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CHRONIC ORAL ADMINISTRATION OF MPEP, AN ANTAGONIST OF $mGlu_5$ RECEPTOR, DURING GESTATION AND LACTATION ALTERS $mGlu_5$ AND A_{2A} RECEPTORS IN MATERNAL AND NEONATAL BRAIN

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Abstract—Antidepressant and anxiolytic drugs are widely consumed even by pregnant and lactating women. The metabotropic glutamate receptor 5 ($mGlu_5$) antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) exerts antidepressant- and anxiolytic-like actions. Given that treatment for anxiety and depression use to be prolonged in time, it is conceivable a possible modulation of metabotropic glutamate receptors (mGlu receptors) after prolonged MPEP exposure, which could also modify adenosine A_{2A} receptors ($A_{2A}R$) since functional cross-talk between them has been reported. Here we report that MPEP crosses placental barrier and reaches neonatal brain through maternal milk using LC–MS/MS methods. Therefore, we analyzed mGlu receptors, mainly $mGlu_5$, and $A_{2A}R$ in both maternal and fetal brain after chronic maternal consumption of MPEP during gestation and/or lactation using radioligand binding, Western-blotting, real-time PCR and phospholipase C (PLC) activity assays. In maternal brain, chronic MPEP consumption caused a significant loss of mGlu, including $mGlu_5$, and $A_{2A}R$ receptors level in plasma membrane. PLC activity assays showed that $mGlu_5$ signaling pathway was desensitized. No variations on mRNA level coding $A_{2A}R$, A_1R and $mGlu_5$ were found after MPEP treatments. In female neonatal brain, maternal consumption of MPEP caused a significant increase in mGlu, including $mGlu_5$, and $A_{2A}R$ receptors level. Neither mGlu receptors nor $A_{2A}R$ were modified in male neonatal brain after maternal MPEP intake. Finally, neither molecular nor behavioral changes (anxiety- and depression-like behavior) were observed in 3-month-old female offspring. In summary, $mGlu_5$ and $A_{2A}R$ are altered in both maternal and female neonatal brain after chronic maternal consumption of MPEP during gestation and/or lactation.

Key words: MPEP, gestation, lactation, $mGlu_5$, A_{2A}, brain.

INTRODUCTION

Evidence accumulated in the last two decades have suggested that a group of receptors named metabotropic glutamate (mGlu) receptors may represent a new pharmacological target for the treatment of CNS disorders such as anxiety and depression. mGlu receptors have been classified into three groups: group I ($mGlu_1$ and $mGlu_5$) which activate phospholipase C (PLC) activity and group II ($mGlu_2$ and $mGlu_3$) and group III ($mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$) which inhibit adenylate cyclase (Hermans and Challis, 2001; Pin and Acher, 2002; Pin et al., 2003). Particularly striking have been the results obtained using the antagonist of $mGlu_5$, 2-methyl-6-(phenylethynyl)-pyridine (MPEP) which have shown that MPEP exerts antidepressant-like and anxiolytic-like actions in several animal models (Spooren et al., 2000; Tatarcynska et al., 2001; Brodking et al., 2002; Li et al., 2006; Belozertseva et al., 2007). $mGlu_5$ is widely distributed in the CNS including brain regions such as hippocampus, olfactory bulb, cortex, striatum, thalamus and cerebellum (Hovelsø et al., 2012).

Although MPEP has been found only to be effective at pre-clinical level it is important to know whether mGlu receptors' density might suffer changes after prolonged MPEP exposure since treatments for anxiety and depression often require prolonged periods of time. In this context, the phenomenon of antagonist-induced receptor regulation is common to many G-protein-coupled receptor (GPCR) including adrenergic, muscarinic, opioid, cannabinoid, histamine, GABAB, serotonine, dopamine and adenosine receptor (Unterwald, 2011). In fact, we have recently reported the upregulation of $mGlu_5$ in male zebrafish brain 24 h after intraperitoneal injection of MPEP (Albasanz et al., 2016). Although the regulation of mGlu receptors follow-

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Abbreviations: ADA, adenosine deaminase; AMPA, (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; $A_{2A}R$, adenosine A_{2A} receptor; a.u., arbitrary units; DHPG, (S)-3,5-dihydroxyphenylglycine; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; EAAT2, excitatory aminoacid transporter 2; GFAP, Glial Fibrillary Acidic Protein; GPCR, G-protein-coupled receptor; L-Glu, L-glutamic; mGlu receptor, metabotropic glutamate receptor; $mGlu_5$, metabotropic glutamate receptor 5; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; NMDA, N-methyl-D-aspartic acid; [^{3}H]PIP_2, phosphatidylinositol-4,5-bisphosphate, [inositol-2-^{3}H(N)]; PLC, phospholipase C; [^{3}H]ZM241385^3, [2-H](4-(2-[7-amino-2(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl]phenol).

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ing prolonged antagonist exposure is less known, Domenici and co-workers (2005) showed that chronic treatment with MPEP (3 mg/kg/day) over two weeks did not modify $mGlu_5$ density in the striatum from adult rats.

Chronic exposure to MPEP could also affect other receptors such as adenosine A_{2A} receptor ($A_{2A}R$). Supporting this idea, Western-blotting analyses have shown the existence of complexes containing $A_{2A}R$ and $mGlu_5$ in striatal membranes from rat brain (Ferré et al., 2002). Furthermore, functional interactions between both receptors have been shown in neostriatal slices and striatal nerve terminals where coactivation of both receptors synergistically increased DAARP-32 phosphorylation and glutamate release (Nishi et al., 2003; Rodrigues et al., 2005). Although $A_{2A}R$ are highly expressed in the striatum, they are also found in other brain regions such as cerebral cortex and hippocampus (Svenningsson et al., 1999). In this context, Tebano and co-workers (2005) have also shown that $A_{2A}R$ and $mGlu_5$ colocalize and functionally interact in the hippocampus from rodent.

Nowadays, antidepressant and anxiolytic drugs are widely consumed by pregnant women. Thus, a recent work carried out in the United Kingdom has estimated that about 3% of women are prescribed antidepressant or anxiolytic medication during the first stage of gestation (Ban et al., 2012). These medicaments can easily cross placental barrier and reach fetal brain where may potentially alter neurotransmitter system and normal brain development in the offspring (El Marroun et al., 2014). To the best of our knowledge, there are no studies examining the effect of chronic MPEP during gestation and lactation on both maternal and neonatal brain. In the present work we show that chronic consumption of MPEP during gestation and/or lactation evoked a significant loss of mGlu receptors in maternal brain associated with a significant $mGlu_5$ receptor down-regulation and group I mGlu desensitization. We also found a significant down-regulation of $A_{2A}R$ in maternal brain in response to chronic MPEP treatment. Concerning neonates, results suggest that maternal consumption of MPEP during gestation or lactation upregulated mGlu receptors, and specifically $mGlu_5$, in female neonates, while no significant variations were found in male neonates. A similar pattern was found when $A_{2A}R$ was studied, suggesting that chronic maternal intake of MPEP during gestation or lactation altered $mGlu_5$ and $A_{2A}R$ in neonatal brain in a gender specific way.

EXPERIMENTAL PROCEDURES

Materials

L-[^{3}H]glutamic acid (49.9 Ci/mmol) and phosphatidylinositol-4,5-bisphosphate, [inositol-2-^{3}H(N)] – ([^{3}H]PIP_2 6.5 Ci/mmol) were from PerkinElmer (Madrid, Spain), [2-^{3}H](4-(2-[7-amino-2(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl]phenol) ([^{3}H]ZM241385 27.4 Ci/mmol) was from Tocris (Bristol, UK) and 2-methyl-6-([3,5 3H] phenylethynyl)pyridine ([^{3}H]MPEP, 60 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). L-glutamate, N-methyl-D-aspartic acid (NMDA), (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), (S)-3,5-dihydroxyphenylglycine (DHPG), kainite and MPEP were from Tocris (London, UK). Calf intestine adenosine deaminase (ADA) and theophylline were purchased from Sigma (Madrid, Spain). Anti-metabotropic glutamate receptor 5 ($mGlu_5$; rabbit polyclonal antibody) was acquired from Chemicon International (Temecula, CA, USA). β-Actin antibody (mouse monoclonal) and anti-Glial Fibrillary Acidic Protein (GFAP) antibody (mouse monoclonal) were from Abcam (Cambridge, UK). Anti-synapsin-I (polyclonal antibody) was from Millipore (Madrid, Spain). Goat anti-rabbit IgG (H + L)-HRP conjugate and goat anti-mouse IgG (H + L)-HRP conjugate were from Bio-Rad (Hercules, CA, USA). All other reagents were of analytical grade and obtained from commercial sources.

Animals

Wistar pregnant rats were obtained from Universidad Autónoma de Madrid (Spain). Wistar pregnant rats, kept on a 12-h light/12-h dark cycle (lights on at 07:00 h) and with free access to food and drinking water, were treated with MPEP (25 mg/L) in the drinking water from gestational day 2 onward during the entire gestational period. Eight control pregnant rats received drug-free tap water. Drinking bottles were pressed immediately before to place them in the cage in order to avoid any loss by leakage. This method allows air to flow into the bottle while it is correctly placed in the cage. Furthermore, we used leakage-proof bottles and no variation was detected. The drinking fluids were changed every 2 days. Tap water was either replaced by MPEP solution immediately after parturition (water + MPEP) ($n = 4$) or kept during lactation period (water + water) ($n = 4$). MPEP solution was replaced by water immediately after parturition (MPEP + water) ($n = 4$) or kept during lactation period (MPEP + MPEP) ($n = 4$). MPEP consumption was estimated daily from the loss of water from the drinking bottles. Fifteen days after parturition mothers and neonates were sacrificed and brains were then removed, frozen in liquid N_2 and stored at −80 °C until experiments were performed. A group of female neonatal brains (water + water and MPEP + MPEP) were dissected, collecting striatal regions and frozen in liquid N_2 and stored at −80 °C.

Once we knew that maternal MPEP intake during gestation and/or lactation induced a similar effect on both $mGlu_5$ and $A_{2A}R$ in female neonates we decided to investigate whether these effects were permanent and whether adult females showed anxiety- or depression-like behavior. For that purpose one pregnant rat was treated with MPEP (25 mg/L) during both periods. Fifteen days after parturition, MPEP solution was replaced by tap water. Offspring were weaned on postnatal day 21. Adult female offspring were assayed on elevated plus maze and forced swim test at the age of 3 months. Finally, rats were sacrificed by decapitation and brains were then removed, frozen in liquid N_2 and stored at −80 °C until experiments were performed.

All experiments followed the Spanish and European laws (Real Decreto 53/2013 and Directive 2010/63/EU))