Effects of Dopamine Denervation on Striatal Peptide Expression in Parkinsonian Monkeys

Brigitte Lavoie, André Parent and Paul J. Bédard

ABSTRACT: In cynomolgus monkeys rendered parkinsonian by systemic injection of MPTP, severe cell losses were noted in the ventrolateral portion of the substantia nigra pars compacta (SNc), compared to a relative sparing of neurons in the ventral tegmental area (VTA) and dorsomedial portion of SNc. Most spared neurons in the SNc-VTA complex were found to contain the calcium binding protein calbindin (CaBP). At striatal levels the dopaminergic (DA) innervation, as visualized by tyrosine hydroxylase immunoreactivity, was markedly reduced in the 'sensorimotor' territory, variably affected in the 'associative' territory, and relatively well preserved in the 'limbic' territory. The immunoreactivity for enkephalin was enhanced and that for substance P was decreased in the sensorimotor territory, whereas the inverse was observed in the limbic territory. The distribution of the two peptides was highly heterogeneous in the associative territory. These findings suggest that the influence of the DA input on peptide expression varies from one striatal territory to the other, and that CaBP may protect midbrain DA neurons from MPTP toxicity.

RÉSUMÉ: Influence dopaminergique sur l'expression des neuropeptides striataux. Des données immunohistochimiques obtenues chez des macaques devenus parkinsoniens suite à l'injection de MPTP ont démontré que la dénervation dopaminergique (DA) striatale était très marquée dans le territoire sensorimoteur, variable dans le territoire associatif, et relativement faible dans le territoire limbique. L'étude de la présence de substance P et d'enképhaline dans les différents territoires striataux a révélé que l'innervation DA avait un effet différent sur l'expression de ces peptides suivant les territoires concernés. De plus nos données suggèrent que la présence de calbindine, une protéine liant le calcium, pourrait protéger les neurones DA du mésencéphale de l'effet toxique du MPTP.

Can. J. Neurol. Sci. 1991; 18: 373-375

The dopaminergic (DA) nigrostriatal pathway is one of the major chemospecific neuronal system involved in Parkinson's disease (PD).[1] Recently, the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been shown to induce a destruction of the nigrostriatal DA pathway and consequently to produce a parkinsonian syndrome in humans[2] as well as in non-human primates.[3-5] Thus, MPTP-injected monkeys have become a widely-used model for studying the pathophysiology of PD. The present immunohistochemical investigation was designed to investigate the effect of MPTP on the DA innervation of the basal ganglia in non-human primates, with a particular attention to the expression of some peptides in the different striatal territories.

Methods

Ten female cynomolgus monkeys (*Macaca fascicularis*) (body weight range 3.6-4.3 kg) were used in this study. Two monkeys were not injected and kept as a control, whereas the other animals received MPTP intravenously in doses of 0.4 to 0.6 mg/kg (total dose range 4-8 mg). These animals became severely bradykinetic and were sacrificed between 1 and 12 months after initial exposure. Their brains were processed according to standard immunohistochemical methods[6,7] for the demonstration of tyrosine hydroxylase (TH-IR), met-enkephalin (ENK-IR), substance P (SP-IR) and the calcium binding protein calbindin (CaBP) on contiguous section. Furthermore, TH and CaBP were visualized on the same sections using double-immunostaining procedures. The TH, ENK and SP antibodies were purchased from Immunonuclear Corp. (Stillwater, MN), whereas the CaBP antibody was generously donated by Dr. M.R. Celio. The number of cells in the substantia nigra pars compacta-ventral tegmental area (SNc-VTA) complex was estimated on 1 out of 4 sections taken throughout the rostrocaudal extent of the midbrain of each animal. The counting was made on both Nissl-stained and TH-immunoreacted sections and the Abercrombie's formula[8] was used to correct for split cell error.

Results

In all MPTP-injected monkeys there was marked cell loss in SNc-VTA complex and the degree of cell loss corresponded to the severity of the striatal denervation. The most prominent loss occurred in SNc, which showed decreases ranging from 80% to over 90% from normal values. Intact nigral TH-IR cells were mostly confined to the dorsal tier of SNc. Cell losses were less severe in VTA ranging approximately from 25 to 40%. Studies

From the Centre de recherche en neurobiologie, Université Laval et Hôpital de l'Enfant-Jésus, Québec
Reprint requests to: André Parent, Ph.D., Centre de recherche en neurobiologie, Hôpital de l'Enfant-Jésus, 1401, 18e Rue, Québec, Québec, Canada G1J 1Z4

on alternate sections stained for TH and CaBP revealed that the distribution of the remaining TH-immunoreactive neurons was strikingly similar to that of the CaBP-positive cells (Figure 1A, B). Furthermore, double-immunostaining procedures applied to single sections showed that the majority of the spared TH-immunoreactive neurons in SNc-VTA complex displayed CaBP-IR (Figure 1C).

In the striatum of PD monkeys the 'sensorimotor' territory (SM), characterized by its inputs from somatosensory and motor cortices which arborize principally in caudal two-thirds of the putamen,[9] was almost devoid of TH-IR fibers and terminals except for a few smooth and linear fibers scattered throughout structure. By comparison the 'associative' territory (AS), as defined by its afferents from associative areas of prefrontal, temporal, parietal and cingulate cortices which terminate mainly in the caudate nucleus and rostral putamen, was less affected. In the 'limbic' territory (LI), comprising nucleus accumbens and part of olfactory tubercle, which are afferented by paralimbic cortices, amygdala and hippocampus, the TH-IR was only slightly reduced by comparison to that in normal monkeys. Also worth noting was the relative sparing of the TH-IR fibers arborizing in the internal segment of the pallidum in all MPTP-injected monkeys. This confirms our previous results suggesting that the nigropallidal DA projection, which arises from a distinct cell

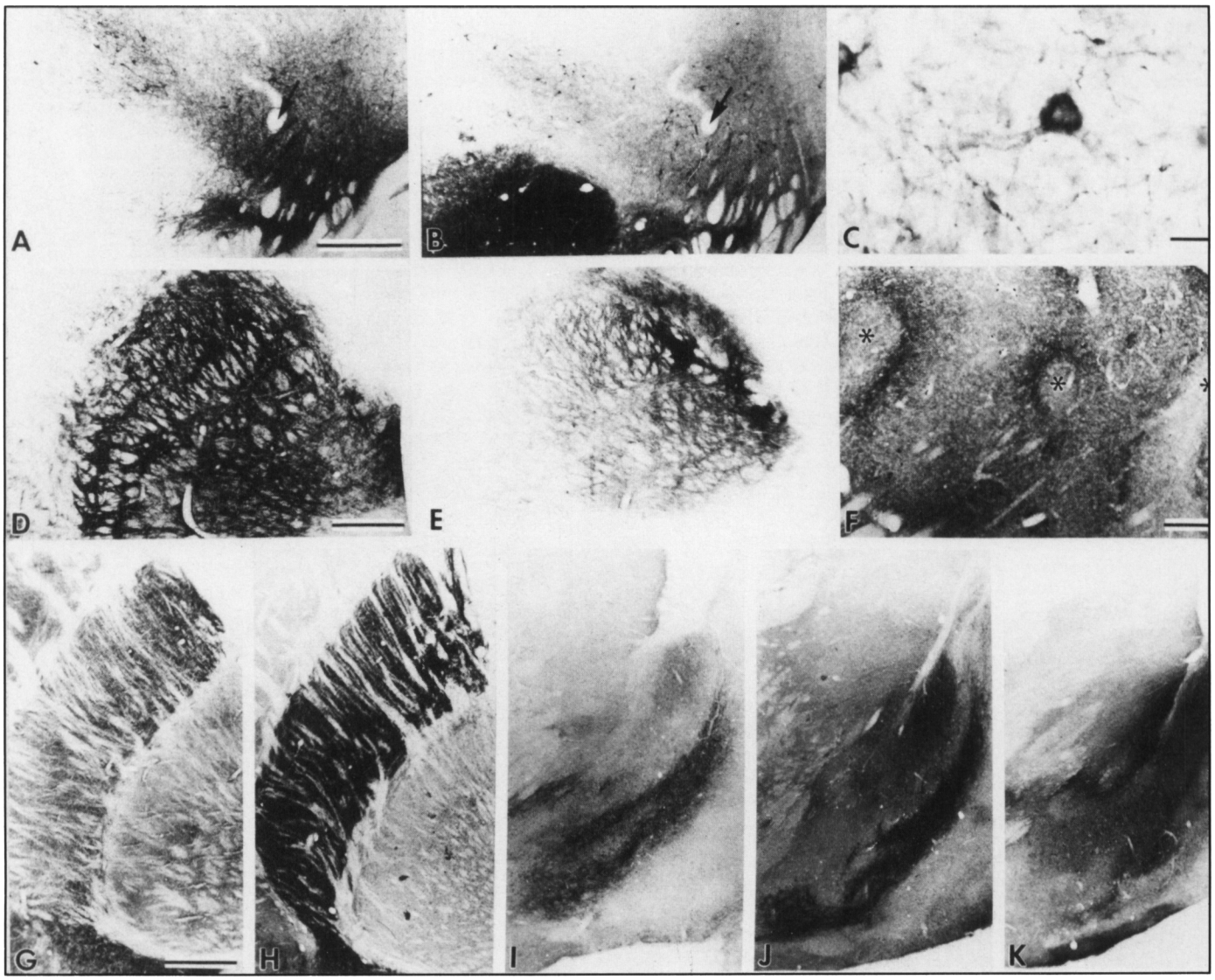

Figure 1 — A, B: Adjacent sections through the midbrain of a PD monkey comparing the distribution of TH-(A) and CaBP-(B) immunoreactive neurons spared by MPTP. In both cases the spared neurons abound in the VTA (lower right) and dorsal tier of SNc (upper left). The arrows point to a blood vessel visible in the two sections. C: Example of a neuron in the dorsal tier of SNc that stained for both TH and CaBP. The presence of CaBP-immunoreactivity is revealed by dark staining granules in the cytoplasm, whereas TH-immunoreactivity occurs in the form of a diffuse and uniform brown staining throughout the cytoplasm and proximal dendrites. D, E: Comparison of patterns of SP fiber immunostaining in the GPi of a normal (D) and a PD monkey (E). F: Examples of 'rimosomes' as seen in the associative striatal territory of PD monkeys. These structures are zones of strong SP neuropil staining that rims the striosomes (asterisks) which are here composed of weakly stained SP cells. G, H: Comparison of patterns of ENK fiber immunostaining in the GPe of a normal (G) and a PD monkey (H). I, J: Comparison of the SP immunostaining seen in the area of nucleus accumbens in normal (I) and PD monkey (H). K: Distribution of TH immunoreactivity in the area of nucleus accumbens in a PD monkey. Scale bars are 0.5 mm in A (also valid for B), 30 μm in C, 1 mm in D (also valid for E), 0.5 mm in F, and 1 mm in G (also valid for H-K).

https://doi.org/10.1017/S0317167100032467 Published online by Cambridge University Press

population in the SNc-VTA complex, is more resistant to MPTP than the nigrostriatal projection terminating in the associative and sensorimotor territories.[10]

In comparison to normal animals, SP-IR was markedly decreased in striatal SM neurons projecting to the internal segment of the pallidum (GPi) in PD monkeys. The SP-IR reduction was particularly obvious in ventral two-thirds of GPi where fibers of SM neurons arborize, whereas SP-IR remained relatively unaffected in the dorsal third of GPi where most fibers of AS neurons are confined (Figure 1 D, E). In contrast, ENK-IR was significantly increased in striatal cells located in SM as well as in their fibers arborizing in the ventral two-thirds of the external segment of the pallidum (GPe) (Figure 1 G, H). In AS itself the intensity and distribution of ENK and SP-IR were highly variable and heterogeneous. Cell patches displaying high ENK-IR or high SP-IR were noted, whereas in some cases patches of weak SP-IR cells corresponding to striosomes, as visualized on adjacent sections stained for CaBP, were rimmed by a dense SP-IR neuropil ('rimosomes') (Figure 1 F). At variance with findings in SM, ENK-IR was decreased and SP-IR increased in LI neurons projecting to the ventral pallidum (Figure 1 I-K).

Discussion

It has recently been shown that, regardless of their location in the midbrain, the DA neurons that are more vulnerable to MPTP are those projecting to the dorsocaudal region of the striatum, whereas the neurons innervating the rostromedial portion of the striatum, including the nucleus accumbens, are much less affected.[5] Indeed, the most severe cell losses after MPTP were found in the ventrolateral portion of SNc, where most of the neurons projecting caudolaterally in the striatum are located, whereas cell losses were much less prominent in the dorsomedial portion of SNc and in VTA, which contains the majority of the neurons projecting rostroventrally in the striatum.[5] The results of the present study are largely in agreement with these findings. Our data further reveal that the DA projection to the basal ganglia in primates in fact consists of several subsystems whose vulnerability to MPTP varies significantly. For instance the DA system arising in the ventrolateral part of SNc and terminating in SM appears highly vulnerable to MPTP, whereas the DA system originating from the VTA and arborizing in LI seems more resistant to the neurotoxin. Likewise the nigropallidal projection remains relatively unaffected by MPTP, while the degree of impairment of the nigrostriatal projection terminating in AS greatly varies from one animal to the other. These differences in vulnerability could be due to a variation in pathological disturbances occurring in the major striatal terminal territories. This would then determine which subpopulations of midbrain DA neurons will eventually be destroyed through retrograde cell degeneration. They may also reflect some unique, intrinsic, biochemical properties of the various DA subpopulations in the midbrain. In agreement with the latter view is the fact that DA neurons that were selectively spared in our PD monkeys displayed CaBP immunoreactivity. Similarly, a relative sparing of CaBP-positive cells compared with CaBP-negative, pigmented neurons was recently noted in the SNc-VTA complex in cases of idiopathic PD.[11] These findings suggest that, through its capacity of binding large quantities of calcium ions, CaBP may protect DA neurons from MPTP toxicity.

In regard to peptides the present findings confirm that DA inhibits the expression of ENK and stimulates that of SP in striatopallidal neurons. However, our study shows that this characteristic genomic effect of DA is obvious mainly on neurons whose cell bodies are located in SM and indicate that DA influence on peptide expression may be different in other striatal territories. Hence, the present data gathered in PD monkeys suggest that the mesostriatal DA projection in primates may in fact be composed of several, distinct, subsystems displaying variable degree of vulnerability to MPTP and exerting different effects on peptide expression in the various striatal territories.

Acknowledgements

The authors express their sincere gratitude to L. Bertrand, S. Bilodeau and C. Harvey for technical and secretarial assistance. This study was supported by MRC grants MT-5781 (to A.P.) and MT-5750 (to P.J.B.). Brigitte Lavoie was the recipient of a Studentship from the FRSQ.

References

1. Agid Y, Javoy-Agid F, Ruberg M.Biochemistry of the neurotransmitters in Parkinson's disease. *In*: Marsden CD, Fahn S: Movement Disorders - 2, Butterworths, London 1987: 166-230.
2. Langston JW, Ballard P.Chronic Parkinsonism in human due to a product of meperidine-analog synthesis. Nature 1983; 219: 979-980.
3. Burns RS, Chiueh CC, Markey SP, et al. A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, Proc Natl Acad Sci (USA) 1983; 80: 4546-4550.
4. Langston JW, Forno LS, Rebert CS, et al. Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) in the squirrel monkey. Brain Res 1984; 292: 390-394.
5. German DC, Dubach M, Askari S, et al. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonian syndrome in *Macaca fascicularis:* which midbrain dopaminergic neurons are lost? Neuroscience 1988; 24: 161-174.
6. Lavoie B, Parent A. Immunohistochemical study of the serotoninergic innervation of the basal ganglia in the squirrel monkey. J Comp Neurol 1990; 299: 1-16.
7. Lavoie B, Smith Y, Parent A. Dopaminergic innervation of the basal ganglia in the squirrel monkey as revealed by tyrosine hydroxylase immunohistochemistry. J Comp Neurol 1989; 288: 36-52.
8. Abercrombie M. Estimation of nuclear population from microtome sections. Anat Rec 1946; 94: 239-244.
9. Parent A. Extrinsic connections of the basal ganglia. Trends in Neuroscience 1990; 13: 254-258.
10. Parent A, Lavoie B, Smith Y, et al. The dopaminergic nigropallidal projection in primates: Distinct cellular origin and relative sparing in MPTP-treated monkeys. *In*: Streifler MB, Korczyn AD, Melamed E, Youdim MBH, eds. Parkinson disease: Anatomy, Pathology and Therapy. Advances in Neurology Vol 53: New York, Raven Press 1990; 111-116.
11. Yamada T, McGeer PL, Baimbridge KG, McGeer EG. Relative sparing in Parkinson's disease of substantia nigra dopamine neurons containing calbindin-D_{28K}. Brain Res 1990; 526: 303-307.