



The Frail Male: Early Life Inflammation Promotes Chloride Loading in Adult Male Mice

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Early Life Inflammation Increases CA1 Pyramidal Neuron Excitability in a Sex and Age-Dependent Manner Through a Chloride Homeostasis Disruption

Gomez CD, Read J, Acharjee S, Pittman QJ. *J Neurosci*. 2019;39(37):7244.

Early life, systemic inflammation causes long-lasting changes in behavior. To unmask possible mechanisms associated with this phenomenon, we asked whether the intrinsic membrane properties in hippocampal neurons were altered as a consequence of early life inflammation. C57BL/6 mice were bred in-house and both male and female pups from multiple litters were injected with lipopolysaccharide (LPS; 100 g/kg, intraperitoneally) or vehicle at postnatal day (P)14, and kept until adolescence (P35-P45) or adulthood (P60-P70), when brain slices were prepared for whole-cell and perforated-patch recordings from CA1 hippocampal pyramidal neurons. In neurons of adult male mice pretreated with LPS, the number of action potentials elicited by depolarizing current pulses was significantly increased compared with control neurons, concomitant with increased input resistance, and a lower action potential threshold. Although these changes were not associated with changes in relevant sodium channel expression or differences in capacitance or dendritic architecture, they were linked to a mechanism involving intracellular chloride overload, revealed through a depolarized γ aminobutyric acid reversal potential and increased expression of the chloride transporter, NKCC1. In contrast, no significant changes were observed in neurons of adult female mice pretreated with LPS, nor in adolescent mice of either sex. These data uncover a potential mechanism involving neonatal inflammation induced plasticity in chloride homeostasis, which may contribute to early life inflammation-induced behavioral alterations.

Commentary

Early life neurological insults, such as inflammation, hypoxia-ischemia, and stress, can affect brain function later in adulthood. Curiously, males appear particularly susceptible to such insults. Male disadvantage refers to a higher risk in males of developing adverse neurological effects following early life hypoxia-ischemia and/or extremely low weight preterm birth.^{1,2} Mechanisms contributing to this gender-specific susceptibility remain unclear. However, it is clear that infection risk is higher during the neonatal period, a critical developmental period of significant neuronal and glial plasticity. Can neonatal infection, even if clinically subtle, exert a lasting effect on neuronal function? And if so, then are males more susceptible? Gomez and colleagues now provide new insights into these questions by revealing changes in adult neuronal activity following an episode of early life inflammation, but only in males.

The authors treated 14-day-old mice of both sexes with a low dose of lipopolysaccharide (LPS, 100 mg/kg), a cell wall component of gram negative bacteria and strong inducer of the immune response. The effect of neonatal LPS exposure

(LPS-exposed) on the excitability of CA1 hippocampal pyramidal neurons was then evaluated in adolescent (35-45 days old) and adult (60-70 days old) male and female mice using electrophysiological approaches. Surprisingly, only CA1 neurons of adult males were hyperexcitable; the input resistance of these neurons was higher, and the action potential threshold was lower. Interestingly, CA1 neurons of adolescent males were unaffected. In addition, early life LPS treatment did not affect CA1 neuronal excitability in female mice of any tested age.

Excessive intracellular chloride concentrations appear to drive the hyperexcitability of LPS-exposed CA1 neurons in adult males. In general, neuronal chloride levels in adult animals are low due to the robust expression of KCC2, the potassium-chloride cotransporter responsible for pumping chloride ions out of cells.³ This function is particularly important for the actions of the primary inhibitory neurotransmitter in the central nervous system (CNS), γ aminobutyric acid (GABA). γ Aminobutyric acid-induced activation of the A-type GABA (GABA-A) receptor generally causes an influx of negatively charged chloride ions and voltage hyperpolarization, an effect that reduces neuronal activity; chloride influx



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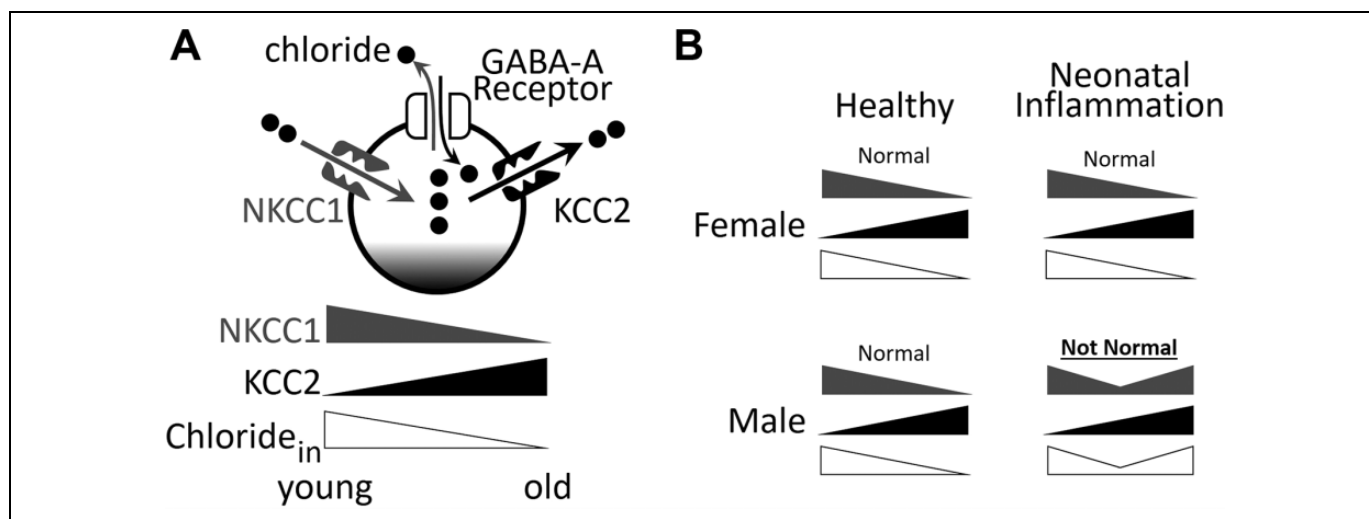


Figure 1. Neonatal inflammation impairs chloride extrusion in adult, male CA1 pyramidal neurons. **A**, Chloride transporters NKCC1 and KCC2 regulate chloride levels in neurons. NKCC1 is primarily expressed during young ages and shuttles chloride into cells. KCC2 is primarily expressed in adulthood and extrudes chloride. The direction of chloride flow through the GABA-A receptor, a ligand-gated chloride channel, is determined by transporter-defined, intracellular chloride levels. **B**, Neonatal inflammation leads to elevated chloride levels in adult CA1 pyramidal neurons, but only in males. Abnormal NKCC1 activity is proposed to promote high chloride levels in adult, male CA1 pyramidal neurons. Shapes representing NKCC1, KCC2, and chloride are similar to those depicted in panel **A**.

occurs because intracellular chloride concentrations are low. Thus, the inhibitory function of GABA-A receptors in adulthood critically depends on a proper chloride gradient across the membrane. In contrast, NKCC1, a cotransporter that moves chloride into cells, is highly expressed in neonates and renders GABA depolarizing because activation of the receptor leads to the efflux of negatively charged chloride ions.⁴ Thus, the ratio of NKCC1 to KCC2 critically defines the actions of GABA on chloride movement across the neuronal membrane (Figure 1A), and of neural network excitability in general. Indeed, increased NKCC1/KCC2 ratios in adulthood are thought to promote neuronal chloride loading and compromised GABAergic inhibitory neurotransmission in several pathological conditions.⁵

The GABA reversal potential refers to a membrane voltage that indicates how a cell will respond to GABA: with excitation or with inhibition. Interestingly, Gomez et al⁶ show that adult male CA1 neurons exposed to LPS as neonates are characterized by a depolarized GABA reversal potential, meaning that GABA depolarizes—possibly even excites—adult CA1 neurons. The authors also show that an unusually high level of NKCC1 activity in LPS-exposed male adult mice underlies the observed depolarized GABA reversal potential. NKCC1 activation normally occurs through phosphorylation of conserved threonine residues, and such activation was elevated in LPS-exposed adult males. Furthermore, treatment of hippocampal brain slices taken from LPS-exposed adult males with the NKCC1 blocker bumetanide reversed the depolarized GABA reversal potential and prevented increased CA1 pyramidal neuron excitability (ie, the increased input resistance and lower action potential threshold). At this point, the authors can only speculate how altered intracellular chloride concentrations

regulate the intrinsic excitability of neurons. While this link has been previously established,⁷ no mechanisms have been identified. The authors suggest that changes in voltage-dependent sodium, potassium, or calcium channels might be involved. Nonetheless, male animals respond to early life inflammation by exhibiting bumetanide-sensitive, abnormal chloride gradients in adulthood that also appear to regulate neuronal excitability (Figure 1B).

But why just males? The answer remains unclear, but may involve sex-dependent differences in when chloride gradients change during development. The switch from depolarizing to hyperpolarizing GABA actions occurs in females around postnatal day 4, an age much younger than the postnatal day 14 that occurs in males.⁸ Thus, the switch would have happened long before exposure to LPS in females whereas it coincided with exposure to LPS in males. If the vulnerability of males is linked to the specific time of exposure, it would raise a possibility that the time of switch in GABA actions creates a window of susceptibility. Lipopolysaccharide treatment at postnatal day 4 may unmask vulnerabilities in female mice.

Inflammation increases CNS vulnerability to subsequent insults.⁹ Lipopolysaccharide can transiently cause fever-like conditions, compromise the blood-brain barrier, and acutely increase susceptibility to hypoxia-ischemia or hyperthermia-induced seizures in neonates.^{10,11} Now, Gomez and colleagues⁶ report that a single, early life exposure to low-dose LPS—a dose that is unlikely to cause acute seizures or trigger neurodegeneration—can also increase the excitability of adult CA1 neurons. Notably, however, the extent of inflammation in the young mice and potential differences between males and females are unknown, as the study did not report this




parameter. Nevertheless, CA1 neurons provide hippocampal output to several brain regions including thalamus, septum, amygdala, and play a critical role in seizure generation and propagation. The potentially depolarizing actions of GABA in LPS-exposed males may make them vulnerable to epileptogenesis following neurological insults. Thus, neonatal inflammation may induce, in a gender specific-manner, latent CNS sensitization that is much longer lasting than previously thought. Undoubtedly, future assessments of seizure susceptibility in LPS-treated males will establish any putative pro-seizure propensity later in life.

Early life injuries in other neurological settings also expose the male disadvantage. Traumatic brain injury (TBI) is a major cause of epileptogenesis. The annual incidence of TBI due to sports, accidents, work-related causes, and other reasons in boys and men is almost double of that in girls and women.¹² The recovery following TBI and impact on cognitive processes also seems to be different between men and women. Several factors, including neuroprotective effects of female reproductive hormones, are likely to contribute to the gender-specific differences in recovery from TBI. Indeed, the neuroprotective effects of hormones generally lower the risk of adverse chronic effects of neurological injury in females. While the mechanisms of the female advantage remain unclear, metabolites of gonadal and adrenal steroid hormones are endogenous compounds with potent anticonvulsant actions, an effect attributed to the functional enhancement of the GABA-A receptor.¹³ While these metabolites are present in the male brain, it remains unclear how the male-specific chloride loading observed by Gomez and colleagues might contribute to hormonal regulation of CA1 excitability.

Clearly, the depolarizing action of adult GABA observed in vitro, if also observed in vivo, has potentially serious ramifications. For example, agents that potentiate GABAergic inhibition represent common anticonvulsant, anxiolytic, and sedative drugs. However, if CA1 neurons are loaded with chloride, these drugs may in fact elevate excitation in adult males who experienced inflammation as neonates. Are males exposed to early live inflammation adversely affected by such drugs? If not, then are the observations of chloride loaded cells localized to just CA1? Clearly, many future studies on the development of the frail male are necessary.

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