When It Comes to GABAergic Responses and Neonatal Seizures—Sex Matters!

Dissociated Gender-Specific Effects of Recurrent Seizures on GABA Signaling in CA1 Pyramidal Neurons: Role of $GABA_A$ Receptors. Galanopoulou AS. *J Neurosci* 2008;28(7):1557–1567. Early in development, the depolarizing $GABA_A$ergic signaling is needed for normal neuronal differentiation. It is shown here that hyperpolarizing reversal potentials of $GABA_A$ergic postsynaptic currents (E_{GABA}) appear earlier in female than in male rat CA1 pyramidal neurons because of increased potassium chloride cotransporter 2 (KCC2) expression and decreased bumetanide-sensitive chloride transport in females. Three episodes of neonatal kainic acid-induced status epilepticus (3KA-SE), each elicited at postnatal days 4 (P4)–P6, reverse the direction of $GABA_A$ergic responses in both sexes. In males, 3KA-SE trigger a premature appearance of hyperpolarizing $GABA_A$ergic signaling at P9, instead of P14. This is driven by an increase in KCC2 expression and decrease in bumetanide-sensitive chloride cotransport. In 3KA-SE females, E_{GABA} transiently becomes depolarizing at P8–P13 because of increase in the activity of a bumetanide-sensitive NKCC1 (sodium potassium chloride cotransporter 1)-like chloride cotransporter. However, females regain their hyperpolarizing $GABA_A$ergic signaling at P14 and do not manifest spontaneous seizures in adulthood. In maternally separated stressed controls, a hyperpolarizing shift in E_{GABA} was observed in both sexes, associated with decreased bumetanide-sensitive chloride cotransport, whereas KCC2 immunoreactivity was increased in males only. $GABA_A$ receptor blockade at the time of 3KA-SE or maternal separation reversed their effects on E_{GABA}. These data suggest that the direction of $GABA_A$-receptor signaling may be a determining factor for the age- and sex-specific effects of prolonged seizures in the hippocampus, because they relate to normal brain development and possibly epileptogenesis. These effects differ from the consequences of severe stress.

COMMENTARY

Neuronal development requires the precise interplay of ionic currents and neurotransmitters, some of which are under hormonal control, that is, they differ by gender (1). The depolarizing action of GABA early in development is well established and likely plays an essential trophic role in neuronal maturation (2,3). Before about postnatal day 14 (P14) in the rat, activation of $GABA_A$ receptors elicits neuronal depolarization rather than hyperpolarization, which emerges later. The early GABAergic depolarization permits calcium influx, which subsequently activates a wide variety of intracellular processes critical for normal neuronal proliferation, migration, and differentiation. This effect is due to differential expression over time of two different chloride (Cl^-) transporters. In the neonatal period, the Cl^- transporter, $Na^+/K^+/2Cl^-$ cotransporter isoform 1 (NKCC1), is predominantly expressed, leading to intracellular Cl^- accumulation and depolarization upon activation of $GABA_A$ receptors. Gradually, over the first 4 weeks of development, the expression of NCCK1 decreases and potassium chloride cotransporter 2 (KCC2) increases. KCC2 transports Cl^- out the cell, resulting in reduction of intracellular chloride concentration so that when $GABA_A$ receptors are activated, Cl^- efflux and hyperpolarization occur.

There has been a recent explosion of research on the developmental regulation of Cl^- as it relates to seizure activity (4). The most vulnerable age at which seizures occur, the first 1–2 weeks of life in the rat and the first couple of months in the humans, have been attributed, at least partially, to this ontogenetic Cl^- regulatory mechanism. In both species, transition from the seizure-prone state correlates with appearance of classic hyperpolarizing GABA responses. Importantly, pharmacologic alteration of these innate responses could lead to novel treatment for human neonatal seizures, for which an ideal therapy does not currently exist. GABAergic drugs, long used to treat human neonatal seizures although often ineffective in suppressing them, could have gender-, age-, and region-specific actions (5). Bumetanide, a diuretic that blocks NKCC1, can prevent excessive GABA depolarization and avert the neuronal hyperexcitability underlying neonatal seizures (6). Therefore, this class of drug shows promise for a potential anticonvulsant effect in neonates.

Until recently, little research has been performed to investigate a relationship between GABA responses and gender. Evidence now exists that males have a longer duration of depolarizing GABAergic responses than females, related to differential timing in the expression of the KCC2 and NKCC1 Cl^- cotransporters (7,8). The current paper describes a number of surprising and potentially important findings, showing that in regard to GABA responses and neonatal seizures, sex does matter! Galanopoulou investigated several aspects of the maturation of the GABAergic system with reference to animal gender. In neonatal rat hippocampal slices, the gramicidin perforated patch-clamp technique was used to study CA1 pyramidal neurons. The author first demonstrated that the GABA

Epilepsy Currents, Vol. 8, No. 6 (November/December) 2008 pp. 166–167
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reversal potential (E_{GABA}) and the difference between the E_{GABA} and E_{GABA} resting potential ($E_{GABA}-V_r$, reflecting the driving force on Cl^- ions) was larger (i.e., more negative) for females than males. In fact, the driving force changed from depolarizing to hyperpolarizing on P14 in males yet was hyperpolarizing at *all* postnatal ages checked (P4–18) for females. If verified, this finding would have profound implications, as it suggests that in postnatal females (at least over P4), GABA elicits only hyperpolarizing responses. The question of whether females *ever* have depolarizing GABA responses is not answered by this or any other study to date. The implications of this finding are that females might have less overall depolarization than males and therefore, less tendency to develop seizures because of more pronounced early $GABA_A$ergic inhibition (9). Previous animal studies used only male rats or did not specify the gender, making any claim for timing of the depolarizing/hyperpolarizing GABA switch uncertain.

In terms of human neonates, these results could explain, in part, the greater propensity of male babies to seize. While definitive data are lacking, extant literature suggests that neonatal males are more prone to neurological disorders of several types than females (10,11). Etiology plays a major role in neonatal seizure occurrence, but male infants suffer a disproportionately higher frequency of many early brain insults, including seizures, independent of other comorbidities (12). It would be important to control for etiology and determine whether a GABA/gender difference plays a role in epilepsy pathogenesis.

To determine the effects of neonatal seizures on $GABA_A$ergic responses as a function of gender, the author induced three episodes of kainic acid (KA) status epilepticus on consecutive days from P4–P6. GABA became hyperpolarizing at an earlier age (around P9 vs P14) in males than females with three KA-induced seizures. Paradoxically, GABA elicited depolarizing responses between P8 and P13 in males before becoming hyperpolarizing at P14. This most surprising result, suggesting a gender-specific shift in $GABA_A$ergic postsynaptic responses that are due to neonatal seizures, requires explanation. Galanopoulou begins this search by examining the expression of the two chloride cotransporters in neonatal rats of each gender. Control females had higher immunoreactivity of KCC2 at earlier ages, corresponding to their earlier GABA hyperpolarizing effects. The three KA seizures affected each gender differently. Males with seizures had greater KCC2 immunoreactivity than controls, again correlating with their enhanced GABA hyperpolarizing response. In pups with seizures, there also were gender-specific effects of the NKCC1 blocker bumetanide on chloride transport: it was decreased in males and increased in females. The GABA antagonist bicuculline reverses the effects of KA seizures on E_{GABA} in rats of both genders, verifying a role of $GABA_A$ receptors in this effect.

Emerging evidence suggests that gender is a critical variable in physiological responses in the developing brain. Investigators have long ignored this issue. However, the possibility that there could be both age- and sex-specific treatments for neonatal seizures is enticing and warrants further exploration.

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