PREGNENOLONE SULFATE ENHANCEMENT OF GABA RELEASE IN THE DEVELOPING RAT CEREBELLUM IS MEDIATED BY PRESYNAPTIC NMDARS AND IS MODULATED BY ETHANOL

Mario Carta, L. Murru, F. Virdis, M.D. Brockmann, R. Marras, E. Sanna, G. Biggio

Dept. Experimental Biology, Sect of Neuroscience, and Center of Excellence for the Neurobiology of Dependence. Cittadella Universitaria, SS 554 Km 4,500, 09042, Monserrato, Cagliari, Italy

The neurosteroid pregnenolone sulfate (Pregs) is thought to have a key role in brain development. Recent findings demonstrate that Pregs, acting on presynaptic NMDARs expressed in neonatal rat hippocampus, enhances glutamate release contributing to synapse maturation. Moreover, it has been shown that ethanol (EtOH) exposure in early postnatal life increases synthesis and local release of Pregs. This enhanced Pregs production may account for abnormal hippocampal wiring and for impairment of cognitive function in rodent model of Fetal Alcohol Syndrome (FAS). Early life exposure to ethanol (EtOH) induces, in the cerebellum, death of Purkinje and granule cells as well as deficits in motor coordination in animal models of FAS. Activation of presynaptic NMDARs present in the terminal of interneurons in the cerebellar cortex facilitates GABA release onto Purkinje cells (PCs). We examined if Pregs was able to modulate GABA release onto PCs and if ethanol could regulate this effect. Whole cell patch clamp recording of miniature inhibitory postsynaptic currents (mIPSCs) were performed from PCs in saggital cerebellar slices prepared from Sprague-Dawley rats (V_{hold}= -65 mV). We found that, in slices prepared from P8 rats, perfusion with Pregs (20 µM) transiently and significantly increased the frequency (242±58 % over control; $p< 0.01$; n=8), but not the amplitude (4±2 % over control; n=8), of mIPSCs. Similar results were obtained in slices from P4 rats, but no effect was observed in slices from P24 rats. Co-perfusion of Pregs (20 µM) with the NMDA-R antagonist DL-AP5, but not the AMPA/KA-Rs antagonist NBQX, blocked the increase in frequency (33±15 % over control; n=5). Finally, when Pregs (20 µM) was perfused with EtOH (50 mM) a larger augmentation of the mIPSCs frequency was observed (1902±671 % over control; $p<0.01$; n=9). This effect was blocked by the application of DL-AP5 (43±41 % over control; n=2). We found that Pregs, acting on NMDARs located on the presynaptic terminal of in the cerebellar cortex, increases the probability of GABA release onto PCs. This effect is age dependent. EtOH enhances the modulatory effect of Pregs with a mechanism that is currently under investigation. Given that GABA at the early developmental stage is excitatory, it is possible that an enhanced GABA-induced depolarization can account for the teratogenic action of EtOH in FAS animals.