DOCTORAL THESIS

Developmental expression of N-methyl-D-aspartate and gamma-aminobutyric acid receptors in the rat basal ganglia

Lau, Wai Kit Jaeger

Date of Award:
2004

Developmental Expression of N-methyl-D-aspartate and Gamma-aminobutyric Acid Receptors in the Rat Basal Ganglia

LAU Wai Kit, Jaeger

A thesis submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Principal Supervisor: Dr. YUNG Kin Lam

Hong Kong Baptist University

February 2004

Abstract

Glutamate is the major excitatory neurotransmitter in the basal ganglia and it is considered as the driving force of the neurons in the basal ganglia. *N*-methyl-*D*-aspartate (NMDA) receptors are a group of important ionotropic glutamate receptors in the basal ganglia that mediate the functions of glutamate. NMDA receptors are therefore known to be involved in the control of movements in the basal ganglia. In contrast, Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the neostriatum. Functions of GABA are mediated by $GABA_A$ and $GABA_B$ receptors in the neostriatum. In order to investigate the developmental expression of NMDA receptor subunits (NR1 and NR2A-D) and GABA receptors subunits ($GABA_A\alpha1$, $\alpha3$ and $\alpha6$; $GABA_B$R1 and R2) in the rat neostriatum (Str) and substantia nigra pars compacta and pars reticulata (SNc and SNr), reverse transcriptase-polymerase chain reaction (RT-PCR) and immunofluorescence were performed. Tissues were obtained from rats at postnatal-day-1 (PND1), day-7 (PND7), and day-14 (PND14) and from adult rats were used.

In the Str, RT–PCR indicated that levels of NR1, NR2A and NR2D mRNAs reached peak levels between PND 7 and PND 14. The levels of NR2B and NR2C mRNAs were increased at PND 7 and remained high in adults. Immunofluorescence combined with image analysis revealed that the levels of NR1 and NR2B immunoreactivity rose progressively in perikarya of striatal neurons. However, levels of NR2A immunoreactivity were higher in striatal neurons at PND 1 and PND 7. In addition, double immunofluorescence revealed that the levels of NR1 immunoreactivity decreased but the levels of NR2A immunoreactivity were the same in parvalbumin (PV)-positive striatal interneurons of PND 14 and adult rats. NR2B immunoreactivity was not detected in PV-positive neurons of PND 14 rats, but intense NR2B labeling was seen in PV-positive neurons of adult rats. In choline acetyltransferase (ChAT)-positive striatal interneurons of PND 14 and adult rats, levels of NR1 and NR2A immunoreactivity were seen to increase and the level of NR2B immunoreactivity remained the same.

In the SN, NR1, NR2A, NR2B and NR2C mRNA levels were highest in PND 14 rats, NR2D mRNA level was highest in PND 7 rats. Highest levels of NR1, NR2A and NR2B immunoreactivity were seen in adult rats. Double immunofluorescence revealed that tyrosine hydroxylase (TH)-positive dopaminergic neurons display peak level of NR1 immunoreactivity in PND1 and PND7 rats. Opposite trend in expression of NR2A and NR2B immunoreactivity was observed in TH-positive dopaminergic neurons. In the SNr, the levels of NR1 immunoreactivity increased in PV-positive GABAergic neurons. However, the levels of NR2A and NR2B immunoreactivity in PV-positive neurons were similar to Str.

In the Str, low levels of the $GABA_A\alpha1$ and $GABA_A\alpha6$ mRNA but high levels of $GABA_A\beta2$ and $GABA_A\beta3$ mRNAs was found during early postnatal period. Immunofluorescence revealed that $GABA_A\alpha1$ immunoreactivity was found in perikarya

of most striatal neurons in young animals. However, $GABA_A\alpha1$ immunoreactivity was only observed in striatal interneurons in PND14 and adult rats. Immunoreactivity for $GABA_A\alpha3$ and $GABA_A\alpha6$ were only observed in PND14 and adult animals. $GABA_BR1$ immunoreactivity was found to be expressed by perikarya of striatal neurons from all ages. In contrast, $GABA_BR2$ immunoreactivity was mainly observed in ChAT-positive striatal interneurons in PND 14 and adult rats. These findings indicated that in adult rats, ChAT-positive striatal interneurons are the major group of neurons that display functional $GABA_B$ receptors.

In the SN, levels of $GABA_A$ mRNAs reached peak levels in the early postnatal period. $GABA_A\alpha1$ immunoreactivity was not found in TH-positive dopaminergic neurons but the levels of $GABA_B\alpha1$ immunoreactivity increased in PV-positive GABAergic neurons. Immunorecativity for $GABA_A\alpha3$ was only observed in perikarya of TH- and PV-positive neurons in adult animals. In contrast, the levels of $GABA_A\alpha6$ and $GABA_BR1$ immunoreactivity of both TH- and PV-positive neurons reached peak levels between PND7 and PND14. However, $GABA_BR2$–positive neurons were only observed in the adult rats of SNc but the levels were the same in SNr of PND 14 and adult rats.

The present results indicate that there are differential patterns of developmental expression of NMDA and GABA receptor subunits in the striatum and the substantia nigra. The present results have provided important implications in development of the glutamate and GABA systems in the rat basal ganglia.

Table of Contents