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# Seizure susceptibility in intact and ovariectomized female rats treated with the convulsant pilocarpine

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**Abstract**

Despite numerous neuroendocrinological studies of seizures, the influence of estrogen and progesterone on seizures and epilepsy remains unclear. This may be due to the fact that previous studies have not systematically compared distinct endocrine conditions and included all relevant controls. The goal of the present study was to conduct such a study using pilocarpine as chemoconvulsant. Thus, age and weight-matched, intact or ovariectomized rats were tested to determine incidence of status epilepticus and to study events leading to status. Intact female rats were sampled at each cycle stage (proestrus, estrus, metestrus, or diestrus 2). Convulsant was administered at the same time of day, 10:00–10:30 a.m. Statistical analysis showed that there was a significantly lower incidence of status on the morning of estrus, but differences were attenuated in older animals. Ovariectomized rats were distinct in their rapid progression to status. These results show that the incidence of status in female rats following pilocarpine injection, and the progression to pilocarpine-induced status, are influenced by reproductive state as well as age. The hormonal milieu present specifically on the morning of estrus appears to decrease susceptibility to pilocarpine-induced status, particularly at young ages. In contrast, the chronic absence of reproductive steroids that characterizes the ovariectomized rat leads to a more rapid progression to status. This dissociation between incidence vs. progression provides new insight into the influence of estrogen and progesterone on seizures.


*Keywords:* Estrogen; Progesterone; Allopregnanolone; Status epilepticus; Estrus; Estrous cycle; Aging

## Introduction

In many women with epilepsy, seizures do not occur randomly but cluster in association with specific stages of the ovarian cycle. This condition, termed "catamenial" epilepsy, is believed to result from the actions of circulating ovarian steroid hormones on the brain. The mechanisms responsible for hormonal modulation of seizure susceptibility, however, remain poorly understood. One way to investigate this problem is to use laboratory animal models, in which interactions between seizures and the ovarian hormones can be studied under more controlled conditions than is possible in human populations. Numerous studies over the last few decades have identified sex and cycle-dependent differences in seizure susceptibility in rodents. These studies have used various methods to induce seizures, including kindling, electroshock, and chemoconvulsant administration (Bujas et al., 1997; Buterbaugh, 1989; Edwards et al., 1999; Finn and Gee, 1994; Frye and Bayon, 1998; Frye et al., 1998; Hoffman et al., 2003; Hom and Buterbaugh, 1986; Hudson and Buterbaugh, 1991; Kalkbrenner and Standley, 2003; Matejovska et al., 1998;

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Nicoletti et al., 1985; Pericic and Bujas, 1997; Persinger et al., 1988; Pesce et al., 2000; Schwartz-Giblin et al., 1989; Slamberova and Vathy, 2000; Tan and Tan, 2001; Tominaga et al., 2001; Valente et al., 2002; Velisek et al., 1999; Wahnschaffe and Loscher, 1992; Woolley, 2000). However, these studies have failed to provide a clear picture of the relationship between ovarian hormone levels and seizure susceptibility. Hence, significant questions remain concerning the roles of these hormones in regulating seizure induction, propagation, and subsequent seizure-associated sequelae, such as neuronal damage.

Of the principal ovarian steroids, estrogen has been suggested to be primarily proconvulsant, whereas it is commonly assumed that progesterone is anticonvulsant. This perspective is based on studies beginning in the 1950s, in both laboratory animals and women with epilepsy, showing that estrogens can exacerbate seizures while progestins decrease seizure frequency (Backstrom, 1976; Logothetis and Harner, 1960; Logothetis et al., 1959; Terasawa and Timiras, 1968). Subsequent work, however, has shown that proconvulsant effects of estrogen, as well as anticonvulsant effects of progesterone, may depend to a large extent on the specific experimental conditions used. For example, although there have been repeated demonstrations that estrogen is proconvulsant (Buterbaugh, 1989; Matejovska et al., 1998; Nicoletti et al., 1985), there is also evidence that this is not the case (Hoffman et al., 2003; Hom and Buterbaugh, 1986; Hudson and Buterbaugh, 1991; Slamberova and Vathy, 2000; Velisek et al., 1999; Woolley, 2000). Furthermore, while estradiol may protect neurons from seizure-induced neuronal damage (Azcoitia et al., 1998; Veliskova et al., 2000), this protective effect may not be mediated by a decrease in seizure severity (Hoffman et al., 2003; Reibel et al., 2000).

One of the major difficulties in evaluating the role of the ovarian steroids is that results from different laboratories have not been consistent. A possible reason for this is that most studies have used ovariectomized rats, treated with a variety of different hormone replacement regimens. These regimens cannot easily be compared because both estrogen and progesterone may have variable effects depending on the dose and duration of treatment (Edwards et al., 1999; Kalkbrenner and Standley, 2003; Schwartz-Giblin et al., 1989; Tominaga et al., 2001).

Intact cycling female rats have also been used to examine the influence of the ovarian steroids on seizures, but this has not necessarily clarified the ways ovarian steroids influence seizures. Again, this may be due at least in part to inconsistencies in the experiments (time of day for the studies, age and body weight of the animals). Most studies have also focused on a few specific endpoints, without consideration of other potential factors that may contribute to seizure susceptibility. For example, in one recent study, susceptibility to pilocarpine-induced seizures was compared in ovariectomized and intact rats, but the intact rats only included animals at the estrous stage of the female reproductive cycle (the "estrous cycle"; Valente et al., 2002). While ovariectomized animals appeared to have a shorter latency to seizures than intact rats, general conclusions regarding the role of the ovarian hormones could not be drawn, since other stages of the reproductive cycle were not included. Other stages could potentially be similar to ovariectomized rats, for example. Tan and Tan (2001) reported increased seizure susceptibility at proestrus compared to estrus. Studies of ovariectomized rats treated with proestrous levels of estradiol also support the hypothesis that seizure susceptibility may be increased at proestrus (Edwards et al., 1999). However, proestrous rats may not *always* be more susceptible to seizures relative to rats examined at other times of the estrous cycle (Finn and Gee, 1994; Pesce et al., 2000; Wahnschaffe and Loscher, 1992), for reasons that presently remain unclear.

The results of the study by Valente et al. (2002) were also surprising because the incidence of status epilepticus was reported not to differ significantly between estrus and ovariectomized rats (Valente et al., 2002). This finding contrasts with data from other studies that have indicated a decreased seizure duration at estrus to seizures induced by the chemoconvulsant kainic acid (Frye and Bayon, 1998; Frye et al., 1998), although seizure threshold in response to other convulsants besides pilocarpine and kainic acid was least at estrus relative to the following day, metestrus (Finn and Gee, 1994).

The present study was undertaken in an attempt to resolve some of this uncertainty, via a systematic comparison of the chemoconvulsant sensitivity of ovariectomized rats and intact rats at different stages of the estrous cycle. Rather than utilize rats treated with different hormone regimens, we focused on the comparison of ovariectomized to intact cycling animals, because cycling rats would provide the best insight into the physiological hormonal parameters that may be most important in regulating seizure susceptibility, free of the possible problems of interpretation associated with hormone replacement. All animals were administered convulsant at the same time of day, mid-morning, to avoid the potential confound of variability due to the circadian rhythm. Furthermore, mid-morning comparisons of cycling rats allowed optimal comparisons of physiological changes of reproductive steroids (e.g., estradiol is elevated during the morning on proestrus but progesterone is not, allowing specific insight into the influence of physiological levels of estradiol). Since age and body weight may also influence the response to convulsants (Bujas et al., 1997; Darbin et al., 2004; Hunter et al., 1989; Pericic and Bujas, 1997; Persinger et al., 1988; Turski et al., 1989), we included animals from a range of ages and body weights in each reproductive condition, to determine whether age and/or body weight might affect the outcome.

Pilocarpine was chosen as the test chemoconvulsant, for several reasons. First, pilocarpine has the ability not only to elicit single seizures but also a state of continuous, severe seizures (status epilepticus). This is potentially important because single seizures and status epilepticus could be