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Andrew W. Zimmerman and Susan L. Connors

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NEUROSCIENCE

Could Autism Be Treated Prenatally?

Treatment of rodent models of autism spectrum disorder with a drug that alters the function of a neurotransmitter ameliorates autistic-like behavior in offspring.

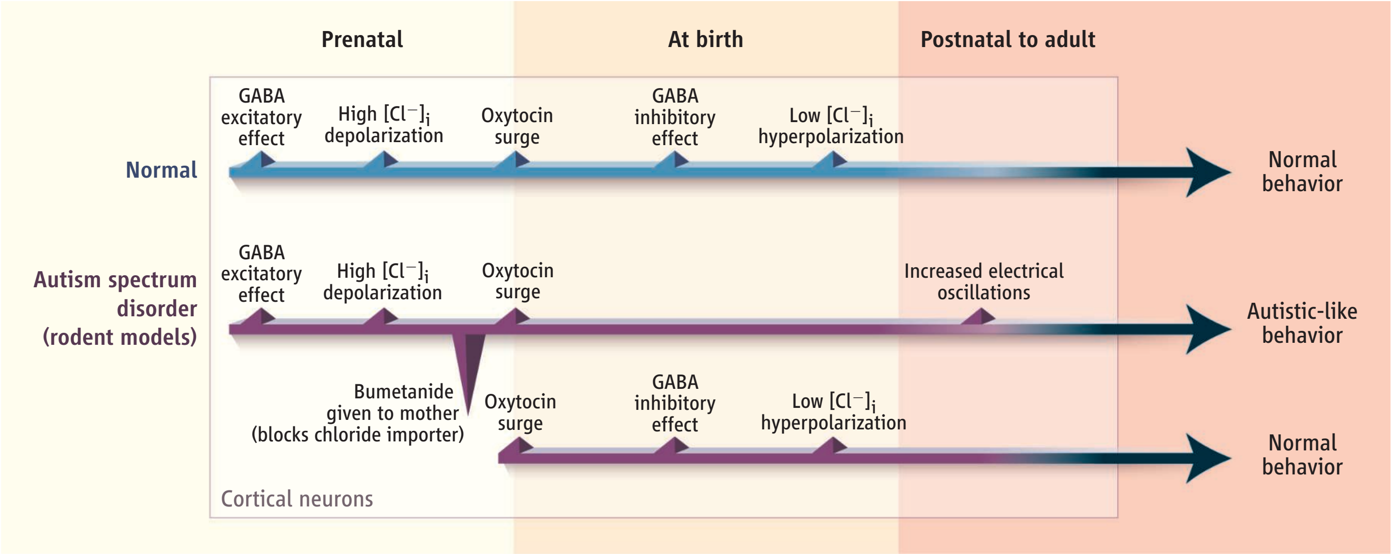
Andrew W. Zimmerman[1] and Susan L. Connors[2]

Autism spectrum disorder (ASD) has presented a conundrum: How can the behavioral signs and symptoms that define the condition arise from different etiologies and lead to so many varied phenotypes? Genetics and the environment, including prenatal and perinatal factors, long have been suspected to interact in the causation of ASD. Evidence for neuronal dysfunction and the frequent development of epilepsy strongly support increased excitatory and decreased inhibitory neuronal activity in ASD. In particular, altered functions of γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter (in the mature brain), have been of interest because GABA's effects are excitatory during prenatal development but become inhibitory at birth (*1*, *2*). What has been unclear is the cellular physiology that underlies this "GABA switch." On page 675 of this issue, Tyzio *et al*. (*3*) show that a defect in this switch is associated with abnormal chloride concentration in neurons in two different animal models of ASD. Normal electrophysiology and behavior can be restored in their offspring by the prenatal administration of the compound bumetanide, which blocks a key chloride transporter. The findings raise the possibility of preventing the autistic phenotype in offspring by predelivery pharmacological treatment.

The switch in GABA activity in cortical neurons results from a shift in intracellular chloride concentration ($[\text{Cl}^-]_i$) that is controlled by two membrane proteins, NKCC1 and KCC2 (a chloride importer and exporter, respectively). Changes in the expression of these chloride transporters lead to a progressive increase in the inhibitory effect of GABA during early brain development. The GABA switch at birth is sensitive to the hormone oxytocin. Maternal oxytocin initiates an abrupt reduction of intracellular chloride and an increase in GABAergic neuron inhibition in the fetal brain. These effects are neuroprotective and play a role in organizing ongoing early brain development (*4*, *5*). The GABA switch is also sensitive to the drug bumetanide, an antagonist of the NKCC1 chloride importer.

Multiple genes have been described that predispose to ASD, such as the causative gene of fragile X syndrome (FRX) [called *Fragile X Mental Retardation 1* (*FMR1*)] and environmental factors such as prenatal exposure to the anticonvulsant valproic acid (VPA). Tyzio *et al*. examined two seemingly unrelated rodent models of ASD: mice lacking the *Fmr1* gene (referred to as FRX mice) and rats exposed to VPA at mid-gestation. The authors found that the GABA switch can be prevented at birth in normal rats by an oxytocin receptor antagonist, thereby producing postnatal "autistic-like" behavioral changes similar to those seen in VPA rats and FRX mice. The behavior changes have been associated with persistently elevated $[\text{Cl}^-]_i$, which drives GABA's excitatory effects as well as oscillations in electrical activity that continue into adulthood. In both FRX mice and VPA rats, the GABA switch is abolished in hippocampal neurons; treatment of cells in both animals with isoguvacine, a GABA receptor agonist, increased, rather than decreased, neuronal excitation. The GABA switch was recently found to be delayed in FRX mice, along with altered expression of the chloride transporters (*6*).

Discovery of a cellular abnormality (change in $[\text{Cl}^-]_i$) that is common to two disparate animal models of ASD implies that the GABA switch can be modified by either a genetic factor or an external factor applied as late as mid-gestation. There may be many ultimate causes for failure of the GABA switch



Treat and switch. The switch from excitatory to inhibitory GABAergic signaling in rodent cortical neurons is mediated by oxytocin during the transition from prenatal to postnatal life. In rodent models of ASD (the FRX mouse and VPA rat), the normal shift from high to low intracellular chloride concentration does not take place but can be restored to normal in both cases by prenatal maternal oral administration of bumetanide.

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at birth and during postnatal brain development. The second half of gestation in humans is a period of rapid development of the cortical GABAergic system that continues into infancy (*7*). During this time, this GABAergic network may be vulnerable to insults at many levels, in addition to genetic susceptibility and epigenetic regulation (*8*). Obstetrical complications, prematurity, and perinatal injuries have been nonspecifically associated with ASD and intellectual disability (*9*), and all might act through one or more parts of the mechanism that shifts $[\mathrm{Cl}^-]_i$, along with the critical effects of oxytocin and its receptor. Abnormalities of GABA signaling have also been associated with neonatal seizures (*10*) and the genetic risk for schizophrenia (*11*).

The use of exogenous oxytocin for the initiation or augmentation of labor in humans has been the focus of much speculation as a possible cause of ASD. For example, oxytocin has been associated with increased odds of ASD, especially in male children (*12*). However, it is possible that pregnancy conditions that lead to the administration of oxytocin may predetermine abnormal development of GABA-associated physiology. It is also possible that improved obstetric and neonatal care allow survival of infants with preexisting brain damage (*13*). Endogenous maternal oxytocin is essential for the switch from excitatory to inhibitory GABA activity in the fetal brain during delivery, but it is not known whether additional exogenous oxytocin during delivery (in animals or humans) may ameliorate abnormally shifting $[\mathrm{Cl}^-]_i$. Unfortunately, it is not possible to measure intracellular $[\mathrm{Cl}^-]_i$ directly in humans. There is strong evidence, however, for abnormal amounts of GABA, GABA receptors, and enzymes that synthesize GABA (GAD65 and 67) in ASD (*2*). The chloride transporters can also be measured in cerebrospinal fluid, and a reduced KCC2/NKCC1 ratio has been reported in Rett syndrome, a neurodegenerative developmental disorder (*14*).

Successful treatment of both the FRX and VPA rodent models of ASD by maternal oral administration of bumetanide 1 day before delivery is the most promising finding of Tyzio *et al.* (see the figure). The authors show that abnormal electrophysiological and behavioral characteristics can be restored by correcting $[\mathrm{Cl}^-]_i$. Treatment with bumetanide has already been shown to ameliorate autistic symptoms in a clinical trial of 3- to 11-year-old children with ASD, which suggests that abnormal $[\mathrm{Cl}^-]_i$ may be a persistent and a treatable feature of ASD beyond infancy (*15*). Given the increased emphasis on early detection of ASD and discovery of its biomarkers, the possibility for perinatal treatment with an agent such as bumetanide is an enticing possibility for the prevention or early treatment of the disorder. However, this would require an accurate way to determine whom to treat because symptoms of ASD often do not appear until the second year of life. With this new insight into a convergent pathogenic mechanism downstream from different etiologies, we may now begin to understand the variability, as well as sameness, among people with ASD and related disorders.

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CHEMISTRY

Envisioning the Bioconversion of Methane to Liquid Fuels

Robert J. Conrado and Ramon Gonzalez

Advances in enzymatic pathways and bioreactor design could allow microorganisms to transform methane into chemicals and fuels.

Efforts to use natural gas in transportation, either directly or by conversion to a liquid fuel, have been spurred by recent increases in available supply and a growing price spread between natural gas and petroleum, especially in the United States (*1*). Conversion of natural gas-to-liquids (GTL) can take advantage of existing engine and delivery infrastructure, but GTL approaches operate on scales similar to that of petroleum refineries and suffer from low energy and carbon efficiencies, as well as high capital cost (*2*). Small-scale methane sources that are often flared or vented and that add greenhouse gas emissions also need an economical route for recovery. Biological methane conversion has the potential to directly activate methane at ambient temperatures and pressures on a scale similar to that of sugar fermentation (*3*) and could circumvent partial oxidation routes used industrially that dominate costs and reduce efficiency. Further process simplification is possible by one-step conversion, producing a single-molecule product and reducing the need for heat integration.

Despite these opportunities, aerobic methanotrophs represent the only available route for methane bioconversion, activating methane to methanol via methane monooxygenase (MMO) (*4*, *5*) and subsequently converting methanol to formaldehyde en route to fuel production. However, aerobic methane bioconversion has two primary challenges: low energy and carbon efficiencies and low-productivity cultures. To access small-scale and time-varying resources, process intensification leading to an order-of-magnitude increase in volumetric productivities is needed and will require technical breakthroughs in three areas—high-efficiency methane activation routes, alternative pathways for conversion of an activated intermediate to a liquid fuel, and high-productivity bioreactors.

High-Efficiency Methane Activation

Direct application of the MMO pathway to activate the strong C–H bonds in methane would face several challenges. One is that MMO requires a reduced electron carrier to activate the dimetal active site, and no energy is captured in the subsequent oxidation of methane to methanol. Thus, the production of a reduced energy carrier

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