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Oxytocin-Mediated GABA Inhibition During Delivery Attenuates Autism Pathogenesis in Rodent Offspring

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We report that the oxytocin-mediated neuroprotective γ-aminobutyric acid (GABA) excitatory-inhibitory shift during delivery is abolished in the valproate and fragile X rodent models of autism. During delivery and subsequently, hippocampal neurons in these models have elevated intracellular chloride levels, increased excitatory GABA, enhanced glutamatergic activity, and elevated gamma oscillations. Maternal pretreatment with bumetanide restored in offspring control electrophysiological and behavioral phenotypes. Conversely, blocking oxytocin signaling in naive mothers produced offspring having electrophysiological and behavioral autistic-like features. Our results suggest a chronic deficient chloride regulation in these rodent models of autism and stress the importance of oxytocin-mediated GABAergic inhibition during the delivery process. Our data validate the amelioration observed with bumetanide and oxytocin and point to common potential locus of analog information storage and pathological, a rich variety of antigens. (v) Pathological conditions that alter [Al], or [Cl], will have secondary effects on both cell volume and [Cl] . This may explain the correlation between magnetic resonance imaging evidence of cytotoxic edema after brain injury and anticonvulsant-resistant seizures, which can occur when increased [Cl] compromises GABA, mediated inhibition (15, 40). Therefore, the magnitude and direction of GABA A R currents at individual synapses are among the wide variety of signaling functions subserved by intra- and extracellular macromolecular networks.

References and Notes

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Supplementary Materials
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A

utism is a developmental disorder characterized by restricted interest and communication impairment generated by genetic and environmental factors. Alterations of oxytocin signals that trigger labor and are instrumental for communication, notably, parental-infant interactions, are important in autism (1). Here, we characterized the cellular and network alterations that occur during the transition from fetal to postnatal life and subsequently in two animal models of autism: rats exposed in utero to valproate (VPA rats) and mice carrying the fragile X mutation (FRX mice). We focused on GABAergic inhibition, as this is deficient in human and animal models of autism, which leads to an imbalance between excitation and inhibition (2–4). In addition, during development, GABAergic currents shift from excitatory to inhibitory (5) because of a reduction

of intracellular chloride concentration ([Cl] i) mediated by a sequential expression of the main chloride importer (Na+–K+–2Cl− cotransporter, NKCC1) and the main chloride exporter, KCC2 (6). Delivery in rodents is fundamental in this sequence, with an abrupt oxytocin-mediated reduction of [Cl] i levels that exerts neuroprotective (7) and analgesic (8) actions on newborns. We report that this sequence is abolished in hippocampal CA3 pyramidal neurons of VPA rats and FRX mice, and its restoration by administering bumetanide to the mother rescues the GABA developmental sequence and the autistic phenotype in rodent offspring.

In naïve rats (Fig. 1A and table S1) [see also (7) and wild-type mice (Fig. 1D and table S1), the driving force of γ-aminobutyric acid type A (GABA A receptor GABA A R (DFGABA A ) was elevated in neonatal brains from normal pups and FRX mice, but was not elevated in fetal brains from normal mice (20)]. In contrast, DFGABA A remained elevated in

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fetal, early postnatal stages, and P15 to P30 in VPA rats (Fig. 1A and table S1) and FRX mice (Fig. 1D and table S1). Acute applications of the specific NKCC1 chloride importer antagonist bumetanide (10 μM) or oxytocin (1 μM) significantly decreased [Cl\textsuperscript{–}], and ΔF\textsubscript{GABA} at P0 in neurons recorded in VPA rats and FRX mice (Fig. 1, B, C, E, and F; and table S2). Therefore, the GABA developmental sequence is abolished in two animal models of autism, with GABA exerting depolarizing actions in a bumetanide and oxytocin-sensitive manner.

The chloride exporter KCC2 is down-regulated after various insults leading to elevated [Cl\textsuperscript{–}], hyperactivity, and more KCC2 down-regulation (10–12). KCC2 was down-regulated in the hippocampi of juvenile VPA rats and FRX mice (fig. S1, A to C, and table S3). In addition, as in epileptic neurons (10), there was a shift of KCC2 labeling from the membrane to the cytoplasm in neurons of VPA rats (fig. S1, D and E, and table S4). Thereby, chloride export is reduced in two animal models of autism, which supports the observed alterations of the polarity of GABA actions.

We next evaluated whether the depolarizing actions of GABA were associated with neuronal excitation. In naïve animals, the specific GABA\textsubscript{A}R agonist isoguvacine (10 μM) inhibited or did not affect spike frequency in cell-attached recordings at P0 (Fig. 1, G to J, and table S5) and P15 (fig. S2, 7FEBRUARY2014VOL343SCIENCEwww.sciencemag.org676).
Fig. 2. Maternal pretreatment with bumetanide before delivery switches the action of GABA from excitatory to inhibitory in offspring in VPA and FRX rodents at P15. (A) Average values of $D_{GABA}$ measured in hippocampal CA3 pyramidal neurons at P15 in control (black), VPA (red), and VPA rats pretreated with bumetanide (blue). Note that pretreatment with bumetanide shifts $D_{GABA}$ from depolarizing to almost isoelectric level. (B) Effects of isoguvacine (10 μM; black bars) in rats: Representative traces of spontaneous extracellular field potentials recorded in hippocampal slices at P15 in control, VPA, and VPA rats pretreated with bumetanide (BUM). Corresponding time courses of spike frequency changes are shown under each trace. (C) Average histograms of normalized spike frequency in rats. Isoguvacine (hatched bars) decreased the spikes frequency in control rats (to 38.9 ± 5.1%; gray); increased it in VPA rats (to 213.5 ± 16.3%; red); and decreased it in VPA rats pretreated with bumetanide (to 82.8 ± 10.7%; blue). (D) The same as in (A) for mice. Wild-type mice (WT, black), FRX mice (red), and FRX mice pretreated with bumetanide (blue). (E) The same as in (B) for FRX mice. (F) The same as in (C) for FRX mice. Wild-type mice (decreased to 67.9 ± 6.1%; gray); FRX mice (increased to 165.8 ± 13.5%; red); FRX mice pretreated with bumetanide (decreased to 80.8 ± 8.2%; blue). Data are presented as means ± SEM. **$P < 0.01$; ***$P < 0.001$.

Fig. 3. Spontaneous activity is increased in VPA and FRX rodents at P15 and restored to control values by maternal pretreatment with bumetanide. Whole-cell voltage clamp recordings of sEPSCs at –70 mV from individual hippocampal CA3 pyramidal neurons in acute brain slices from P15 VPA rats or FRX mice and respective control and bumetanide or SSR126768A pretreated animals. (A and C) Representative traces of sEPSCs recorded from rats (A) and mice (C). Note that maternal pretreatment of animals with bumetanide decreases sEPSCs frequency in both models, whereas treatment with SSR126768A increases spontaneous activity of neuronal networks in rats and mice. (B) Average values of sEPSCs frequencies in rats: Control rats (gray) and VPA rats (red), VPA rats with maternal pretreatment with bumetanide (blue) and SSR126768A-treated rats (orange). (D) The same as (B) for mice. Wild-type mice (gray), FRX mice (red), FRX mice with maternal pretreatment with bumetanide (blue), and SSR126768A-treated mice (orange). One-way analysis of variance (ANOVA) Fisher’s least significant difference as a post hoc test. Data are presented as means ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. 

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A and C, and table S7) and in field-potential recordings at P15 (Fig. 2, B and E, and table S11). In contrast, isoguvacine increased spike frequency in neurons of VPA rats and FRX mice in cell-attached recordings at P0 (Fig. 1, G to J) and P15 (Fig. S2, B and D, and Fig. 1, H and J) and in field-potential recordings at P15 (Fig. 2, B, C, E, and F). Hence, GABA excites newborn and juvenile neurons recorded in VPA rats and FRX mice.

We next determined whether excitatory GABA is associated with enhanced network activity. In hippocampal slices of VPA rats and FRX mice, there was bursting activity at P0 and a fourfold increase of the frequency of glutamatergic spontaneous excitatory postsynaptic currents (sEPSCs) at P0 (fig. S3, A to C and F to H; and tables S6 to S8) and P15 (fig. S3, D, E, I, and J; and table S9). Application of bumetanide restored control glutamatergic sEPSC frequency at P0 (fig. S3, B and G, and table S7) and P15 (fig. S3, E and J, and table S9). Therefore, developing networks of VPA rats and FRX mice are hyperactive with bumetanide-sensitive enhanced glutamatergic activity likely due to GABAergic excitation impinging on principal cells.

We then tested the hypothesis that restoring low [Cl]– and inhibitory GABA actions during delivery rescues naïve electrophysiological features in juvenile offspring. We treated pregnant VPA rats and FRX mice females orally 1 day before delivery with bumetanide (2 to 2.5 mg/kg in drinking water) and recorded neuronal activity in offspring at P15. Bumetanide pretreatment restored control DfGABA values (Fig. 2, A and D, and table S10), suppressed the excitatory actions of isoguvacine (Fig. 2, B, C, E, and F; fig. S4; and tables S11 and S12), and significantly reduced ongoing activity and frequency of glutamatergic sEPSCs (Fig. 3 and table S9). Thus, elevated [Cl]– and excitatory GABA actions at birth produce long-term effects in juvenile VPA rats and FRX mice that can be restored by maternal pretreatment with bumetanide.

As the perinatal excitatory-to-inhibitory shift of GABA is mediated by oxytocin receptors (7), we tested the effect of a selective oxytocin receptor antagonist SSR126768A in naïve rodents. Pretreatment of naïve mothers one day before delivery with SSR126768A in drinking water produced in juvenile rats elevated DfGABA (fig. S5A and table S13), excitatory GABA actions (fig. S5B and table S14), and exacerbated glutamatergic activity (Fig. 3 and table S9). Therefore, blocking oxytocin signals during delivery in naïve animals produces actions similar to those observed in the VPA rats and FRX mice and stresses the importance of the oxytocin-GABA link.

We then used behavioral tests to determine whether treatment of mothers with bumetanide shortly before delivery prevents autistic-like behaviors in offspring. The isolation-induced ultrasonic vocalizations that pups emit when separated from their mothers (9, 13) were reduced in P4 VPA rats with fewer calls and shorter total call durations than age-matched control rats. In addition, FRX mice (P8) had a higher probability of emitting downward and chevron calls than age-matched wild-type mice (Fig. 4A and table S16). Maternal pretreatment with bumetanide rescued this
behavioral alteration in VPA rats (Fig. 4A and table S16) and FRX mice (Fig. 4B and table S16). Furthermore, offspring of naïve mothers pretreated with SSR126768A to block oxytocin signals had an increased probability of emitting downward calls (P8, mice) and a longer latency to reach home bedding than age-matched control pups in the nest-seeking test (P9 rats) (fig. S5C and table S15). Therefore, bumetanide restores naïve behavior in VPA rats and FRX mice, and blocking oxytocin signaling produces behavioral alterations and autistic-like features.

Finally, as alterations of gamma oscillations have been observed in patients with autism (14), we tested whether similar changes occur in vivo in VPA rats. With intracranial electroencephalographic (EEG) recordings in the hippocampal CA3 region, hyperactivity was observed in VPA (P15) but not in age-matched naïve rats. These included enhanced network oscillation power in a broad spectrum of frequencies, including gamma but excluding fast ripples and very low (δ) frequencies. Maternal pretreatment with bumetanide restored physiological values in offspring (Fig. 4, C to E, and table S17). Therefore, the polarity of GABA actions during delivery exerts long-term effects on brain oscillations in VPA rats.

During parturition, the human fetus is subjected to an important stress associated with a high surge of catecholamine levels. This adapts neonates to extrauterine life by promoting lung maturation and increased cardiovascular performance and blood flow to the brain (15). However, in rodents, elevated catecholamine levels produce KCC2 down-regulation, elevated [Cl−] levels, excitatory GABA, and neuronal hyperactivity (16, 17) that are prevented during delivery by oxytocin (7). Similar deleterious alterations are observed in epilepsies and other pathologic conditions (10–12, 18). It is noteworthy that complicated deliveries have elevated catecholamines in umbilical cord blood and have been associated with an increased prevalence of autism (15–20).

Whether GABA exerts excitatory actions in humans with autism is not known. However, in keeping with this hypothesis, agents that act through GABA (benzodiazepines and pheno-barbital) produce paradoxical effects in patients with autism (21) and experimental epilepsy in rodents (10). Note also that rodent KCC2 activity is altered by autism-linked genetic mutations. Oxytocin improves information processing by exciting GABAergic interneurons and inhibiting their target pyramidal neurons (22). An excitatory shift of this link will enhance glutamatergic drive in neurons and thereby affect information-processing in the developing brain.

To conclude, our observations suggest that in addition to triggering labor and inducing trust, empathy, and parental-infant relationships in humans (23), oxytocin signals might exert a protective action during delivery, preventing deleterious effects of enhanced activity. Further investigations are needed to better understand the links among pregnancy complications, cesarean sections, and autism (16, 17). In conclusion, our results validate the clinical actions of bumetanide (24) and oxytocin (25) and emphasize the importance of investigating how and when developmental sequences are disrupted in animal models of autism in order to develop novel therapeutic avenues (26).

References and Notes
9. Materials and methods are available as supplementary materials on Science Online.

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Supplementary Materials
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