Stirring the Pot With Estrogens

Estradiol Acutely Suppresses Inhibition in the Hippocampus Through a Sex-Specific Endocannabinoid and mGluR-Dependent Mechanism.

Huang GZ, Woolley CS. *Neuron* 2012;74:801–808.

The steroid 17β-estradiol (E2) is well known to influence hippocampal functions such as memory, affective behaviors, and epilepsy. There is growing awareness that in addition to responding to ovarian E2, the hippocampus of both males and females synthesizes E2 as a neurosteroid that could acutely modulate synaptic function. Previous work on acute E2 actions in the hippocampus has focused on excitatory synapses. Here, we show that E2 rapidly suppresses inhibitory synaptic transmission in hippocampal CA1. E2 acts through the α form of the estrogen receptor to stimulate postsynaptic mGluR1-dependent mobilization of the endocannabinoid anandamide, which retrogradely suppresses GABA release from CB1 receptor-containing inhibitory presynaptic boutons. Remarkably, this effect of E2 is sex specific, occurring in females but not in males. Acute E2 modulation of endocannabinoid tone and consequent suppression of inhibition provide a mechanism by which neurosteroid E2 could modulate hippocampus-dependent behaviors in a sex-specific manner.

Commentary

Estrogens, $GABA_A$ receptors ($GABA_AR$), metabotropic glutamate receptors (mGluRs), and endogenous cannabinoids, such as anandamide, are well known neuromodulators with sex-specific effects on brain development and function. Huang and Woolley link these four signaling pathways by proposing a new feedback loop through which 17β-estradiol triggers an mGluR1-mediated increase in the retrograde release of anandamide, which activates cannabinoid receptors 1 (CB1R) and suppresses $GABA_AR$-IPSCs. The 17β-estradiol regulation of $GABA_AR$-IPSCs appears to involve coupling of estrogen receptor α (ERα) with mGluR1 and occurs in ovariectomized female, but not in castrated or gonadally intact male rats. The study involves postnatal day (PN) 47 to 57 rats, which are considered as late pubertal or early adults. These findings highlight once again the complex and sex-specific interactions among signaling systems that affect hippocampal activity. The current study was conducted in seizure-naïve rats, with unclear implications for the hippocampus of a rat exposed to seizures.

17β-estradiol is well known for its sex-specific, cell-type, age, and activity-dependent effects (1-4). Indeed, the authors here demonstrate the predilection for 17β-estradiol to reduce $GABA_AR$-IPSCs in ovariectomized females but not in gonadally intact or castrated males. The use of nM concentrations of 17β-estradiol was intended to parallel the high local concentrations of the neurosteroid, which range between 5 to 10 nM in the adult male hippocampus (5). It is, however, surprising that the ERβ-preferring agonist diarylpropionitrile, at doses that can activate both ERα and ERβ (500 nM), was completely inactive (6). ER/mGluR coupling occurs in hypothalamic neurons and glial cells (3), does not require presynaptic glutamate release (1), and may involve ERα or ERβ. The type of mGluR involved (mGluR1/5 or mGluR2/3) determines the downstream effects (3), which can be sex-specific (1). For instance, in early postnatal hippocampal cultures, ERα/mGluR1 may trigger phosphorylation of cAMP-responsive element binding protein (pCREB), whereas ER(α or β)/mGluR2/3 coupling may reduce pCREB (1).

The authors hypothesize that 17β-estradiol may regulate presynaptic GABA release, although potential effects on postsynaptic $GABA_ARs$ are also worth investigating, given the lasting suppression of $GABA_AR$-IPSC. However, the search for a presynaptic messenger led to fruitful links to endocannabinoid signaling, since the 17β-estradiol effects seemed to be mediated by retrograde release of anandamide. Although the sex-specific mechanisms are not explored here, these are likely to be tissue-specific, since potentiation of anandamide-induced vasorelaxation by 17β-estradiol occurs in mesenteric arteries of male but not of female rats (7).

CB1R-mediated regulation of $GABA_AR$ signaling, via 2-arachidonoylglycerol (2-AG) and/or anandamide, has been reported in the hippocampus of rodents of either undetermined sex (8-10) or of young male rats (11). Interestingly, here, only anandamide mediates the 17β-estradiol–induced suppression of $GABA_AR$-IPSCs. Inhibition of fatty acid amide hydroxylase (FAAH), which catabolizes anandamide, by URB 597 masked the 17β-estradiol effect on $GABA_AR$-IPSCs, whereas blockade of 2-AG breakdown had no such effect. This is not necessarily surprising considering that 17β-estradiol may increase anan-

Epilepsy Currents, Vol. 13, No. 3 (May/June) 2013 pp. 129–131

damide synthesis or inhibit its catabolizing enzyme, FAAH (12, 13). The inactivity of 17β-estradiol, when the FAAH inhibitor URB 597 is applied, could then be due to the occupancy of its target by URB 597, yet this possibility is not further investigated. In further support, the plasma levels of anandamide correlate nicely with the levels of estrogen during the menstrual cycle in women (13). It is tempting to cite here the clinical studies that find increased frequency of certain types of seizures during menstrual cycle periods with increased estrogen/progesterone serum level ratios (14) and question whether this anandamide-induced decrease in $GABA_AR$ signaling might be one of the mechanisms involved in the observed increased seizure frequency. But then, why isn't the frequency of other focal-onset seizure types increased as well.

Could this 17β-estradiol/anandamide signaling-induced disinhibition of the hippocampus reveal a new mechanism underlying the pro-convulsant actions of high 17β-estradiol levels? In support of this possibility, FAAH(-/-) mice show increased seizure severity in the bicuculline and kainic acid models, which was further accentuated by concomitant anandamide administration, without any reported sex differences (15). In contrast, in PN21-24 old rats of undetermined sex, FAAH inhibition had antiseizure effects in the kainic acid model (16). The rest of the existing studies on anandamide effect on seizures have been done in males and are difficult to compare with the authors' findings. Rather, the existing studies tend to suggest dose and model-specific effects, as well as the involvement of additional signaling pathways (17-20).

It is also unclear how significant this pathway may be in disinhibiting an epileptic hippocampus, where (a) GABA may be depolarizing rather than hyperpolarizing in certain hippocampal regions (21), (b) endocannabinoids may also reduce the excitatory postsynaptic currents (22), and (c) the circuitry of connections and the involved signaling pathways may be undergoing drastic changes from normal controls.

Overall, this is an elegant study, which incrementally unravels an exciting sequence of events leading to the sex-specific regulation of $GABA_AR$ IPSCs in the hippocampus by 17β-estradiol and endocannabinoids. Further experiments will be needed to clarify the relevance of the current findings not only to epilepsy but also to normal cognitive processes, drug addiction, or other neurological disorders involving the hippocampal structures and endocannabinoid signaling. The relevance of these *in vitro* observations to an intact, healthy or diseased organism that undergoes continuous changes through time, remains unclear. Deciphering these issues is always a challenge in any research that investigates activity-related functions.

by Aristea S. Galanopoulou, MD PhD

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