The Absence of Information about Hormones and Absence

Hormonal Regulation of Absence Seizures

Persad V, Ting Wong CG, Cortez MA, Wang YT, Snead OC 3rd

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A time course study that examined the effects of the female estrous cycle on the chronic slow spike-and-wave discharges (SSWDs), $GABA_B$-receptor ($GABA_BR$) binding, and $GABA_BR$ protein expression was conducted in Long-Evans hooded rats treated during development with a cholesterol synthesis inhibitor AY9944 (AY). In addition, a pharmacologic study using the hormones progesterone, 17β-estradiol, mifepristone (intracellular progesterone-receptor antagonist), tamoxifen (intracellular estrogen-receptor antagonist), and allopregnanolone (progesterone metabolite) was performed to determine their effects on AY-induced seizures. The data indicate that a significant increase occurs in both the duration of SSWDs and $GABA_BR$ binding in the AY model during the proestrus stage of the estrous cycle, the stage during which the levels of progesterone are at their highest. No changes in $GABA_BR1a$ or R2 protein levels were observed. In addition, the administration of both progesterone and allopregnanolone exacerbated seizures in the AY model, whereas 17β-estradiol attenuated the SSWD duration. Neither mifepristone nor tamoxifen blocked the effects of progesterone and 17β-estradiol, respectively, on SSWD duration in the AY model, suggesting that these two sex hormones are working in a manner independent of their intracellular receptors. These data suggest an important role for steroid hormones in the regulation and maintenance of AY-induced atypical absence seizures.

COMMENTARY

Gender differences appear to exist in absence seizures and in spike-and-wave discharges, but there is an absence of explanations for why this might be the case. Authors of a recently published article have begun to examine the topic by addressing hormonal regulation of absence seizures, by using female rats. The model is interesting because it involves administration of an antagonist of cholesterol biosynthesis during development—and nothing else. Thus, four injections of AY9944 are administered every 6 days from postnatal days 2 to 20, resulting in animals with absence seizures throughout life. AY9944 blocks the last step in cholesterol synthesis, the conversion of 7-dehydrocholesterol to cholesterol, by inhibiting the enzyme 3$\tilde{\beta}$-hydroxysterol Δ^7-reductase. Interestingly, the seizures in this model are not classic absence seizures but are atypical, that is, the seizures may include movement or may originate in hippocampus. The method for this model suggests a novel hypothesis for epileptogenesis. By blocking cholesterol formation, steroid hormone biosynthesis is impaired. However, cholesterol also has important actions in development of the brain that could have led to the absence phenotype, such as has been demonstrated by the interactions between cholesterol and hedgehog proteins (1).

The experiments by Persad and colleagues addressed how hormone administration influenced seizure frequency by using two approaches: (a) exogenous hormones were administered, and (b) seizures were examined during the estrous cycle (i.e., during natural hormonal fluctuations). First, progesterone and its metabolite allopregnanolone were studied. Previously, it had been shown that progesterone and allopregnanolone worsen absence seizures in the WAG/Rij rat, a strain that is genetically predisposed to absence seizures. These results may be explained by the ability of allopregnanolone to increase $GABA_A$-receptor inhibition, because previous studies of $GABA_A$-receptor agonists (e.g., muscimol, THIP) showed increased seizures in the AY9944 model (2). Conversely, $GABA_A$-receptor antagonists (e.g., bicuculline, picrotoxin) ameliorated seizures in the AY9944 model (2). In the present study, the authors found that seizures in the AY9944 model were increased by progesterone and allopregnanolone injection. These studies highlight how important progesterone, via allopregnanolone, is to seizure frequency, which is most likely because of its ability to regulate GABAergic function.

Next, the relative effects of endogenous progesterone and intraperitoneal injection of the hormone were compared. Surprisingly, the effects were distinct. At proestrus, seizure frequency was the highest relative to the following day (i.e., estrus) and also high relative to the subsequent days (i.e., diestrus 1 and diestrus 2, which appear to have been pooled). The authors interpreted these results as consistent with the effects of exogenous administration, because progesterone reaches a peak

on proestrus. However, progesterone peaks on proestrus quite late in the day, in the early evening. A key aspect of the study was that seizures of intact female rats were examined between 10 AM and 2 PM on proestrus, when progesterone is actually only beginning to increase. If one compares 10 AM and 2 PM on proestrus with the same time on the next day (estrus), serum progesterone levels are unlikely to be very different. The metabolite allopregnanolone might actually be higher on the morning of estrus, yet seizures had decreased frequency at that time. Without confirmation of hormone levels, data interpretation is difficult because of substantial variability among normal cycling female rats. Thus, data from the intact rats do not appear to be consistent with the effects of exogenous administration of progesterone or allopregnanolone.

Why would the data from intact rats be so different from those concerning effects of hormone injection? This finding may be a classic example of a pharmacologic versus physiologic effect. Pharmacologically, the use of supraphysiologic doses of progesterone and allopregnanolone impaired seizures. However, the physiologic levels, at least as examined in the intact rats in this study, may not have been anticonvulsant. Importantly, endogenous progestins might have anticonvulsant effects, if the point in the cycle when progesterone is highest were examined (i.e., the evening of proestrus).

Other pharmacologic experiments in the study by Persad and colleagues addressed the potential role of estrogen. Administration of estrogen reduced seizures, leading to the conclusion that estrogen was inhibitory in this model. However, only one dose was administered, which makes the results difficult to interpret because the estrogen dose-response relation is often bell-shaped (3,4). Furthermore, high concentrations of estrogen can induce progesterone-receptor expression (5). Indeed, the dose of estrogen was extremely high (1.5 mg). Thus, a lower dose might have exacerbated seizures, and indeed, the data from intact rats supported this possibility, because seizure frequency was highest between 10 AM and 2 PM on proestrus.

Additional experiments examined the potential role of reproductive hormones on $GABA_B$ receptors. These experiments are interesting, given that $GABA_B$ receptors clearly modulate absence seizures and little is known about how hormones might regulate them. It has been shown that $GABA_B$-receptor binding is influenced by the estrous cycle in neocortex, hippocampus, and hypothalamus, although the effects in neocortex peaked at a different cycle stage than did those elsewhere, suggesting that the issue is complex (6). In the present study, $GABA_B$-receptor levels and binding were examined in neocortex across the estrous cycle. Binding was altered but receptor levels were not. Thalamic levels would have been interesting to evaluate, given that the $GABA_B$ receptors in this structure play such an important role in absence seizures. Clearly, it will be important to clarify how $GABA_B$ receptors as well as $GABA_A$ receptors are influenced by hormones.

The authors also addressed whether hormone action was mediated by classic nuclear steroid-hormone receptors or membrane receptors, which is a timely topic because membrane receptors, particularly for estrogen, are increasingly appreciated for their role in neurobiology. However, the choice of drugs examined makes it difficult to draw conclusions. For example, tamoxifen was chosen as an estrogen-receptor antagonist, but tamoxifen is a receptor antagonist only in specific areas of the body, such as the breast. Tamoxifen is one of a class of agents, termed selective estrogen receptor modulators (SERMs), and in the uterus and bone, it is estrogenic. In the CNS, tamoxifen actions appear to vary with brain region, although it has potential to be estrogenic (7,8). Similarly, mifepristone (RU486) is not an ideal progesterone-receptor antagonist, because it is a well-known antagonist of glucocorticoid receptors. It also may be progestin-like under certain conditions (9), can be antiestrogenic or estrogenic (10–12), and can influence androgen receptors (13,14).

In summary, the authors address highly significant issues and use an intriguing model of absence epilepsy. Epilepsy research will certainly benefit from studying how hormonal modulation of GABA-receptor function may be relevant to hormone-sensitive seizures.

by Helen E. Scharfman, Ph.D.

References

1. Porter JA, Young KE, Beachy PA. Cholesterol modification of hedgehog signaling proteins in animal development. Science 1996;274:255–259.
2. Smith KA, Bierkamper GG. Paradoxical role of GABA in a chronic model of petit mal (absence)-like epilepsy in the rat. Eur J Pharmacol 1990;176:45–55.
3. Emons G, Knuppen R, Ball P, Catt KJ. Biphasic modulation of pituitary sensitivity to GnRH by oestrogens: the effects of A- and D-ring substitution on LH release in cultured pituitary cells. Acta Endocrinol (Copenh) 1984;107:317–327.
4. Wide JK, Hanratty K, Ting J, Galea LA. High level estradiol impairs and low level estradiol facilitates non-spatial working memory. Behav Brain Res 2004;55:45–53.
5. Etgen AM. Effects of body weight, adrenal status, and estrogen priming on hypothalamic progestin receptors in male and female rats. J Neurosci 1985;5:2439–2442.
6. Al-Dahan MI, Tehrani MHJ, Thalmann RG. Regulation of gamma-aminobutyric acid B (GABAB) receptors in cerebral cortex during the estrous cycle. Brain Res 1994;640:33–39.
7. Cyr M, Thibault C, Morissette M, Landry M, Di Paolo T. Estrogen-like activity of tamoxifen and raloxifene on NMDA receptor binding and expression of its subunits in rat brain. Neuropsychopharmacology 2001;25:242–257.

8. McMillan PJ, LeMaster AM, Dorsa DM. Tamoxifen enhances choline acetyltransferase mRNA expression in rat basal forebrain cholinergic neurons. Mol Brain Res 2002;103:140–145.
9. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 2000;21:55–89.
10. McDonnell DP, Goldman ME. RU486 exerts antiestrogenic activities through a novel progesterone receptor A form-mediated mechanism. J Biol Chem 1994;269:11945–11949.
11. Jeng MH, Langan-Fahey SM, Jordan VC. Estrogenic actions of RU486 in hormone-responsive MCF-7 human breast cancer cells. Endocrinology 1993;132:2622–2630.
12. Dibbs KI, Sadovsky Y, Li XJ, Koide SS, Adler S, Fuchs AR. Estrogenic activity of RU 486 (mifepristone) in rat uterus and cultured uterine myocytes. Am J Obstet Gynecol 1995;173:134–140.
13. Slayden OD, Nayak NR, Burton KA, Chwalisz K, Cameron ST, Critchley HO, Baird DT, Brenner RM. Progesterone antagonists increase androgen receptor expression in the rhesus macaque and human endometrium. J Clin Endocrinol Metab 2001;86:2668–2679.
14. Narvekar N, Cameron S, Critchley HO, Lin S, Cheng L, Baird DT. Low-dose mifepristone inhibits endometrial proliferation and up-regulates androgen receptor. J Clin Endocrinol Metab 2004;89:2491–2497.