Amino Acids (1998) 14: 17–23

Amino
Acids

The role of NMDA receptors in the slow neuronal degeneration of Parkinson's disease

L. D. Loopuijt and **W. J. Schmidt**

Department of Neuropharmacology, University of Tübingen, Federal Republic of Germany

Accepted September 26, 1997

Summary. Parkinson's disease is a disorder, in which neurons of various neuronal systems degenerate. Furthermore, in such degenerating neurons, the cytoskeleton seems to be affected. In this respect, Parkinson's disease resembles Alzheimer's disease. Since it has been shown, that elevated levels of intracellular calcium can disrupt the cytoskeleton and that the stimulation of glutamate (NMDA) receptors can cause high intracellular concentrations of calcium, it has been suggested, that the stimulation of glutamate receptors plays a role in the slow degeneration in Alzheimer's and Parkinson's disease. In case of the degeneration of the dopaminergic nigrostriatal system in Parkinson's disease, neurons that contain calcium binding protein appear to be less vulnerable than the neurons that lack it, suggesting that calcium binding protein might protect these neurons from degeneration by preventing that cytosolic calcium concentrations increase excessively. And, since there is in the nigrostriatal system a glutamatergic afferent pathway (the prefrontonigral projection) and since dopaminergic nigrostriatal neurons contain postsynaptic NMDA receptors, glutamatergic excitation may play a role in the degeneration of the nigrostriatal system in Parkinson's disease. If so, it may be possible to protect the neurodegeneration of these dopaminergic neurons by NMDA receptor antagonists.

Keywords: NMDA receptors – Excitotoxicity – Chronic neuronal degeneration – Nigrostriatal neurons – Parkinson's disease

Introduction

Chronic degeneration in various neurodegenerative diseases is under the influence of many different factors: reduced utilization of glucose, deficits in mitochondria and energy supply (Beal, 1992; Greene and Greenamyre, 1995a; 1995b) and free radicals and oxidative stress (Youdim et al., 1994, German et al., 1996). In acute neurodegeneration, there is ample evidence, that overexcitation of neurons by means of excitatory amino acids, such as glutamate and aspartate, as well as high intracellular calcium, can result in the death of

neurons (Choi, 1992). However, also slow (chronic) neurodegeneration is thought to be the result of stimulation of glutamate receptors by their endogenous agonists. For example, if cells are challenged by a reduced glucose utilization or mitochondrial deficit, they will degenerate after stimulation of glutamate receptors or intracellular calcium concentration at levels, that are not toxic to healthy neurons (Greene and Greenamyre, 1995b). Thus, several factors can interact in order to cause slow neuronal death. Therefore, the common path leading to degeneration may be the stimulation of excitatory amino acid receptors (or excessive intracellular calcium) together with downstream events.

Alzheimer's disease

Although the two most common neurodegenerative diseases, Alzheimer's and Parkinson's disease, differ substantially in clinical symptoms and pathologic changes of the brain, these two diseases also have many features in common. Both diseases are primarily diseases of the cytoskeleton of a few aging cell types. Pathological changes in Alzheimer's disease mainly consist of neurofibrillary tangles and neuropil threads. Neurons, that contain these pathological structures will ultimately degenerate. The mechanism of this degeneration is still unknown, but it is likely that neurofibrillary tangles and/or neurofibrillary threads play an important role (Braak et al., 1994a, 1996; Goedert, 1993; Iqbal et al., 1994). It appears, that neurons in which neurofibrillary tangles are formed ultimately will degenerate. After the death of these neurons, neurofibrillary tangles remain in situ. Neurofibrillary tangles consist for its major part, if not exclusively, out of tau, a microtubulin-associated protein (Goedert, 1993). In the normal brain tau is phosphorylated at six to eight sites. In Alzheimer's disease however, it is phosphorylated at more sites (there are 17 positions available). It has been hypothesized, that in Alzheimer's disease, there has been an abnormal phosphorylation of tau, implying deregulation of some phosphorylation-dephosphorylation mechanism (Goedert, 1993). In this highly phosphorylated state, tau cannot bind as efficiently to microtubules as before, causing destabilization of microtubules. Such a destabilization will result in impairment of vital cellular processes, such as rapid axonal transport and leading to degeneration of the affected nerve cells.

The subpopulation of neurons, that is affected in Alzheimer's disease include neurons in the entorhinal cortex, the CA2 region of the hippocampus and the long corticocortical connections. These neurons are glutamatergic and also receive glutamatergic input (Huntley et al., 1994). After stimulation of glutamatergic NMDA receptors, there is an increase in intracellular Ca^{2+} concentration (Mattson et al., 1992). This increase is probably due to the opening of NMDA coupled Ca^{2+} channels by means of the stimulation of NMDA receptors. Furthermore, there is evidence that Ca^{2+} can disrupt the cytoskeleton (Mattson et al., 1992). Finally, exposure of neurons to β-amyloid, the protein that can be found in neurofibrillary tangles and neuropil threads, sensitises cells to glutamate toxicity (Mattson et al., 1992; Le et al., 1995).

These lines of evidence taken together, make it likely that excitatory amino acids play a role in the degeneration of neurons in Alzheimer's disease.

Parkinson's disease

The pathological hallmark of Parkinson's disease is the degeneration of dopaminergic neurons in the substantia nigra, pars compacta. However, this is not the only affected region. Other affected areas include: the hypothalamus (Gibb, 1988; Bethlem and den Hartog Jager, 1960), the entorhinal cortex, locus coeruleus, hippocampus, raphe nucleus (Gibb, 1988; Bethlem and den Hartog Jager, 1960), amygdala (Braak et al., 1994b) (Table 1). Many pathways of the autonomic nervous system appear to be involved (Gibb, 1988; Bethlem and den Hartog Jager, 1960). Transsynaptic degeneration or a combination of transsynaptic and parallel degeneration occur (Agid, 1991). However, it is not known, in which region the degeneration is initiated.

At the cellular level, in regions with degeneration of neurons, Lewy bodies can be found. These pathological structures are located within the cell bodies of living neurons and consist of accumulations of cytoskeletal proteins (Gibb, 1988; Goldman et al., 1983; Galloway et al., 1988). This is an indication, that also in Parkinson's disease, as in Alzheimer's disease, neuronal degeneration might be triggered by disorganization of the cytoskeleton. However, the composition between Lewy bodies in Parkinson's disease and paired helical filaments (the main components of neurofibrillary tangles) in Alzheimer's disease differs: Lewy bodies contain tubulin, various neurofilament proteins, microtubulin-associated proteins 1 and 2 (MAP-1 and MAP-2), but not tau (Galloway et al., 1988), while tau is the principal component of neurofibrillary tangles in Alzheimer's disease (see above). Furthermore, Lewy bodies only

Table 1. Affected brain areas in Parkinson's disease*

Region	Area
Cerebral cortex	Temporal, frontal, anterior cingulate and insular cortex
Diencephalon	Hypothalamus, especially lateral, posterior, tuberomammillary and paraventricular nuclei; thalamus; nucleus basalis of Meynert; amygdala
Brainstem	Substantia nigra; ventral tegmental area; Edinger-Westphal nucleus; periaquaductal gray; locus coeruleus; superior central nucleus; dorsal vagal nucleus
Spinal cord	Intermediolateral column; Onufs nucleus
Sympathetic ganglia	Cervical; thoracic; lumbar; sacral
Parasympathetic myenteric plexus	Esophagus

* Table adapted from Gibb, 1988. Data from Bethlem and Den Hartog Jager, 1960; Qualman, 1984; Hunter, 1985; Braak et al., 1994b.

can be found within living neurons. In Parkinson's disease, neurons with apoptotic changes, undergoing programmed cell death, have been shown in the substantia nigra. However, they do not contain Lewy bodies (Anglade et al., 1997). It was therefore hypothesized that Lewy bodies might represent a transient stage of vulnerable neurons (Anglade et al., 1997).

Selective neuronal vulnerability in Parkinson's disease

Since, in Parkinson's disease, the degeneration of the substantia nigra, pars compacta, gives rise to its main clinical symptoms, this neuronal system has been extensively studied. What attracts attention after inspection of human postmortem tissue of Parkinson patients, is the fact, that not all dopaminergic neurons degenerate at the same time, but that degenerating and healthy dopaminergic neurons reside side by side (Gibb and Lees, 1991). It appeared, that there are two different types of dopaminergic neurons in the substantia nigra, pars compacta, of which one population contains calcium binding protein and the other does not (Gerfen et al., 1987b). The cells that lack calcium binding protein preferentially degenerate in Parkinson's disease (Yamada et al., 1990; Hirsch et al., 1992; German et al., 1992). This indicates, that calcium binding protein protects against neurodegeneration, probably by binding part of the accumulating intracellular Ca^{2+}. However, these two populations of neurons differ in more respects than only their calcium binding protein content. They belong to a different circuitry, so that they have a different population of target neurons (Gerfen et al., 1987a). From these different types of target neurons, they might each receive different neurotrophic compounds, that may influence their survival differently. Moreover, the diameter of their axons differs (Gerfen et al., 1987a), implying, that the amount of neurofilament protein content differs between these populations (Lasek et al., 1983). Finally it might be possible, that there is another kind of neurofilament protein present in the respective populations (Hof and Morrison, 1995). Therefore, this difference in vulnerability also could be carried back to differences in the sort or the amount of neurofilament protein and/or retrogradely transported trophic factor.

Glutamatergic stimulation of NMDA receptors of dopaminergic neurons in the substantia nigra, pars compacta

The substantia nigra receives glutamatergic afferents *in vivo*. Firstly, the prefrontal cortex projects to the substantia nigra, pars compacta and reticulata (Nitai and Kita, 1994; Kornhuber et al., 1984). Furthermore, there also is a glutamatergic pathway from the subthalamic nucleus to the substantia nigra, pars reticulata (Nauta and Cole, 1978; Hammond et al., 1978). But these fibers seem to avoid pigmented (dopaminergic) neurons (Nauta and Cole, 1978) and there is only direct evidence for synaptic contact between subthalamonigral nerve endings and nigrothalamic neurons (Bevan et al., 1994). Thus the

subthalamonigral fibers do not seem to have much impact on dopaminergic compacta neurons, while the afferentation from the prefrontal cortex is not very dense, when compared to the corticostriatal afferentation (Nitai and Kita, 1994).

Nevertheless, there is evidence for NMDA (Mercuri et al., 1992; Albin et al., 1992; Christoffersen and Meltzer, 1995) and AMPA (Albin et al., 1992; Christoffersen and Meltzer, 1995) receptors on dopaminergic pars compacta neurons, although at relatively low levels. In addition, NMDA receptor subunits NMDAR1, NMDAR2C and NMDAR2D have been shown to be present in the substantia nigra, pars compacta (Standaert et al., 1994). However, the distribution of NMDA receptors subunits has not been compared between calcium binding protein positive and negative neurons of the pars compacta, so that we cannot decide at the moment whether this discrepancy in survival can be correlated with the presence or absence of (a specific subtype of) NMDA receptor.

Thus it seems possible, that the degeneration in the substantia nigra, at least partly, depends on stimulation of glutamate receptors. In line with such a hypothesis is the fact, that, under some circumstances, NMDA receptor antagonists protect from damage, inflicted by the dopaminergic drug amphetamine (Sonsalla et al., 1989), while this might also be the case for MPP^+-induced damage (Turski et al., 1991), although this is controversial (Sonsalla et al., 1992). Moreover, in the striatum, apoptotic (programmed) cell death can be blocked by glutamate antagonists or can be inhibited by removal of glutamatergic cortical afferents (Mitchell et al., 1994). Also in the substantia nigra apoptotic cell death has been described (Anglade et al., 1997).

Therefore, the use of glutamate receptor antagonists might be a possible way to prevent nigral dopaminergic cells in Parkinson's disease. Indeed, promising results have been obtained with NMDA receptor antagonists as neuroprotective agents (Kornhuber et al., 1994).

Acknowledgement

Supported by the Deutsche Forschungsgemeinschaft SFB 430.

References

Agid Y (1991) Parkinson's disease: pathophysiology. Lancet 337: 1321–1324

Albin RL, Makowiec RL, Holingsworth ZR, Dure IVLS, Penney JB, Young AB (1992) Excitatory amino acid binding sites in the basal ganglia of the rat: a quantitative autoradiographic study. Neurosci 46: 35–48

Anglade P, Vyas S, Javoy-Agid F, Herrera MT, Michel PP, Marquez J, Mouatt-Prigent A, Ruberg M, Hirsch EC, Agid Y (1997) Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. Histol Pathol 12: 25–31

Beal MF (1992) Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? Ann Neurol 31: 119–130

Bethlem J, Den Hartog Jager WA (1960) The incidence and charateristics of Lewy bodies in idiopathic paralysis agitans (Parkinson's disease). J Neurol Neurosurg Psychiatry 23: 74–80

Bevan MD, Bolam JP, Crossman AR (1994) Convergent synaptic input from the neostriatum and the subthalamus onto identified nigrothalamic neurons in the rat. Eur J Neurosci 6: 320–334

Braak E, Braak H, Mandelkow EM (1994a) A sequence of cytoskeleton changes related to the formation of neurofibrillary tangles and neuropil threads. Acta Neuropathol 87: 554–567

Braak H, Braak E, Yilmazer D, de Vos RAI, Jansen ENH, Bohl J, Jellinger K (1994b) Amygdala pathology in Parkinson's disease. Acta Neuropathol 88: 493–500

Braak H, Braak E, Yilmazer D, de Vos RAI, Jansen ENH, Bohl J (1996) Pattern of brain destruction in Parkinson's and Alzheimer's diseases. J Neural Transm 103: 455–490

Choi DW (1992) Excitotoxic cell death. Neurobiology 23: 1261–1276

Christoffersen CL, Meltzer CT (1995) Evidence for N-methyl-D-aspartate and AMPA subtypes of the glutamate receptor on substantia nigra dopamine neurons: possible preferential role for N-methyl-D-aspartate receptors. Neurosci 67: 373–381

Galloway PG, Grundke-Iqbal I, Iqbal K, Perry G (1988) Lewy bodies contain epitopes both shared and distinct from Alzheimer neurofibrillary tangles. J Neuropathol Exp Neurol 47: 654–663

Gerfen CR, Herkenham M, Thibault J (1987a) The neostriatal mosaic: II. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems. J Neurosci 7: 3915–3934

Gerfen CR, Baimbridge KG, Thibault J (1987b) The neostriatal mosaic: III. Biochemical and developmental dissociation of patch-matrix mesostriatal systems. J Neurosci 7: 3935–3944

German AM, McGowan A, O'Neill C, Cotter T (1996) Oxidative stress and apoptosis in neurodegeneration. J Neurol Sci 139 [Suppl]: 45–52

Gibb WRG (1988) The neuropathology of Parkinsonian disorders. In: Jankovic J, Toloa E (eds) Parkinson's disease and movement disorders. Urban and Schwarzenberg, Baltimore Munich, pp 205–223

Gibb WRG, Lees AJ (1991) Anatomy, pigmentation, ventral and dorsal subpopulations of the substantia nigra, and differential cell death in Parkinson's disease. J Neurol Neurosurg Psychiatry 54: 388–396

Goedert M (1993) Tau protein and the neurofibrillary pathology of Alzheimer's disease. Trends Neurosci 16: 460–465

Goldman JE, Yen S-H, Chiu F-C, Peress NS (1983) Lewy bodies of Parkinson's disease contain neurofilament antigens. Science 221: 1082–1084

Greene JG, Greenamyre JT (1995a) Characterization of the excitotoxic potential of the reversible succinate dehydrogenase inhibitor malonate. J Neurochem 64: 430–436

Greene JG, Greenamyre JT (1995b) Exacerbation of NMDA, AMPA and L-glutamate excitotoxicity by the succinate dehydrogenase inhibitor malonate. J Neurochem 64: 2332–2338

Hammond C, Deniau JM, Rizk A, Feyer J (1978) Electrophysiological demonstration of an excitatory subthalamonigral pathway in the rat. Brain Res 151: 235–244

Hof PR, Morrison JH (1995) Neurofilament protein defines regional patterns of cortical organization in the Macaque monkey visual system: a quantitative immunohistochemical analysis. J Comp Neurol 352: 161–186

Hunter S (1985) The rostral mesencephalon in Parkinson's disease and Alzheimer's disease. Acta Neuropathol 68: 53–58

Huntley GW, Vicker JC, Morrison JH (1994) Cellular and synaptic localization of NMDA and non-NMDA receptor subunits in neocortex: organizational features related to cortical circuitry, function and disease. Trends Neurosci 17: 536–543

Iqbal K, Alonso AC, Gong CX, Khatoon S, Singh TJ, Grundke-Iqbal I (1994) Mechanism of neurofibrillary degeneration in Alzheimer's disease. Mol Neurobiol 9: 119–123

Kornhuber J, Kim J-S, Kornhuber M, Kornhuber HH (1984) The corticonigral projection: reduced glutamate content in the substantia nigra following frontal cortex ablation in the rat. Brain Res 322: 124–126

Kornhuber J, Weller M, Schoppenmeyer K, Riederer P (1994) Amantadine and memantine are NMDA receptor antagonists with neuroprotective properties. J Neural Transm [Suppl] 43: 91–104

Lasek RJ, Oblinger MM, Drake PF (1983) The molecular biology of neuronal geometry: the expression of neurofilament genes influences axonal diameter. Cold Spring Harbor Symp Quant Biol 48: 731–744

Le W-D, Colom LV, Wie W, Smith G, Alexianu M, Appel SH (1995) Cell death induced by β-amyloid 1-40 in MES 23.5 hybrid clone: the role of nitric oxide and NMDA-gated channel activation leading to apoptosis. Brain Res 686: 49–60

Mattson MP, Cheng B, Davis D, Bryant K, Lieberburg I, Rydel RE (1992) Beta-amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicitity. J Neurosci 12: 376–389

Mercuri NB, Stratta F, Calabres P, Bernard G (1992) A voltage-clamp analysis of NMDA-induced responses on dopaminergic neurons of the rat substantia nigra zona compacta and ventral tegmental area. Brain Res 593: 51–56

Mitchell IJ, Lawson S, Moser B, Laidlaw SM, Cooper AJ, Walkinsaw G, Waters CM (1994) Glutamate-induced apoptosis results in a loss of striatal neurons in the Parkinsonian rat. Neurosci 63: 1–5

Naito A, Kita H (1994) The cortico-nigral projection in the rat: an anterograde tracing study with biotynilated dextran amine. Brain Res 637: 17–32

Nauta HJW, Cole M (1978) Efferent projections of the subthalamic nucleus: an autoradiographic study in monkey and cat. J Comp Neurol 180: 1–16

Qualman SJ, Haupt HM, Young P, Hamilton SR (1984) Esophageal Lewy bodies associated with ganglion cell loss in achalasia. Similarity to Parkinson's disease. Gastroentrology 87: 848–856

Sonsalla PK, Nicklas WJ, Heikkila RE (1989) Role of excitatory amino acids in methamphetamine-induced nigrostriatal dopaminergic toxicity. Science 243: 398–400

Sonsalla PK, Zeevalk GD, Manzino L, Giovanni A, Nicklas WJ (1992) MK-801 fails to protect against the dopaminergic neuropathology produced by systemic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice or intranigral 1-methyl-4-phenylpyridinium in rats. J Neurochem 58: 1979–1982

Standaert DG, Testa CM, Young AB, Penney JB Jr (1994) Organization of N-methyl-D-aspartate glutamate receptor gene expression in the basal ganglia of the rat. J Comp Neurol 343: 1–16

Turski L, Bressler K, Rettig K-J, Löschmann P-A, Wachtel H (1991) Protection of substantia nigra from MPP^+ neurotoxicity by N-methyl-D-aspartate antagonists. Nature 349: 414–418

Youdim MBH, Ben-Sacher D, Riederer P (1994) The enigma of neuromelanin in Parkinson's disease substantia nigra. J Neural Transm [Suppl] 43: 113–122

Authors' address: Dr. L. D. Loopuijt, Department of Neuropharmacology, Med. Naturw. Forschungszentrum, Universität Tübingen, Ob dem Himmelreich 7, D-72074 Tübingen, Federal Republic of Germany.

Received August 25, 1997