aptara | EPC | epc270 | Dispatch: June 23, 2008 | CE: PEK
Journal | MSP No. | No. of pages: 3 | PE: Cindy

CURRENT LITERATURE IN BASIC SCIENCE

GABA REGULATES STEM CELL PROLIFERATION BEFORE NERVOUS SYSTEM FORMATION

Histone H2AX-Dependent $GABA_A$ Receptor Regulation of Stem Cell Proliferation. Andäng M, Hjerling-Leffler J, Moliner A, Lundgren TK, Castelo-Branco G, Nanou E, Pozas E, Bryja V, Halliez S, Nishimaru H, Wilbertz J, Arenas E, Koltzenburg M, Charnay P, El Manira A, Ibañez CF, Ernfors P. *Nature* 2008;451(7177):460–464. Stem cell self-renewal implies proliferation under continued maintenance of multipotency. Small changes in numbers of stem cells may lead to large differences in differentiated cell numbers, resulting in significant physiological consequences. Proliferation is typically regulated in the G1 phase, which is associated with differentiation and cell cycle arrest. However, embryonic stem (ES) cells may lack a G1 checkpoint. Regulaton of proliferation in the "DNA damage" S/G2 cell cycle checkpoint pathway is known for its role in the maintenance of chromatin structural integrity. Here we show that autocrine/paracrine γ-aminobutyric acid (GABA) signalling by means of $GABA_A$ receptors negatively controls ES cell and peripheral neural crest stem (NCS) cell proliferation, preimplantation embryonic growth and proliferation in the boundary-cap stem cell niche, resulting in an attenuation of neuronal progenies from this stem cell niche. Activation of $GABA_A$ receptors leads to hyperpolarization, increased cell volume and accumulation of stem cells in S phase, thereby causing a rapid decrease in cell proliferation. $GABA_A$ receptors signal through S-phase checkpoint kinases of the phosphatidylinositol-3-OH kinase-related kinase family and the histone variant H2AX. This signalling pathway critically regulates proliferation independently of differentiation, apoptosis and overt damage to DNA. These results indicate the presence of a fundamentally different mechanism of proliferation control in these stem cells, in comparison with most somatic cells, involving proteins in the DNA damage checkpoint pathway.

GABA, the principal inhibitory neurotransmitter in the brain, is a versatile molecule that not only plays a role in synaptic transmission but also provides important signaling cues in the developing brain. The signaling function occurs through anion-permeable $GABA_A$ receptors whose effects on neuronal excitability are dependent upon the developmental stage: excitatory in immature neurons because of higher intracellular Cl^- concentration ($[Cl^-]_i$) and inhibitory in adults because of a progressive, developmentally programmed loss of $[Cl^-]_i$ (1,2). Recent studies show that GABA stimulates neurons even before synapses have been formed, suggesting that the consumption of GABA-acting drugs, including antiepileptic drugs, may affect neuronal migration and produce misplaced neurons (3). $GABA_A$ receptor-mediated events act to regulate neural stem cell proliferation both in the developing cortex (4) and in the adult subventricular zone (5), supporting the hypothesis that GABA regulates the formation of neurons and cortical units, such as achieving the balance between excitation and inhibition in the neocortex.

As described in the commentary by Mathews in this issue, the $[Cl^-]_i$ gradient is created by the expression of the $Na^+/K^+/Cl^-$-cotransporter isoform 1 (NKCC1) or the reduced expression of the K^+/Cl^- cotransporter (KCC). NKCC1 imports Cl^- and is expressed from the embryonic stage until the first postnatal week, whereas KCC2 exports Cl^- and is weakly expressed at birth and upregulated as the brain matures. These temporal patterns of transporter expression correspond to the switch from GABA being excitatory to inhibitory. In the embryonic cortex and the adult neurogenic regions, GABA depolarizes neuronal stem cells because they have high $[Cl^-]_i$ (4–7).

The Andäng et al. study reviewed here proposes that the GABAergic signaling system plays a role in the generation of embryonic stem cells. Through a variety of in vitro and in vivo studies, the authors demonstrate that mouse embryonic and neural crest stem cells express glutamic acid decarboxylase and functional $GABA_A$ receptors. They also show that stem cells possess the machinery to synthesize and respond to GABA long before the formation of the nervous system: GABA *hyperpolarizes* embryonic stem cells and decreases proliferation. Two important questions raised by this study will be discussed: How can GABA hyperpolarize stem cells and then depolarize immature neurons a few days later? By what mechanism do these actions occur, since the co-transporter that extrudes chloride is not expressed at this early stage? This issue is of importance considering the large number of antiepileptic drugs that exert their action by GABA receptors and are used during pregnancy by women with epilepsy.

Epilepsy Currents, Vol. 8, No. 5 (September/October) 2008 pp. 1–3
Blackwell Publishing, Inc.

Andäng and colleagues note that embryonic stem cells have a relatively depolarized resting membrane potential of −26 mV and a hyperpolarizing $GABA_A$ receptor-mediated response (E_{GABA}) of –78 mV. However, other studies have found depolarizing GABA responses in embryonic neuronal stem cells as well as in the adult hippocampus and subventricular zone (4,5,7). The discrepancy may be explained in several ways. For instance, embryonic stem cells were recorded in cell culture medium, whereas the neuronal stem cells were recorded in standard artificial cerebrospinal fluid. Without knowing the external Cl^- concentration one cannot extrapolate the internal Cl^- concentration from E_{GABA}. Furthermore, the current elicited by application of GABA was not completely blocked by the $GABA_A$-receptor antagonist, bicuculline. The residual current, carried through another channel, may have contributed to the reversal potential seen in this study. While the authors used voltage-sensitive dyes and Ca^{2+} imaging to confirm the nondepolarizing effects of GABA, a direct measurement of the Cl^- gradient would have given an unequivocal determination of the effect of GABA on embryonic stem cell membrane potential. The investigators' use of invasive recording techniques (i.e., intracellular, perforated, or whole cell recordings) may also present problems, as recent studies indicate that basic parameters, particularly the resting membrane potential, cannot be adequately measured in immature neurons by these recording techniques because of their high input resistance that produces large leak currents through the seal between electrode and cell (8). The disparity in outcomes is highly significant (over 30mV) and will lead to major differences in estimations of E_{GABA} and in the resting membrane potential (less than 30mV), which is most likely underestimated. The issue is more than theoretical. Indeed, extensive investigations suggest that the chloride extruder co-transporter KCC2 is functional after delivery and induces the postnatal depolarizing to hyperpolarizing shift in the actions of GABA (1). If GABA hyperpolarizes stem cells and depolarizes immature neurons, a mechanism, such as KCC2 or some other Cl^- transporter, must be present for chloride removal at an early embryonic stage. This putative, undetermined system would have to be eliminated later when neurons develop, migrate, and form coherent patterns.

Andäng et al. suggest that GABA signaling activates the phosphatidylinositol-3-OH-kinase-related kinase (PIKK) family of proteins that phosphorylate histone H2AX—a critical factor in the S/G2 DNA-damage checkpoint complex. Activation of this pathway by GABA hyperpolarization leads to an accumulation of cells in S-phase without influencing cell survival, apoptosis, or overt DNA damage. Their proposed model provides an intriguing mechanism by which GABA can regulate stem cell proliferation, given that a previous study on neuronal stem cells of the developing cortex demonstrated that GABA depolarization was found to inhibit DNA synthesis and cell cycle progression (4). While these two mechanisms are not mutually exclusive, to determine the effect of GABA on cell survival, the authors assayed the amount of cleaved Poly(ADP-ribose) polymerase-1 (PARP-1), an early marker of apoptosis. The use of other more appropriate markers of cell death, such as TUNEL and caspase-3, would reinforce the conclusions and exclude possible negative effects of GABA on survival of embryonic stem cells. Also, the suggestion of a GABA-induced hyperpolarization that (via Cl^- influx) induces cell swelling to activate the DNA-damage checkpoint pathway remains to be directly shown.

The novel finding that GABA can regulate embryonic stem cell proliferation has important implications for studies of development and disease. Controlling embryonic stem cell cycle progression by autocrine/paracrine GABA secretion provides a negative feedback mechanism that regulates embryo growth. GABA levels correlate with the stem cell pool size, and an increase in GABA concentration provides a signal to slow proliferation. The proposed GABA activated DNA-damage checkpoint pathways may shed light on developmental pathological conditions, such as tumor formation. Interestingly, in the developing nervous system, this pathway may be a key regulator for creating a balance between excitatory and inhibitory neuron generation. Inhibitory GABAergic and excitatory glutamatergic neurons arise from two distinct progenitor populations in the brain, the ganglionic eminences and cortical ventricular zone, respectively (9–12). During corticogenesis, the inhibitory interneurons from the ganglionic eminences migrate tangentially to the cortex (9,12) where they may tonically release GABA and provide paracrine GABA signaling of neuronal stem cells that regulate the generation of excitatory neurons (4,13,14). If this GABA signaling is disrupted, increased generation of excitatory neurons might lead to a hyperexcitable cortical circuit, making the brain more prone to epilepsy. The role of GABA signaling is becoming more intriguing. Understanding the diverse developmental roles of GABA may further an understanding of the pathophysiology of a variety of developmental disorders and epilepsy.

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