

MASTER'S THESIS

Glutamate receptors in an animal model of Parkinson's disease

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Glutamate Receptors in an Animal Model
of Parkinson's Disease

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Abstract

Basal ganglia are a group of subcortical nuclei in the forebrain that are associated with movement of the body. Degeneration of dopaminergic neurons in the basal ganglia, i.e., the neurons in the substantia nigra pars compacta, is the cause of one of the major dysfunction of the basal ganglia, namely Parkinson's disease. In order to develop better treatments for Parkinson's disease, neurotransmitter glutamate and its receptors are implicated as the targets. There is a complicated family of glutamate receptors described so far. The major objectives of the present thesis were to investigate the precise cellular localization of nine glutamate receptor subunits and subtypes (N-methyl-D-aspartate receptors: NMDAR1 and NMDAR2B; α -amino-3-hydroxy-5-methyl-4-isoxazole propionate receptors: GluR1-4; kainate receptors: GluR5/6/7; and metabotropic glutamate receptors: mGluR1 α and mGluR2/3) in the neostriatum and the substantia nigra, the two major areas of the basal ganglia that are known to be affected by the neuropathology of Parkinson's disease. Using an animal model of Parkinson's disease, the 6-hydroxydopamine-lesioned rats, the changes of the glutamate receptors during the course of the motor disorder were then investigated.

In the first part of study, double immunocytochemistry and immunofluorescence was employed to reveal the precise cellular localization of immunoreactivity for glutamate receptor subunits in subpopulations of neurons in the rat substantia nigra. In the substantia nigra pars compacta, around 90 % of the tyrosine hydroxylase-immunoreactive neurons are found to express immunoreactivity for GluR1, GluR2, GluR2/3, NMDAR1 and GluR5/6/7. Only about 60 % of tyrosine hydroxylase-positive neurons were found to express NMDAR2B immunoreactivity. In the substantia nigra pars reticulata, subpopulations of neurons that displayed strong immunoreactivity for parvalbumin and GABA transaminase were found to express immunoreactivity for GluR1, GluR2, GluR2/3, GluR4, NMDAR1, NMDAR2B and GluR5/6/7. These results indicate that the compacta neurons are the major neuronal elements that express most of the ionotropic glutamate receptor subunits in the region. In addition, subpopulations of neurons in the reticulata that express strong immunoreactivity for parvalbumin and GABA transaminase are the major neurons that express most of the ionotropic glutamate receptors.

In the second part of study, immunocytochemistry was employed to localize the precise cellular distribution of immunoreactivity for glutamate receptor subunits and subtypes in the neostriatum. Immunoreactivity for GluR2, GluR2/3, NMDAR1, NMDAR2B and GluR5/6/7, as well as GluR1 was primarily found in neurons that resembled the medium-sized spiny neurons in the neostriatum. Immunoreactivity for GluR1 and GluR4 was primarily found in interneurons. Immunoreactivity for mGluR1 α and mGluR2/3 was mainly found in the neuropilar elements of the neostriatum.

Based on the data obtained above, immunofluorescence was employed to investigate the changes of glutamate receptor immunoreactivity in the substantia nigra of the unilaterally 6-hydroxydopamine-lesioned rats. Lesioned rats were perfuse-fixed at different time periods (2 weeks, 3 months and 1 year of lesion). In the lesioned rats after 2 weeks, 3 months and 1 year of lesion, immunoreactivity for GluR1, GluR2, GluR2/3, NMDAR1, NMDAR2B, GluR5/6/7 was found to be depleted in the substantia nigra pars compacta. In addition, immunoreactivity for GluR1, GluR2, GluR2/3, GluR4, NMDAR1, NMDAR2B, GluR5/6/7, mGluR1 α and mGluR2/3, which was indicated by the immunofluorescence

intensity, was found to be unchanged between the non-lesioned and lesioned substantia nigra pars reticulata. These results confirm that the compacta neurons are the major neuronal populations in the rat substantia nigra that display most of the ionotropic glutamate receptors as immunoreactivity for these glutamate receptors are depleted by the loss of dopaminergic neurons in the compacta. Immunoreactivity for the glutamate receptors in the reticulata may not be modified after the lesion.

In addition, based on the above data, immunofluorescence was also employed to study the changes of glutamate receptor immunoreactivity in the neostriatum after the lesion. Similarly, lesioned rats were perfuse-fixed after the three different time periods (2 weeks, 3 months and 1 year of lesion). Immunoreactivity for GluR1, GluR2, GluR2/3 was found to decrease in terms of immunofluorescence intensity in the neostriatum of 2 week-lesioned rats. Immunoreactivity for GluR1 was also found to decrease in the neostriatum of 3 month-lesioned rats. The present study demonstrates that GluR1, GluR2 and GluR2/3 but not the other glutamate receptors are down regulated in the region during the initial stage of lesion. In later stages of lesion, the receptor immunoreactivity is found to be similar between the lesioned and non-lesioned neostriatum. These results indicate that there is a differential effect of the dopamine denervation to different glutamate receptors in the neostriatum and the changes in receptor immunoreactivity are time dependent.

Last but not least, in order to correlate the aftermath changes of glutamate receptors after the lesion at the cellular level to those at the subcellular level, GluR1 immunoreactivity was revealed in the neostriatum. In the three age groups of lesioned animals, the percentage of GluR1-immunoreactive dendritic spines was found to decrease in the lesioned neostriatum. The present qualitative observations indicate that there may be subcellular changes occur in the neuronal elements that are likely to be spiny neurons after the dopamine denervation.

In summary, results of the present thesis as a whole indicate that there is differential localization of glutamate receptors in the neostriatum and in the substantia nigra. After the 6-hydroxydoapmine-lesioned, there are cellular and subcellular changes of glutamate receptors in the regions that may have implication in future therapeutic applications in treatments of Parkinson's disease.

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