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Research Report

Neuroprotection against excitotoxic brain injury in mice after ovarian steroid depletion

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ABSTRACT

Ovarian steroid hormones influence not only seizure phenomena, but also the neuronal cell death that follows. In the present study, we applied two models of ovarian steroid loss, ovariectomy and chemically-induced ovarian failure, to evaluate kainate-induced seizure activity and the susceptibility of hippocampal neurons to seizure-induced neurodegeneration. Young adult female FVB/NJ mice were ovariectomized with (OVX+E, $n=6$) or without (OVX, $n=8$) estrogen replacement. A separate group of females received the ovotoxin, 4-vinylcyclohexene diepoxide (VCD, $n=8$) to deplete ovarian follicles. Mice underwent kainate-induced status epilepticus and were evaluated for seizure activity (3 h) and delayed hippocampal neuronal injury (7 days). While there were no differences in latency or duration of severe seizures among control, OVX and VCD-treated mice, OVX+E mice exhibited seizures of a significantly longer duration. However, both VCD-induced ovarian failure and OVX led to a dramatic reduction in the extent of excitotoxic cell death, with slightly greater effects observed in VCD-treated mice. Estradiol administration to OVX mice also exerted a significant neuroprotective effect against kainate-induced cell death. These results support and extend earlier findings suggesting that the hormonal milieu may have differential effects on seizure susceptibility that are separate and distinct from those influencing hippocampal neuronal vulnerability. Collectively, these findings highlight the complex interactions among the loss of ovarian steroid hormones, estrogen replacement, seizures, and seizure-induced cell death.

1. Introduction

Epilepsy is a neurodegenerative disorder that afflicts more than 50 million people (Browne and Holmes, 2001). While the prevalence of epilepsy is similar for men and women, it is estimated that nearly 1,000,000 women of childbearing age in the United States currently suffer from epilepsy (Jacobs et al., 2001). Epilepsy in women presents special problems resulting from the complex and significant interplay of seizure disorders with reproductive function (Zahn et al., 1998). In particular, the ovarian cycle influences seizures in many women with epilepsy. Seizure frequency can vary across the menstrual cycle (catamenial epilepsy), during puberty when ovarian cyclicity is first established, or at menopause when cyclicity ceases (Bauer, 2001; Morrell, 2002; Herzog et al., 2004; Maguire et al., 2005).

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Abbreviations: ANOVA, analysis of variance; E, estrogen; E2, 17-β estradiol; KA, kainic acid; OVX, ovariectomy; S.E.M., standard error of the mean; SHAM, sham ovariectomy; VCD, 4-vinylcyclohexene diepoxide

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Similarly, studies in young adult animals have demonstrated that gonadal hormones can influence both seizure initiation and propagation. The effects of gonadal steroids on seizure susceptibility have been shown in adult ovariectomized female rats in several studies (Gazzaley et al., 1996; McEwen, 1996; MacLusky et al., 1997; Edwards et al., 1999; Hoffman et al., 2003). In particular, progesterone exerts anticonvulsive effects in both acute and chronic epilepsy models (Spiegel and Wycis, 1945; Landgren et al., 1978; Nicoletti et al., 1985; Bäckström and Rosciszewska, 1997; Herzog, 1999; Hoffman et al., 2003), while estrogen, by contrast, lowers the seizure threshold and facilitates seizures (Buterbaugh, 1989; Frye, 1995; Murphy et al., 1998; Woolley and Schwartzkroin, 1998; Frye and Bayon, 1999; Woolley, 1999; Hoffman et al., 2003). Estrogen has been shown to potentiate the behavioral effects of kainic acid (Azcoitia et al., 1999a; Hoffman et al., 2003) and facilitates kindling from the anterior neocortex (Buterbaugh, 1989).

In contrast to estrogen's pro-convulsant effects, substantial evidence from *in vivo* animal studies has indicated that estradiol can act as a neuroprotectant against hippocampal neuronal loss induced by excitotoxins (Azcoitia et al., 1999a, 2001; Reibel et al., 2000). Pretreatment with estradiol attenuates hippocampal damage in the hilus of the dentate gyrus and in the CA1 and CA3 hippocampal regions following severe long-lasting seizures (Reibel et al., 2000; Velišková et al., 2000; Galanoupoulou et al., 2003; Hoffman et al., 2003; Kalkbrenner and Standley, 2003).

Ovariectomy (OVX) is the most common animal model for studying the effects of ovarian steroids on brain function. However, OVX causes a precipitous decline in estrogen and progesterone, eliminates all ovarian secretions, and severs important nerve connections (Aguado and Ojeda, 1984). Recently, a chemically-induced model of ovarian steroid loss has been developed. In 2002, Mayer et al. reported that the industrial chemical 4-vinylcyclohexene diepoxide (VCD), selectively kills the small primordial and primary ovarian follicles in rats and mice, leading to a gradual decline in reproductive activity. Importantly, evidence for VCD's lack of toxicity to other tissues or organ systems was published in an early work by the National Toxicology Program (1986, 1989). Thus, VCD treatment provides an important opportunity to study how a gradual decline in ovarian hormones modifies normal and pathologic processes in the brain. However, the effects of the VCD-induced model of ovarian failure on seizure susceptibility and seizure-induced cell loss have yet to be evaluated. In the present study, we compared the effects of OVX and VCD on seizure susceptibility and seizure-induced cell death in female mice induced by the kainate model of temporal lobe epilepsy. Our data suggest that the loss of ovarian steroid hormones plays an important functional role in neuronal viability following excitotoxic injury.

2. Results

2.1. *Effect of VCD on ovarian cycles*

VCD has previously been shown to disrupt estrous cyclicity by acting as an ovarian toxin, resulting in the loss of primary and primordial follicles (Springer et al., 1996a,b; Borman et al., 1999; Kao et al., 1999). As shown in Fig. 1A, all vehicle-treated females maintained regular cycles throughout the study, averaging 4.9±0.2 days. VCD treatment caused a gradual disruption of estrous cyclicity with VCD-treated females averaging cycle lengths of 8.5±0.9 days (Fig. 1A). Ovarian failure, as assessed by a persistent state of diestrus, was observed 26.2±3.2 days after the onset of dosing. Likewise, in post-mortem histologic sections of the ovary, VCD treatment significantly depleted the number of oocytes (Fig. 1B). Ovaries from vehicle-treated female controls averaged 2.2±0.7 oocytes/mm^2. By contrast, VCD-treated females had only 0.1±0.1 oocytes/mm^2 ($p<0.05$). However, the area of the ovarian sections in VCD females (0.6±0.1 mm^2) was not significantly different from that of controls (0.9±0.1 mm^2, $p>0.05$). Thus, in agreement with previous studies in other strains of mice (Mayer et al., 2002; Thompson et al., 2002;

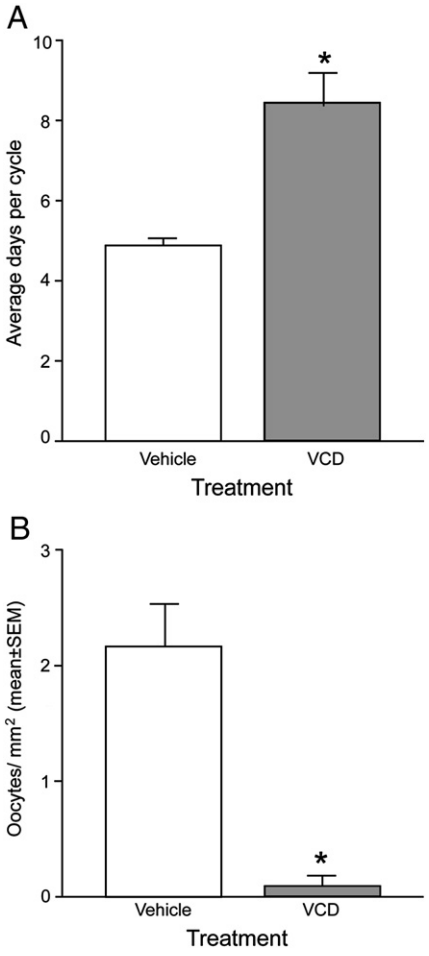

Fig. 1 – VCD causes a dose-related inhibition of estrous cyclicity and induces follicular depletion. (A) Daily administration of 320 mg/kg VCD for 15 days eliminated estrous cyclicity by day 11. Note the significant increase in average days per cycle in VCD-treated mice, as compared to normally cycling ovary-intact mice. ($F=29.513$; $p=0.002$). (B) VCD also significantly depleted oocytes in ovarian sections, compared with vehicle-treated control females. Bars represent mean±S.E.M. *$p<0.05$.