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Glutamate neurotransmission is affected in prenatally stressed offspring

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ABSTRACT

Previous studies from our laboratory have shown that male adult offspring of stressed mothers exhibited higher levels of ionotropic and metabotropic glutamate receptors than control rats. These offspring also showed long-lasting astroglial hypertrophy and a reduced dendritic arborization with synaptic loss. Since metabolism of glutamate is dependent on interactions between neurons and surrounding astroglia, our results suggest that glutamate neurotransmitter pathways might be impaired in the brain of prenatally stressed rats. To study the effect of prenatal stress on the metabolism and neurotransmitter function of glutamate, pregnant rats were subjected to restrain stress during the last week of gestation. Brains of the adult offspring were used to assess glutamate metabolism, uptake and release as well as expression of glutamate receptors and transporters. While glutamate metabolism was not affected it was found that prenatal stress (PS) changed the expression of the transporters, thus, producing a higher level of vesicular vGluT-1 in the frontal cortex (FCx) and elevated levels of GLT1 protein and messenger RNA in the hippocampus (HPC) of adult male PS offspring. We also observed increased uptake capacity for glutamate in the FCx of PS male offspring while no such changes were observed in the HPC. The results show that changes mediated by PS on the adult glutamatergic system are brain region specific. Overall, PS produces long-term changes in the glutamatergic system modulating the expression of glutamate transporters and altering synaptic transmission of the adult brain.

1. Introduction

Glutamate (Glu) is the principal excitatory neurotransmitter in the mammalian central nervous system, participating in the integration of brain function and in synaptic plasticity, memory and learning processes. In glutamatergic neurons, de novo synthesis of neurotransmitter Glu has an obligatory requirement of neuron–glia interactions since its precursor glutamine (Gln) can only be synthesized in astrocytes due to the exclusive glial localization of the enzymes pyruvate carboxylase and glutamine synthetase. The former enzyme, pyruvate carboxylase, acts as an anaplerotic pathway for replenishment of tricarboxylic acid (TCA) cycle intermediates which is the prerequisite for production of Gln (Schousboe et al., 2013). This anaplerosis must be coupled to cataplerosis which is not fully understood (Sonnewald, 2014). Subsequent to transfer of Gln from astrocytes to the glutamatergic neurons, these cells convert Gln to Glu in the reaction catalyzed by phosphate activated

Abbreviations: FCx, frontal cortex; HPC, hippocampus; Glu, glutamate; TCA, tricarboxylic acid; PAG, phosphate activated glutaminase; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, *N*-methyl-D-aspartate; KA, kainate; GDH, glutamate dehydrogenase; LC-MS, liquid chromatography–mass spectrometry; C, control; PS, prenatal stress; PND, postnatal day; RT-qPCR, real time reverse transcription polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride; GPV, glial plasmalemmal vesicles; SYN, synaptosomes; TBOA, threo-β-benzyloxyaspartate; DHT, dihydrokainate; vGluTs, vesicular glutamate transporters; QAR, quantitative receptor autoradiography.

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glutaminase (PAG) in the neuronal mitochondria (Schousboe et al., 2013). When Glu is released to the synaptic cleft, it binds to postsynaptic ionotropic receptors, i.e. those activated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), *N*-methyl-D-aspartate (NMDA) and kainate (KA), and to metabotropic receptors (mGluR 1–8), thus, mediating and modulating synaptic transmission. Subsequently, Glu diffuses in the synaptic cleft and it is internalized by excitatory amino acid transporters (EAATs), primarily localized in surrounding astrocytes (Danbolt, 2001; Gegelashvili and Schousboe, 1997; Zhou and Danbolt, 2013). In the astrocytes, Glu is transformed to Gln through the activity of the enzyme glutamine-synthetase or oxidatively metabolized via conversion to α-ketoglutarate in the glutamate dehydrogenase (GDH) reaction (Schousboe et al., 2013). The trafficking of these amino acids between neurons and glia is named the "glutamine–glutamate cycle" which plays a major role in the maintenance of the Glu pools in cells, in particular the neuronal neurotransmitter pool (Waagepetersen et al., 2005). The significance of this cycle is to secure a rapid removal of Glu from the synaptic space by astrocytic uptake, subsequent replenishment of the neurotransmitter pool in the glutamatergic vesicles and to some extent provision to neurons of a metabolic substrate, i.e., Gln can be a potential fuel (Daikhin and Yudkoff, 2000; Popoli and Pepponi, 2012).

Among glutamate receptors, those activated by NMDA and AMPA play critical roles in associative memory and remodeling of synapses in the hippocampus (HPC) and frontal cortex (FCx). Disruption of these receptors is known to cause cognitive deficits and learning impairments (Lewis, 1997). To date, variations of AMPA and NMDA receptor levels and function have been found in mood disorders (Chen et al., 2010). In addition to this, subunit dysfunction has also been found in neurodegenerative conditions and neuropsychiatric disorders, such as schizophrenia (Coyle et al., 2003). Another major point of regulation for glutamatergic neurotransmission is the EAATs. These transporters show a regional cell type-specific pattern of expression. There are five different types of EAATs: EAAC1, EAAT4 and EAAT5 are predominantly expressed in neuronal extra-synaptic locations while glutamate transporter 1 (GLT-1) and glutamate/aspartate transporter (GLAST) are expressed in glia (Danbolt, 2001). Neuronal transporters appear to contribute less significantly to glutamate uptake than the glial transporters (Gegelashvili et al., 2000). Both, protein levels and kinetics of these transporters are regulated by glutamate receptors, glutamate release and other factors related to glutamatergic neurotransmission (Gegelashvili and Schousboe, 1997). Another well described transporter for Glu is the vesicular glutamate transporter (vGluT). This carrier mediates Glu uptake into synaptic vesicles at the glutamatergic neuronal terminals (Herzog et al., 2006). There are three isoforms of vGluT (1, 2 and 3) that differ in their expression profiles. In the adult brain, vGluT-1 predominates in cerebral cortex, cerebellum and hippocampus and vGlut-2 is present in diencephalon, brainstem and spinal cord. vGluT-3 is the less expressed isoform and is found in non glutamatergic neurons like GABAergic or cholinergic ones. The presence of vGluT in neurons has been taken as a phenotypic trait of glutamatergic neurons with a fundamental role in glutamatergic transmission (Wojcik et al., 2004).

Central nervous system responses to stress are often dependent on the individual's adaptation to her/his environment. In adults, such responses are greatly influenced by previous experiences to stress, especially during the prenatal period (Charil et al., 2010; Finlay and Zigmond, 1997). In this sense, fluctuations in the uterine environment might be transmitted to the fetuses, inducing long term modifications in the structure and function of diverse tissues, increasing the risk of developing diseases in adult life (Cottrell and Seckl, 2009). It was reported that rats exposed to different types of stress during pregnancy produce offspring that show increased vulnerability to anxiety, depression, drug seeking behavior and learning deficits (Maccari and Morley-Fletcher, 2007; Weinstock, 2008). Moreover, phenotypes resembling schizophrenia, hypersensitivity to amphetamine, disrupted social behavior, impaired stress axis regulation and aberrant prefrontal expression of genes involved in synaptic plasticity were also reported (Koenig et al., 2005; Lemaire et al., 2000). The neurochemical basis of many of these disorders has been linked to impairments of the dopaminergic system (Baier et al., 2012), as well as to the serotonergic and noradrenergic systems (Suzuki et al., 2010). In addition, during the last years new evidence has emerged showing that maternal stress leads to malfunction of the glutamatergic system. Yaka et al. (2007) found a synaptic reduction in the GluR1 subunit of the AMPA receptor in the hippocampus of prenatally stressed rats. Similar results were reported in the same area with impaired function of NMDA receptors and reduced long term potentiation (Markham et al., 2010; Son et al., 2006). In prefrontal cortex, prenatal stress seems to affect the modulation of glutamatergic response and NMDA receptor subunits (Fumagalli et al., 2009).

Previous studies from our laboratory have shown that adult offspring of stressed rats exhibited higher levels of ionotropic (NMDA) and metabotropic (mGlu III) glutamate receptors in frontal cortex, striatum and hippocampus compared to control rats (Berger et al., 2002). These animals also show long-lasting astroglial reaction and a reduced dendritic arborization with synaptic loss (Barros et al., 2006a). Since glutamatergic functions are based on a tight relation between neurons and surrounding astroglia, our results suggest that the glutamate neurotransmitter pathway might be impaired in the brain of prenatally stressed offspring. With this hypothesis in mind we addressed a broad analysis of the effect of maternal stress on the glutamatergic synapse of the offspring covering the main steps of the neurotransmitter metabolism. We evaluated glutamate metabolism using [1-^{13}C]glucose injection combined with liquid chromatography–mass spectrometry (LC–MS) analysis of brain extracts, as well as glutamate release and the expression of NMDA and AMPA receptors. We also evaluated the expression and function of the transporters vGluT-1, GLT1 and GLAST in adult male and female offspring of prenatally stressed rats in two brain areas: FCx and HPC. Main findings point out that the expression of the transporters is changed by PS, showing a higher level of vesicular vGluT-1 in the frontal cortex (FCx) and elevated levels of GLT1 protein and messenger RNA in the hippocampus of adult male PS offspring. We provide novel data about the long-lasting consequences of prenatal stress on one of the major neurotransmission systems of the brain.

2. Materials and methods

2.1. Animal care

Virgin female Wistar rats weighing 250 g were obtained from outbred rats from the animal facility at the University of Buenos Aires. A maximum of five dams were housed per cage with ad libitum access to standard rat chow (Asociación de Cooperativas Argentinas, Buenos Aires, Argentina) and water. A constant light/dark cycle, with lights on at 06:00 and off at 18:00, and a temperature of 21–25 °C were maintained. Females were individually mated with a sexually experienced male Wistar rat. The day on which vaginal plug was found was designated as the first day of pregnancy. Care was taken to minimize the number of animals used. All procedures were in agreement with the standards for the care of laboratory animals as outlined in the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (Facultad de Medicina, Universidad de Buenos Aires).

2.2. Prenatal stress

Pregnant dams (n = 8) were randomly assigned to either the control (C) or the prenatal stress (PS) group and were individually