SOCIETÀ ITALIANA DI FARMACOLOGIA

MODELLO PER INVIO RELAZIONE DI METÀ E FINE PERIODO

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TIPOLOGIA DI BORSA RICEVUTA: Borsa di ricerca SIF per soggiorno all'estero

TIPOLOGIA DI RELAZIONE : relazione di metà periodo

TITOLO DELLA RELAZIONE: Synaptic plasticity in Valproic acid model of autistic spectrum disorders

RELAZIONE:

Autistic Spectrum Disorder (ASD) is a heterogeneous set of neurodevelopmental disabilities. ASD is characterized by altered social interaction, compromised verbal and nonverbal communication, stereotyped and repetitive behaviours, often associated with comorbid features, such as social and generalized anxiety. Both genetic and environmental factors are involved in the etiology of ASD. Many of the ASD-associated genes are involved in brain development, cortical organization, synaptogenesis and neurotransmission. Among the environmental factors involved in the pathogenesis of ASD, it has been well documented that prenatal exposure to the antiepileptic drug valproic acid (VPA) is associated with increased risk of neurodevelopmental delay and autistic symptoms in the offspring. Indeed, when given during gestation, VPA not only increases the risk for various congenital malformations (8,10), but also induces core autistic symptoms in the offspring, i.e., impaired communication, reduced sociability and stereotyped behaviours.

The altered social behaviour displayed by VPA-exposed rats may be due to either a deficit in social reward processing or to a more general inability to properly understand and respond to social signals, which has been hypothesized to be the consequence of abnormal activity of the brain reward circuit in social contexts (1, 14). The most important brain areas involved in reward and reward-related learning are the ventral tegmental area (VTA), the nucleus accumbens (NAc), and the prefrontal cortex (PFC), that form mesocorticolimbic (MCL) pathway and which is involved in motivation and reward-related behavior. The NAc is a major target of the mesolimbic dopaminergic system and the overall impact of NAc output on behaviour depends on relative activity of D1- vs D2-expressing medium spiny neurons (MSNs), therefor given the important role of NAc dopamine in rewarding forms of social interaction such as social play (13), we addressed the role of the NAc in the social impairment displayed by VPA-exposed rats (500 mg/Kg) by performing behavioural, neurochemical electrophysiological experiments in this brain area. Along this line, previous studies in our laboratory have

shown that prenatal exposure to VPA causes selective deficits in the social domain in the rat offspring such as reduced play responsiveness, sociability and social discrimination compared with SAL-exposed animals, confirm here that adolescent rats prenatally exposed to VPA show decreased social reward-related behaviours. Moreover since the activation of D1 and D2 receptors in corticolimbic regions is also important for the expression of social behavior in rodents (4, 16, 13, 9), we hypothesized that the social deficit displayed by VPA-exposed rats may be due to changes in dopaminergic neurotransmission in corticolimbic brain areas. We found that, compared to control animals, adolescent rats prenatally exposed to VPA showed increased expression of D2 dopamine receptors in the NAc. Starting from this evidence, we found that NAc MSNs of VPA-exposed animals show a significant depolarization of the resting membrane potential and increased excitability indicating higher firing probability in conditions of normal synaptic excitation (3). These changes are likely caused by altered Kir current density, known to determine the hyperpolarized value of membrane potential in normal MSNs (~-85 mV) and to affect their AP discharge pattern (11). Collectively, these findings suggest an increase in intrinsic excitability of MSNs of VPA-exposed animals. Proper activity of direct and indirect pathways underlies proper motor learning as well as the acquisition of reward-related behaviours. Moreover, since changes in local and distant connectivity in the brain have been proposed as a possible cause of autistic behaviour (4), we investigate the synaptic transmission in VPA rats model and, in preliminary data, we found that VPA-exposed rats show an increases in glutamatergic transmission. This suggests a perturbation of the excitatory/inhibitory in VPA-exposed rats. Moreover NAc is characterized by endocannabinoid (eCB)-mediated long term depression (LTD) originally discovered in the NAc/ventral striatum (15). The mesocorticolimbic eCB system modulates a vast array of synaptic functions (15) and plays a key role in brain development. eCB dysfunction is implicated as a major causal factor synaptopathies linked to the NAc (7, 12, 6) and it has been recently involved in ASD (17). For the first part of this project I investigate (1) excitatory/inhibitory synaptic transmission in Saline and VPA-exposed rats (2) eCB-mediated long term depression in Saline and VPA-exposed rats (3) synaptic properties of Baso-lateral amygdala (BLA)-NAc pathway.

MATERIALS AND METHODS

Animals

On gestational day 12.5, females Wistar rats received a single intraperitoneal injection of either VPA or saline (SAL) (500mg/Kg). The experiments were carried out on the male offspring during adolescence (PNDs 30–40) and adulthood (PNDs 90–95). One male pup per litter from different litters per treatment group was used in each experiment.

Virus injection

Microinjection needles (32G) were connected to a 10 µL Hamilton syringe and filled with purified, concentrated adeno-associated virus (1.98x1013 infectious units per mL) encoding hChR2-EYFP under control of the CaMKIIα promoter (University of Pennsylvania, Philadelphia, 133 USA). Mice were anesthetized with 150 mg/kg ketamine and 50 mg/kg xylazine and placed in a stereotaxic frame. Microinjection needles were bilaterally placed into basolateral amygdala (Coordinates: AP=±1.9mm; ML=±4.6mm; DV=±7.5mm) and 250 nL virus was injected with a speed of 100 nL/min. The needles were left in place for an additional 5 min to allow for diffusion of virus particles away from injection site.

Slice preparation

Adolescent male rats (P30-P40) were deeply anesthetized with isoflurane and sacrificed. The brain was sliced (300 µm) on the coronal plane in a sucrose-based solution at 4°C (in mm as follows: 87 NaCl, 75 sucrose, 25 glucose, 2.5 KCl, 4 MgCl2, 0.5 CaCl2, 23 NaHCO3 and 1.25 NaH2PO4). Immediately after cutting, slices containing the NAc were stored for 1 h at 32°C in a low calcium ACSF that contained (in mm) as follows: 130 NaCl, 11 glucose, 2.5 KCl, 2.4 MgCl2, 1.2 CaCl2, 23 NaHCO3, 1.2 NaH2PO4, and were equilibrated with 95% O2 5% CO2 and then at room temperature until the time of

recording.

Whole-Cell Patch Clamp and Extracellular field Recordings in Acute Brain Slices

For whole-cell patch-clamp experiments, neurons were visualized using an upright microscope with infrared illumination. The intracellular solution was based on K+gluconate (in mM as follows: 145 K+ gluconate, 3 NaCl, 1 MgCl2, 1 EGTA, 0.3 CaCl2, 2 Na+ ATP, and 0.3 Na+GTP, 0.2 163 cAMP, buffered with 10 HEPES). The pH was adjusted to 7.2 and osmolarity to 290-300 mOsm. Electrode resistance was 4-6 MOhm. Current-voltage (I-V) curves were made by a series of hyperpolarizing to depolarizing current steps for evaluate MSN excitability. Excitatory/ Inhibitory post-synaptic currents were recorded in presence of GABAZINE (GABA-A antagonist) and NBQX and APV (AMPA-NMDA antagonists) respectively. While miniature events were recorded in presence of TTX, a voltage-dependent sodium channels blocker. Both spontaneous/miniature post-synaptic currents were recorded at -70 mV.

For extracellular field experiments, the recording pipette was filled with ACSF. The glutamatergic nature of the field EPSP (fEPSP) was systematically confirmed at the end of the experiments using the ionotropic glutamate receptor antagonist CNQX (20 μM), which specifically blocked the synaptic component without altering the non-synaptic component.

RESULTS

Previous preliminary experiments have shown that VPA-prenatal exposure modified NAc-MSN synaptic transmission in VPA-exposed rats, based on these results in the first part of the research I recorded both spontaneous (s)/miniature (m) excitatory(E) post-synaptic currents (s/mEPSCs) and inhibitory (I) post-synaptic currents (s/mIPSCs) in control rats (PND 30-40). I found that VPA-prenatal exposure significantly reduces the amplitude of sEPSCs and increases the frequency of mEPSCs in VPA-NAc-MSN compared to control group. Moreover cumulative distribution function of mEPSCs in exposed rats was different compared control group. These data can indicate alterations in connectivity and synaptic strength in VPA-exposed rats. In the second part of experiments I studied eCB-LTD in control rats (PND 30-40) in which low frequency stimulation protocol produces significant depression. eCB-LTD was analyzed by comparing fEPSP amplitudes before and after 40 min after 10 Hz stimulation train. Finally I recorded both sIPSCs/ mIPSCs in control rats. Unfortunately in both eCB-LTD and sIPSCs/ mIPSCs experiments I used only control animals because of COVID-19 emergency. I could not conclude these experimental groups with VPA-exposed rats since all researches have been suppressed starting from March the 16. At the end of the emergency I will record sEPSCs/ mEPSCs, sIPSCs/ mIPSCs and eCB-LTD in VPA-exposed rats. Secondly, I will record EPSCs evoked by local photostimulation of ChR2-positive glutamatergic terminals for study pathway specific plasticity to understand if VPA-prenatal exposure causes synaptic changes in pathway specific manner.

References

1.Chevallier C, Kohls G, Troiani V, Brodkin ES, Schultz RT. The social motivation theory of autism. Trends in cognitive sciences. 2012;16(4):231-9. Epub 2012/03/20.

2.Cazorla M, Shegda M, Ramesh B, Harrison NL, Kellendonk C. Striatal D2 receptors regulate dendritic morphology of medium spiny neurons via Kir2 channels. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2012;32(7):2398-409. Epub 2012/03/08.

3.Iezzi D, Schiavi S, Manduca A, Leone S, Melancia F, Carbone C, Petrella M , Mannaioni G, Masi A, Trezza V. Reward-Related Behavioral, Neurochemical and Electrophysiological Changes in a Rat Model of Autism Based on Prenatal Exposure to Valproic Acid. Front. Cell. Neurosci., 25 October 2019

4.Geschwind and Levitt. Autism spectrum disorders: developmental disconnection syndromes. Current opinion in Neurobiology,2007

5. Gunaydin LA, Grosenick L, Finkelstein JC, Kauvar IV, Fenno LE, Adhikari A, et al. Natural neural projection dynamics underlying social behavior. Cell. 2014;157(7):1535-51. Epub 2014/06/21.

6.Jung K-M, Sepers M, Henstridge CM, Lassalle O, Neuhofer D, Martin H, Ginger M, Frick A, DiPatrizio NV, Mackie K,

Katona I, Piomelli D, Manzoni OJ (2012) Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. Nat Commun 3:1080.
7.Kasanetz F, Deroche-Gamonet V, Berson N, Balado E, Lafourcade M, Manzoni O, Piazza PV (2010) Transition to addiction is associated with a persistent impairment in synaptic plasticity. Science 328:1709–1712.
8. Kini U, Adab N, Vinten J, Fryer A, Clayton-Smith J. Dysmorphic features: an important clue to the diagnosis and severity of fetal anticonvulsant syndromes. Archives of disease in childhood Fetal and neonatal edition. 2006;91(2):F90-5. Epub 2005/10/22.
9. Kopec AM, Smith CJ, Ayre NR, Sweat SC, Bilbo SD. Microglial dopamine receptor elimination defines sex-specific nucleus accumbens development and social behavior in adolescent rats. Nature communications. 2018;9(1):3769. Epub 2018/09/27.
10. Kozma C. Valproic acid embryopathy: report of two siblings with further expansion of the phenotypic abnormalities and a review of the literature. American journal of medical genetics. 2001;98(2):168-75. Epub 2001/02/27.
11. Kreitzer AC. Physiology and pharmacology of striatal neurons. Annual review of neuroscience. 2009;32:127-47. Epub 2009/04/30.
12.Lafourcade M, Larrieu T, Mato S, Duffaud A, Sepers M, Matias I, De Smedt-Peyrusse V, Labrousse VF, Bretillon L, Matute C, Rodríguez-Puertas R, Layé S, Manzoni OJ (2011) Nutritional omega-3 deficiency abolishes endocannabinoid-mediated neuronal functions. Nat Neurosci 14:345–350.
13. Manduca A, Lassalle O, Sepers M, Campolongo P, Cuomo V, Marsicano G, et al. Interacting Cannabinoid and Opioid Receptors in the Nucleus Accumbens Core Control Adolescent Social Play. Frontiers in behavioral neuroscience. 2016;10:211. Epub 2016/12/03.
14. Pellissier LP, Gandia J, Laboute T, Becker JAJ, Le Merrer J. mu opioid receptor, social behaviour and autism spectrum disorder: reward matters. British journal of pharmacology. 2018;175(14):2750-69. Epub 2017/04/04.
15.Robbe D, Gérard A, Séverine C, Joe B, Manzoni OJ. Role of P/Q-Ca2+ Channels in Metabotropic Glutamate Receptor 2/3-Dependent Presynaptic Long-Term Depression at Nucleus Accumbens Synapses. The Journal of Neuroscience, June 1, 2002, 22(11):4346–4356
16. Robinson DL, Heien ML, Wightman RM. Frequency of dopamine concentration transients increases in dorsal and ventral striatum of male rats during introduction of conspecifics. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2002;22(23):10477-86. Epub 2002/11/27.
17.Zamberletti E, Gabaglio M, Parolaro D (2017). The endocannabinoid system and autism spectrum disorders: insights from animal models. Int J Mol Sci 18 10.3390/ijms18091916.

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