemical labelling. Sections were incubated for 1 h in PBS (0.1 M, pH 7.4) containing 0.2% gelatin and 0.3% Triton X-100 (PBS-G-T). They were then sequentially incubated for 18 h in PBS-G-T containing monoclonal anti-TH antibody (Incstar) diluted 1:2000; for 1 h in PBS-G-T containing horse anti-mouse immunoglobulin-G antibody (Vector; UK) diluted at 1:500; for 2 h in PBS-G-T containing avidin-biotin-peroxidase complex (Vector, UK) diluted 1:500. The enzymatic activity of this complex was then revealed by 3,3'-diaminobenzidine (DAB, Sigma Fast). The sections were finally dehydrated in graded ethanols and methylcyclohexane, and coverslipped. TH positive (TH⁺) neurons were then counted at the six anatomical levels previously selected where SNc presented its optimal development (between interaural line +2.96 mm and +3.8 mm; [39]). The total number of TH⁺ neurons in SNc was calculated over the six anatomical levels using a previously described correction method [1]. TH⁺ cells were counted in the lateral lesioned region defined by a 80% reduction in TH_{im} and DAT_{bind}, and in the medial region, which is not affected by the lesion in terms of DAT_{bind} TH_{im} density. The second sections were Nissl-stained for histological reference.

2.8. Tissue DA, DOPAC and HVA determination

The brains were rapidly removed after decapitation, frozen by immersion in isopentane at -30°C and stored at -80°C. Coronal sections (500 µm thick) were prepared at -30°C. The slice on which the anterior commissure could be seen was selected. Two samples were punched with a truncated needle (1.3 mm outer diameter) from the lateral and the medial regions of the CP, following the inferior limit of the corpus callosum. The punches were collected in 75 µl of 0.1 N perchloric acid and 12.5 µl EDTA 0.8 mM. After sonication (30 s) and centrifugation (10 000×g, 10 min), 20 µl of the supernatant were mixed with an internal standard (epinine) and injected into a C18 reverse-phase microcolumn (Spheri5, RP-18, 220×2.1 mm, Brow-

nee Labs). The mobile phase consisted of 40 mM KH₂PO₄ (pH 4.5), 0.26 mM octane sulphonic-acid, 15 mg/l EDTA and 11% methanol (vol/vol), and the flow rate was 0.20 ml/min. The liquid chromatography system (TSP, France), was coupled with an electrochemical detector (Waters 460, Millipore) (LCED) with a working electrode set at 0.8 V. Proteins were evaluated using the micro BCA Protein Assay (Pierce, Biorad, France). For each region, concentrations of DA and metabolites in CP were expressed as a percentage of values obtained in control.

2.9. TH activity

The rate of DA synthesis in CP was determined by measuring DOPA accumulation with LCED after in vivo inhibition of DOPA decarboxylase by 3-hydroxybenzylhydrazine, 2 HCl (NSD-1015). Thirty minutes after the i.p. injection of 100 mg/kg NSD 1015, rats were decapitated, their brains rapidly removed and the tissue samples were prepared as described in the previous section. Before LCED, the samples were loaded on alumina microcolumns (50 mg) for purification. The columns were then washed twice with 150 µl of 0.1 M Tris buffer solution and the catecholamines were eluted with 150 µl perchloric acid (0.1 M). The out flow was analyzed by LCED using a C18 reverse-phase column (Spheri5 ODS1 C18, 4.6×250 mm, Waters). The mobile phase consisted of 100 mM KH₂PO₄ (pH 3), 0.4 mM octane sulphonic acid, 0.1 mM EDTA, with a flow rate of 2 ml/min. TH enzyme activity was expressed with reference to the amount of tissue protein in the sample.

2.10. Voltammetry

Carbon fiber electrodes made and electrochemically treated as previously described [19,20] were used for Differential Normal Pulse Voltammetry (DNPV) and Differential Pulse Amperometry (DPA). The electrodes measured 250 µm in length and 8 µm in diameter. They were connected to a Biopulse (Radiometer, France) and calibrated using a standard solution of DA prepared in PBS, in the presence of ascorbic acid (100 µM). The values of the oxidation potentials (versus an Ag/AgCl reference electrode) were, respectively, -60 mV for ascorbic acid and +90 mV for DA. Because the oxidation potentials for DA and DOPAC are too close to be correctly differentiated, extracellular DA was measured by inhibiting the DOPAC production with 75 mg/kg pargyline (i.p.) an inhibitor of monoamine oxidase known to increase the basal extracellular DA concentration. In the present report, both lesioned and control rats were treated with pargyline to avoid any bias of the result whatever the technique used (DNPV or DPA).

2.11. Extracellular DA in CP

Rats were anaesthetized with 1% fluothane (Halothane, Belamont) in pure O₂ and maintained in a David Kopf stereotaxic device. Electrodes were implanted in the CP in a vertical plane situated 8.2 mm anterior to the interaural axis, the horizontal plane being defined by the interaural axis and the incisor bar. The measurements were alternatively performed either at 2 or at 4 mm from the lateral position. Five depth levels (−3, −3.5, −4, −4.5, −5 in mm under the cortical surface) were explored and the values obtained at each of these levels have been averaged. A given electrode was used for only one lateral measurement and then changed for each additional measurement. For each vertical movement of the electrode, a stabilization period of about 10 min (four scans) was allowed before measurement.

2.12. DA fiber stimulations

A bipolar stimulating electrode was implanted in the medial forebrain bundle (MFB) where DA fibers pass. The electrode was located 1.3 mm lateral to the interhemispheric plane and 4.0 mm anterior to the interaural axis. The depth (about 8.2 mm under the cortical surface) was adjusted for each experiment so that the response was maximal in the ipsilateral CP. Electrical stimulation consisted of a train (500 ms) of square pulses (40 Hz, 0.5 ms, 300 μ A) repeated every second for a duration of 20 s; This stimulation was repeated every 6 min. The response was observed using DPA in the ipsilateral CP at 4 mm in depth and characterized by both the amplitude of DA overflow and the time taken to return to the prestimulated state.

2.13. Statistics

The statistical significance of the differences between means obtained from treated and control groups was analyzed using an unpaired two-tailed Student's *t*-test.

3. Results

3.1. Nigral lesions

An important loss in the number of TH⁺ neurons (72±5%) was observed in the lesioned lateral SNc. In this area, the densities of DAT_{bind} and TH_{im} were reduced to below 20% of that observed in controls. The surface area of the reduced densities of DAT_{bind} and TH_{im} correspond to 39±2 and 40±3%, respectively, of the total nigral surface area appearing on the same coronal plane (Fig. 1). In the medial region, which is not affected by the lesion in terms of DAT_{bind} and TH_{im} densities, a neuronal loss of only 10±7% was measured.

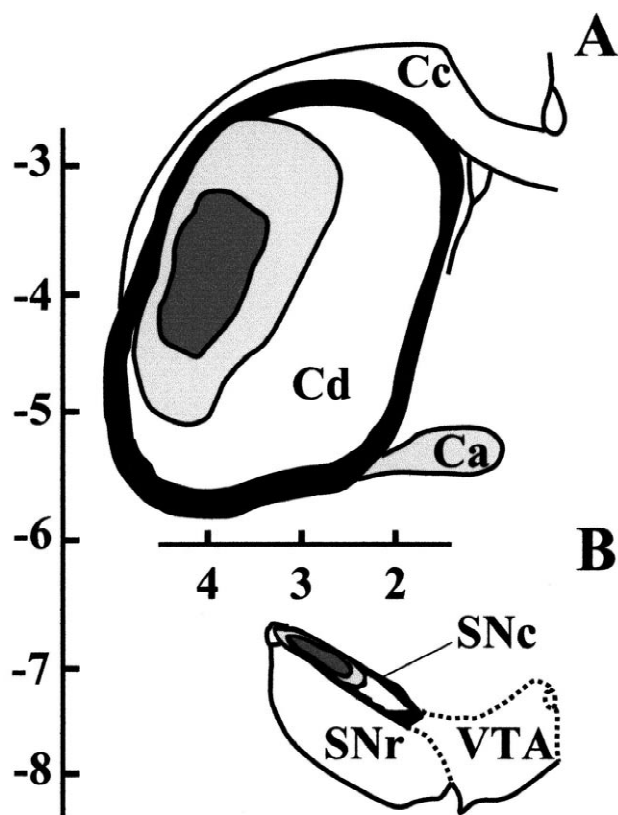


Fig. 1. Schematic representation of the striatal denervation (A) and nigral lesion (B). The deep gray areas correspond to the mean denervated regions. They are surrounded by a light gray area which corresponds to the inter-individual standard deviation (S.D.). The thick external limit (black) of SN and CP represents the S.D. of the mean area of each structure. Depth is given in mm and in reference to the cortical surface (from −3 to −8). Laterality from the interhemispheric plane is given in mm. Cc, corpus callosum; Cd, caudate nucleus; Ca, anterior commissure; VTA, ventral tegmental area.

3.2. Lateral region of CP

The surface area of CP denervation, evaluated by both DAT_{bind} and TH_{im}, was found to be 35±2 and 31±2% of the total CP surface area, respectively (Fig. 1). The optical densities for DAT_{bind} and TH_{im} decreased to 14 and 20% of the controls, respectively. The values of DA, DOPAC and HVA concentrations were greatly reduced (13±2, 40±4, and 56±4% of control values, respectively, Fig. 2). TH activity of the enzyme was not significantly modified (Fig. 3).

In control animals, when the absolute values from the different depths were averaged, the extracellular concentrations of DA and DOPAC were 7.3±0.6 nM and 8.3±0.7 μ M, respectively. In lesioned rats (Fig. 4), the extracellular DA was slightly increased (135±3% of controls), while DOPAC was reduced (26±6% of controls).

During an electrical stimulation of MFB, the extracellular DA concentration increased to 90 nM in control animals and the return back to the basal level took about

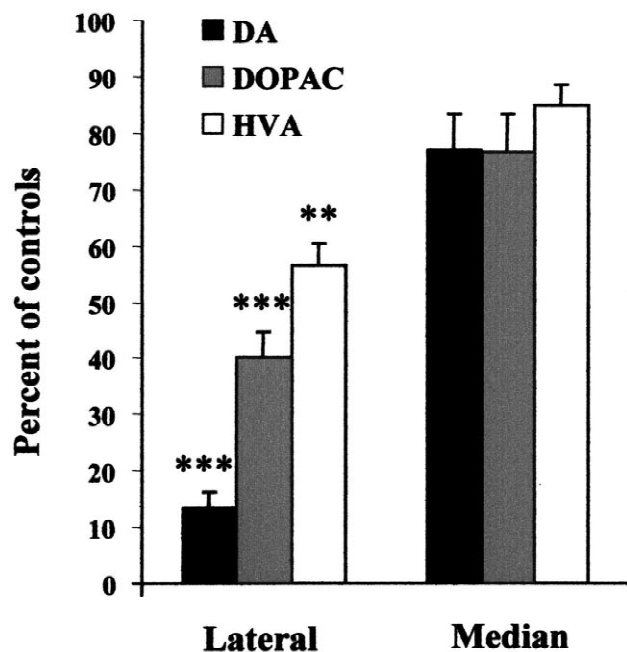


Fig. 2. Effects of partial unilateral nigrostriatal lesion on tissue DA and metabolites in CP. Striatal tissue dopamine and metabolites were assayed on tissue samples, taken at the level of the anterior commissura in median (lat 2–3) and lateral (lat 3–4) regions. Biochemical analysis were performed by LCED coupled with electrochemical detection. Results from lesioned animals ($n=7$) are expressed as a percentage of values found in the control group ($n=9$) for the same regions. Statistical analysis used the Student's *t*-test. * $P<0.1$; ** $P<0.01$; *** $P<0.001$.

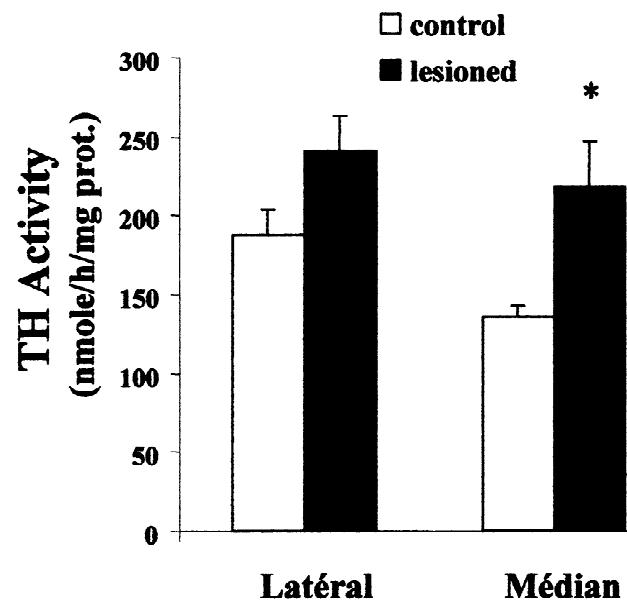


Fig. 3. Effects of partial nigral lesion on TH activity in CP. Each bar represents the mean (\pm S.E.M.) TH activity (nmol/h per mg prot.), in the lateral and median regions. The rate of striatal DA synthesis was determined by measuring DOPA accumulation using LCED after inhibition of Dopa decarboxylase with NSD 1015. Statistical analysis used the Student's *t*-test. * $P<0.05$.

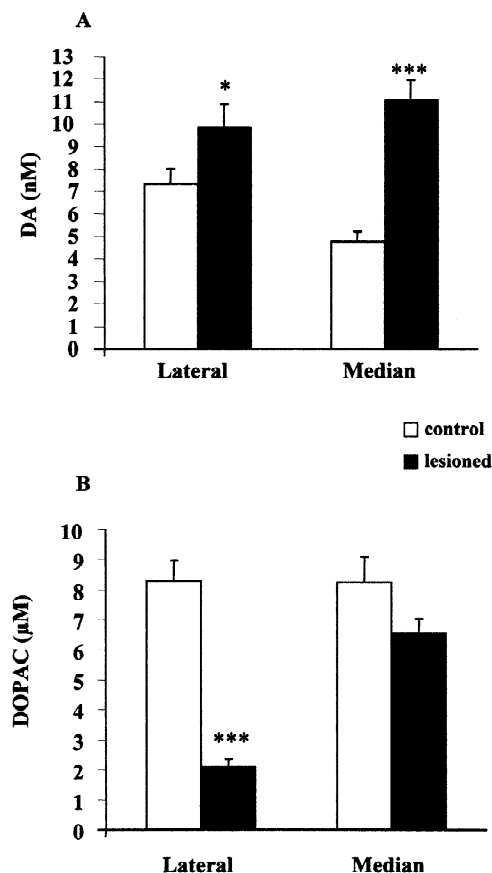


Fig. 4. Effects of partial unilateral nigral lesion on extracellular concentrations of DA and DOPAC, in CP. The extracellular concentrations in both CP of the lesioned group (DA, $n=13$; DOPAC, $n=18$) are expressed in nM or μ M after in vitro calibration of electrodes and compared with controls (DA, $n=8$; DOPAC, $n=8$).

80 s. In lesioned animals, no significant differences were observed in the amplitude of the response (Fig. 5) or the time for DA clearance (data not shown).

3.3. Medial region of CP

In the medial CP, DAT_{bind} and TH_{im} were slightly reduced (76 and 83% of controls, respectively). Tissue DA, DOPAC and HVA levels were also slightly decreased (77 ± 6 , 76 ± 7 and $85\pm3\%$ of controls, respectively, Fig. 2). In contrast, TH activity was increased ($161\pm21\%$; Fig. 3). When the absolute values from the different depths were averaged, extracellular concentrations of DA and DOPAC in control rats in the medial region were 4.7 ± 0.5 nM and 8.2 ± 0.9 μ M, respectively. In lesioned animals, extracellular DA was notably increased ($235\pm36\%$ of control values) and DOPAC slightly decreased ($76\pm4\%$).

In control rats, electrical stimulation of MFB enhanced the extracellular DA concentration up to 95 nM, followed by a return back to the basal level in about 80 s. In the lesioned rats, the amplitude of increase was only 80% of controls (n.s., Fig. 5) and the time needed to clear the

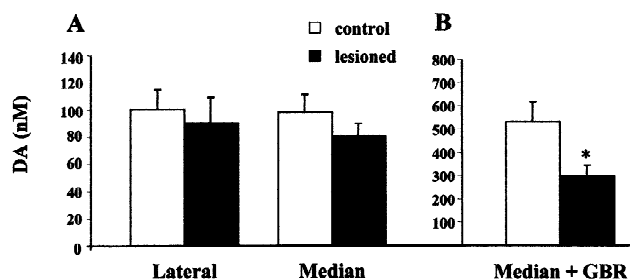


Fig. 5. (A) Effects of partial unilateral nigral lesion on electrically-evoked DA overflow in CP. Electrical stimulation of MFB consisted of a train (500 ms) of square pulses (40 Hz, 0.5 ms, 300 μ A.) repeated every second during 20 s. During the stimulation, evoked striatal DA release was detected with DPA in median and lateral regions of the ipsilateral CP. Homologous regions were explored in the control group. Results are expressed in nM, after in vitro, pre-calibration of carbon fiber micro-electrodes. Statistical analysis used the Student's *t*-test and no significant differences were found between lesioned ($n=8$) and control ($n=12$) animals. (B) Effects of GBR 12909 (20 mg/kg, i.p.) on the electrically-evoked DA overflow in the median region. The difference in the evoked DA signal amplitude between lesioned ($n=8$) and control animals ($n=12$) was analysed using the Student's *t*-test. * $P<0.05$.

excess amine from the extracellular space was unchanged (data not shown).

In the medial region of CP, the electrically-evoked DA overflow was increased after i.p. injection of 20 mg/kg GBR12909 ($544\pm 84\%$ of controls). In the lesioned rats, this increase was only $370\pm 59\%$ (Fig. 5).

4. Discussion

The most striking observation in our study is the change of DA metabolism in a region where denervation probably does not exceed 10–20%. It was also observed that extracellular DA increases at the terminal level of the spared DA neurons after partial lesion of the dopaminergic bundle. It is finally suggested that a shift occurs in the mechanism of the DA release rather than an enhancement of the normal mode of release.

Consistent with the laterally restricted nigral lesion, the denervated region in the CP was situated laterally and corresponded well with the nigral lesion. A drastic reduction of DA stores, DOPAC and HVA in the lateral CP attested to a severe DA denervation, and this was confirmed by the reduction of DAT_{bind} and TH_{im} to less than 20% of controls. The unchanged TH activity measured in spite of the important DA denervation, the moderate increase in extracellular DA (135% of controls) and the important reduction of the DA storage (13% of controls) indicated a large increase in DA turn-over in this region in agreement with several reports [2,3,22,46]. The reduction of extracellular DOPAC (26% of controls) was moderate and similar to the reduction of tissue DOPAC (40% of controls) as there is no dynamic mechanism releasing DOPAC.

In the medial region of the CP, the reductions of DAT_{bind} and TH_{im} (76 and 83% of controls, respectively) were in line with the 10% cell loss in the medial SNc. The reduction of specific DA-metabolism markers (DA, DOPAC and HVA) suggests that the amplitude of the DA denervation could be about 10–20% in our experimental conditions. Extracellular DA was however increased in this region when measured with voltammetry. The maintenance of the extracellular DA in the CP of partially lesioned rats has sometimes been observed [40]. This was confirmed by a maintained 3-methoxytyramine content (product of the extracellular catabolism of DA) unless the denervation exceeds 80% [4]. Contralateral increases have also been reported [41,52].

What could be the nature of the mechanism of release tonically enhanced, particularly in view of the fact that the denervation in this region did not exceed 20%? The enhanced DA release is unlikely to be due to a tonic increase of the electrical activity of the DA neurons. In fact, a denervation more than 80% is needed to modify the firing activity of DA neurons after nigrostriatal partial lesion [25]. The enhanced DA release could also be the result of an enhanced amount of amine released per pulse. This does not seem to be the case for the following reasons: (1) In the presence of GBR12909, stimulated DA overflow was reduced when compared to the same stimulation in controls. This effect of GBR12909 has already been reported by others [24,47]. In the multipulse stimulation used here, the amplitude of the amine overflow is directly related to the difference between the amine released during the pulse and the amine uptaken during the interpulse [45]. Since it can be expected that 25 mg/kg GBR12909 completely saturates uptake sites in both the lesioned and control rats, this result suggests a reduction of the DA release per pulse in the lesioned animals as has been already proposed [17]; (2) in the absence of GBR12909, the amplitude of the amine released by electrical stimulation remained unchanged. Thus, in lesioned animals, uptake of the extracellular amine should be reduced as often proposed [15,18,24,32,54] but only compensating the reduced release. Extracellular DA could be responsible for the reduced release per pulse through an action on the preterminal D2 receptors [35,49]. Thus the firing evoked DA release is unlikely to be responsible for the sustained increase of the extracellular DA observed after weak DA denervation.

The enhanced DA release is correlated with modifications of DA synthesis. Several authors have already emphasized that in 6-OHDA lesioned rats, DA release is more affected by inhibition of DA synthesis than in controls [21,34,44]. This is in line with a reduced amine storage in the DA terminals and increased TH activity [23,43,44].

The present report shows that DOPAC efflux is reduced after lesion. Since DOPAC is considered as originating from the cytoplasmic DA [11,29,42,51], this suggests that

neosynthesized cytoplasmic molecules are more involved in DA release than amine storage and catabolism.

The use of pargyline could have produced a bias in the extracellular DA measurements. Pargyline, blocking the cytoplasmic MAO-A enzyme, could have increased the cytoplasmic DA concentration. Since the control animals were treated in the same way the results confirm that the increase in tonic DA release is related to increased basal synthesis. This could enhance a mechanism of release involving cytoplasmic DA but remaining independent of the firing of the DA cells. The reverse transport of DA by the DA uptake carrier might be proposed to mediate this effect [31]. Indeed, its high sensitivity to extracellular glutamate has been reported [27,48] and glutamatergic neurotransmission seems to be activated in the striatum after partial lesion [26,50]. It was recently reported that daily treatment with glutamate receptor antagonists is able to block neurochemical compensation of partial 6-OHDA lesion [16]. Also, local application of NMDA or kainate in the CP, increases levels of extracellular DA simultaneously with a reduced ability of DA terminals to release DA in response to MFB stimulation [38]. Finally glutamate has been observed to enhance TH enzyme activity in various models [10,14,30]. It can thus be hypothesized that, after partial and chronic lesions of the SNc, a tonic glutamatergic control might increase DA synthesis and, through the activation of the reverse transport of the amine, enhance extracellular DA.

In conclusion, this study underlines the presence of profound alterations of DA metabolism and DA release after slight DA denervation. Furthermore, it suggests that the lesion may induce separate mechanisms in CP, maintaining both high extracellular DA and normal DA transmission at synapses.

Acknowledgements

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