Molecular Biological and Neurochemical Studies in a Parkinson’s Disease Model

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Abstract

Parkinson's disease (PD) is a motor disorder that is characterized by a depletion of dopaminergic neurons in the substantia nigra pars compacta of the basal ganglia. After dopamine denervation, glutamatergic pathways to the striatum (Str) and within the basal ganglia become overactive. Glutamate and its receptors have been said to involve in the pathogenesis of PD. Administration of the ionotropic glutamate receptor antagonists may have direct antiparkinsonian action in animal models of PD. Therefore, the major objectives of the present study were to characterize the changes of ionotropic glutamate receptor subunits in the 6-hydroxydopamine (6-OHDA)-lesioned rats, a model of PD. In addition, the effects of administration of antisense oligonucleotides specific for N-methyl-D-aspartate (NMDA) receptor one (NR1) subunit (ANR1) were also investigated using normal and 6-OHDA-lesioned rats. Another important issue in the study is whether it is possible to interfere with the course of neuronal injury that eventually lead to PD. Thus, investigation was conducted to examine the metabolic changes following 6-OHDA lesion in the substantia nigra (SN). These studies could provide background information for designing protective means against injury to the dopaminergic neurons.

The present study was divided into four parts. The first part aimed to identify the changes in levels of mRNA expression of ionotropic glutamate receptors were conducted in the rat Str from unilaterally 6-OHDA lesioned rats. When compared with the contralateral Str without lesion, level of NR1 mRNA was significantly higher (+27.95%) but the level of GluR1 mRNA was significantly lower (-15.41%) in the lesioned side. No significant modulations was observed in other NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate acid (AMPA) receptor subunits. In addition, modulations of levels of glutamate receptor proteins were investigated by immunofluorescence with image analysis. The intensity of GluR1 immunoreactivity was found to decrease (-23.35%) in the lesioned side. At the cellular level, a decrease in intensity of GluR1 immunoreactivity was found in perikarya of presumed medium spiny neurons (-16.49%) but not in the parvalbumin-positive striatal interneurons. However, NR1 immunoreactivity was found to be unchanged in the Str ipsilateral to the lesion. These results indicate that there are differential modulations of different ionotropic glutamate receptors in the Str after the 6-OHDA lesion and the glutamate receptors are likely to be involved in the pathogenesis of PD.

In the second part of the study, a single dose of ANR1 was applied unilaterally in the Str of normal rats in order to investigate the effects of ANR1 on the gene expression of NMDA receptor subunits gene. After 1 day of ANR1 treatment, ipsilateral rotation behaviors that were induced by apomorphine were found in the treated animals. Reductions in the levels of expression of NR1 (-20.6%) and NR2A (-19.7%) mRNAs
were found in the ANR1-treated striatal tissues by RT-PCR. There was no change in the levels of NR2B, NR2C and NR2D mRNAs. After two days, Western blotting experiments showed that there were decreases in the levels of expression of NR1 (-27.6%) and NR2A (-19.2%) proteins in the ANR1-treated striatal tissues. In addition, NR1 immunoreactivity was found to decrease in intensity in the ANR1-treated Str. At the cellular level, intensity of NR1 immunoreactivity in perikarya of presumed medium spiny neurons was found to decrease. These results indicate that a single dose of ANR1 modifies the expression of NR1 mRNA and protein in neurons in the Str. The modification in the expression of NR1 has differential effects in the expression of NR2 subunits. Gene expression of the native NR subunits is likely to be a dynamic process. The change in gene expression of the NR subunits in the Str may have a profound effect on the motor behaviors of rats.

Thirdly, a single dose of ANR1 was administrated into the Str ipsilateral to the lesion in 6-OHDA-lesioned rats. After one days of injections, the apomorphine-induced rotation was significantly attenuated (number of turn per minute: 0.81 ± 0.48). After two days of treatment, a significantly reduction in number of rotation was still observed (numbers of turns per minute: 4.0±0.5). A slight increase in the levels of NR1 mRNA expression (+ 3.251%) was shown by RT-PCR in the ANR1-treated Str. However, no significant change was found in the expression of NR1 proteins by western blotting experiments. Furthermore, no observable changes of NR1 immunoreactivity were seen in the Str of the lesioned side. At the cellular level, a reduction of NR1 immunoreactivity was seen in perikarya of presumed medium spiny neurons (- 28.63%). These results as a whole indicate that ANR1 has efficacy in antiparkinsonian effects in modulation of motor behaviors of parkinsonian animals and block the abnormal gene expression of NR1 receptor after the lesion. ANR1 is therefore a potential agent for treatments of PD.

In the last part of this study, attempt was made to identify the cause of cell injury in the 6-OHDA-lesioned rats by examining the expression of metallothionein-I (MT-I) and zinc (Zn) ions. Intense MT-I immunoreactivity was found to be highly expressed in the astrocytes of the SN after the lesion. In addition, Zn was found to accumulate neuronal elements of the SN after the lesion. These results indicate that MT-I and Zn ions may be involved in the cell death mechanisms of the dopaminergic neurons in the SN.

Results of the present study as a whole can provide evidence that glutamate receptors, MT-I and Zn ions are involved in the pathogenesis of PD. Blockage of NR1 receptor by ANR1 is useful in reduction of symptoms of PD and may be a potential agent for therapy of PD in the future.
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