Z Epileptol 2015 · 28:246–253
DOI 10.1007/s10309-015-0001-x
Published online: 30. June 2015

E. Taubøll[1,2] · K. Heuser[1] · L. Sveberg[1] · S. Svalheim[1]

[1] Department of Neurology, Oslo University Hospital – Rikshospitalet, Oslo, Norway
[2] University of Oslo, Oslo, Norway

Experimental models for the study of hormonal changes in epilepsy

Introduction

The interactions between hormones, epilepsy, and antiepileptic drugs (AEDs) are complex. While there is ample evidence that hormones influence epilepsy, it is also apparent that epileptic activity influences hormones in both women and men. In addition, AEDs may disturb endocrine function. The clinical importance of these interactions is primarily related to the effects on reproductive hormones and is the focus of this article. Reproductive endocrine dysfunction is common among women and men with epilepsy [1]. Menstrual disorders, polycystic ovaries, and infertility have been described among women with epilepsy [1–3], while reduced potency and sperm abnormalities have been found in men [1, 4–5]. Sexual problems [6] and endocrine changes have been described frequently in both sexes [1–3]. There are also, however, interactions with other hormones, especially thyroid hormones (see [7]).

The close interplay between hormones, epilepsy, AEDs, psychosocial factors, comorbid conditions, and other non-epilepsy medication means that studying the effects of each factor in isolation can be problematic. However, animal models and experimental models using human tissue allow us to investigate the independent effects of the various factors.

Reproductive endocrine effects of epilepsy

The impact of the epilepsy itself on endocrine function has been recognised and discussed for many years. Clinical studies have shown that epilepsy itself affects the secretion of pituitary hormones, thereby affecting the secretion pattern, rhythmicity, and levels of the peripheral sex steroid hormones [1, 8]. Even laterality of epileptic activity may be important, as there are some indications that left-sided temporal foci increase the occurrence of polycystic ovaries in women, while right-sided foci increase the frequency of hypogonadotropic hypogonadism [9].

Anatomical and neurophysiological studies of tissue from animals and humans have shown that there is a close connection between the temporolimbic system and the hypothalamus, which controls the neuroendocrine system. The amygdala, in particular, has extensive direct reciprocal connections with regions of the hypothalamus that are involved in the regulation, production, and secretion of gonadotropin-releasing hormones (GnRH). In line with this, early studies demonstrated how amygdala-kindling in male cats led to hyposexuality [10], and limbic seizures elicited in female rats resulted in interruption of the estrous cycle and altered mating behavior [11]. Stimulation of the corticomedial amygdala can induce ovulation and uterine contractions in several species [12–13]. Bilateral amygdalectomy in adult male rats and cats has been found to cause marked degeneration of the testes [14], and bilateral ablations of the amygdala in adult female deer mice can induce anovulatory cycles and polycystic ovaries [15]. In addition, bilateral amygdalectomy in female monkeys induces amenorrhea and hypogonadal vaginal changes [16]. These studies demonstrate the close interactions between neuronal hyperactivity or hypoactivity in the temporolimbic brain areas and reproductive endocrine function.

A series of experiments conducted by Edwards and co-workers [17–18] showed the effects of seizures on reproductive functions in both female and male rats. In females, amygdala-kindled seizures halted ovarian cyclicity and also caused high serum estradiol concentrations, increased pituitary weight, theca cell hyperplasia, and polyfollicular ovaries consisting of many cystic follicles, as well as follicles in various stages of growth and atresia. Progesterone treatment, which is used to restore cyclicity, was effective in only 5 of the 28 animals that had stopped cycling, while all sham-kindled controls that had stopped cycling regained cyclicity. For intact male animals, amygdala-kindled seizures resulted in an increase in serum testosterone, estradiol, and prolactin, which was accompanied by a significant increase in testis, epididymis, and pituitary weight, as well as a significant decrease in prostate weight. Maximal electroshock seizures (MES) caused a short-term reduction in serum testosterone concentrations and in testis, epididymis, and prostate weight. These findings demonstrate that both focal limbic (amygdaloid) seizures and generalized (MES) seizures disturb the normal repro-

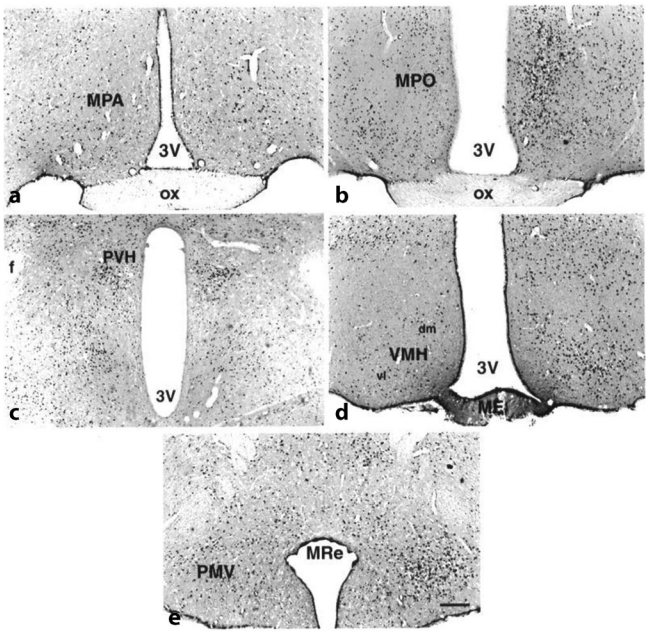

Fig. 1 ▲ Photomicrographs of hypothalamic nuclei from a right-amygdala-stimulated rat. a, c Similar numbers of Fos-ir neurons on the left and right-hand sides of the medial preoptic area (MPA) and paraventricular hypothalamic nucleus (PVH), areas not involved in reproductive endocrine function. b, d, e Laterally asymmetric, ipsilaterally predominating numbers of Fos-ir neurons in the medial preoptic nucleus (MPO), ventrolateral part of the ventromedial hypothalamic nucleus (VMH), and in the ventral premamillary nucleus (PMV), areas that are involved in reproductive function and reproductive endocrine secretion. (Copyright John Wiley & Sons Inc. [19])

ductive physiology in rats, independently of AEDs or other confounding factors.

The demonstration that different endocrine responses depend on the laterality of the epileptic activity provides a firm indication of the direct influence of epilepsy on endocrine function. Silveira and co-workers [19] reported asymmetric activation of hypothalamic regions after unilateral amygdala-stimulated seizures. By studying Fos-immunoreactivity in different hypothalamic areas, they identified three areas that are prominently involved in reproductive function, including the medial preoptic nucleus (MPO), the ventrolateral part of the ventromedial hypothalamus (VMHVL), and the ventral premammillary nucleus (PMV). These areas showed significantly greater and more asymmetric, ipsilaterally predominating induction of Fos following unilateral amygdala-stimulated seizures than other regions investigated, which are not involved in reproductive endocrine function (◘ **Fig. 1**). Asymmetric activation of the hypothalamus could be the basis for the occurrence of such reproductive endocrine disorders in patients with left-sided or right-sided temporal lobe epilepsy, since there is a clear asymmetry in the reproductive functions of the hypothalamus, including asymmetric content of GnRH [20]. These animal studies provide a definitive demonstration of a direct effect of epilepsy and epileptic discharges on reproductive endocrine function.

Effects of hormones on epilepsy

Both female and male sex steroid hormones influence brain excitability. Among the female sex steroid hormones, progesterone and its metabolites are anticonvulsant, while estrogens are mainly proconvulsant. Androgens are also mainly anticonvulsant, but their effects are more varied, probably due to the metabolism of androgens to, among others, estradiol.

Estrogens

Application of estrogen directly to the cortex has potent epileptogenic effects [21], while topical application of estrogen has even been used as a model of focal epilepsy (see [22]). Estradiol also reduces the electroshock threshold, increases paroxysmal spiking in epileptogenic foci in cats and rabbits, facilitates kindling, and potentiates seizures induced by different chemoconvulsants (see [23]).

Of considerable importance for the generally excitatory effect of estrogens is their ability to enable the rapid increase in responses of neurons to the excitatory effect of glutamate [24–26]. This potentiating effect is thought to primarily influence NMDA, but also non-NMDA types of glutamate receptors (see [26]). The excitatory responses to glutamate are increased by estradiol in a dose-dependent manner [24–25]. Typically, enhancement of glutamate excitation occurs within seconds after onset of local steroid application, indicative of an effect on membrane-bound glutamate receptors.

GABA-ergic mechanisms do not seem to be affected as an acute response [24, 27], but estrogens do affect the GABA-ergic system over time. Prolonged exposure (more than 24 hours) to estradiol suppresses GABA-ergic inhibition of hippocampal neurons that may be related to decreased GABA release at inhibitory synapses (see i.e. [28]). Also, it is assumed that estradiol decreases GABA synthesis by reducing the activity of glutamate decarboxylase (GAD) [29–31], although this was not observed in all studies [32].

Estradiol has also been found to alter brain morphology by increasing dendritic spine density via an NMDA receptor-dependent mechanism and altering the pattern of hippocampal synaptic connectivity [26, 33–35]. Estradiol selectively increased neuronal sensitivity to synaptic input mediated by the NMDA type of glutamate receptor, while responses mediated by the AMPA receptor were not affected. These continuous, plastic changes in morphology that are related to spine density and neuronal sen-

sitivity to glutamate are probably of major importance with respect to the effect of estrogens on brain excitability.

Estrogens may be anticonvulsant in particular circumstances. As with other peripheral sex steroid hormones, estrogens also exert their effect at intracellular receptors, the estrogen receptors, ERα and ERβ. Of these, the effect of ERβ is the most important for the non-reproductive effects of the hormone, and some experiments have found that low doses of estrogen may actually reduce seizures by acting at ERβ (see [36]), while long-term estrogen exposure may decrease the susceptibility to kainate-induced seizures in some cases (see [28]).

Progesterone and its metabolites

Progesterone and its metabolites have been shown to increase the electroshock threshold [37, 81], protect against audiogenic and pentylenetetrazol (PTZ)-induced seizures, inhibit kindling, and also to protect against electroshock-induced seizures (see i. e. [23]).

The main mechanisms by which progesterone and its metabolites exert their effect on brain excitability are non-classical, being related to membrane receptors. Progesterone receptors are widely distributed in the brain (see [38]) and progesterone circulating in the blood gains rapid, and relatively unrestricted, access to all parts of the nervous system [39–40]. Neural tissue is able to convert progesterone to more potent antiepileptogenic progesterone metabolites within the brain (see [41]).

The main effects of progesterone and its metabolites are considered to be related to an enhanced postsynaptic GABA-ergic effect, as evidenced by several lines of investigation. Progesterone and its metabolites increase the inward chloride current induced by GABA [37, 42], increase the binding of muscimol [37, 43], stimulate the binding of flunitrazepam [44], and displace TBPS binding to the GABA receptor complex [44–45]. The effects on GABA-ergic inhibition of 3α-5α-THP, the most potent antiepileptogenic metabolite of progesterone, were investigated by Majewska et al [42], and dose-dependent increases in both peak amplitude and duration of the inward chloride current induced by GABA were demonstrated. In line with this, the effects of both progesterone and 3α-5α-THP on recurrent GABA-ergic inhibition have also been observed in hippocampal slices from rats [82].

The mechanisms by which the effect on GABA-ergic induced chloride influx is exerted and exactly where the steroid binds to the GABA-A receptor complex have been discussed. In the mid-1980 s the effect on chloride influx was shown to be largely related to an increase in the effective open time of the chloride channels [46]. However, although this may be the most important mechanism, later studies indicated that an increase in opening frequency may also be involved [47]. The binding site for progesterone and its metabolites within the GABA-A receptor complex is unique and dif-

Abstract · Zusammenfassung

Z Epileptol 2015 · 28:246–253 DOI 10.1007/s10309-015-0001-x

E. Taubøll · K. Heuser · L. Sveberg · S. Svalheim

Experimental models for the study of hormonal changes in epilepsy

Abstract

Reproductive endocrine dysfunction is common among both women and men with epilepsy. The reasons for this are multifactorial and bidirectional; epilepsy can affect hormones, and hormones can affect seizures. Furthermore, several antiepileptic drugs (AEDs) can have endocrine side-effects, while psychosocial factors and co-morbidity add further complexity. Animal models and experimental models using human tissue or cell lines provide new approaches to investigating the independent effects of the epilepsy itself, hormonal effects, and the effects of AEDs, in isolation and without confounding factors. This paper reviews the literature regarding animal studies and selected experiments using human cell lines related to reproductive endocrine function in epilepsy. By comparing results from clinical and experimental studies and by developing appropriate animal models, several mechanistic questions regarding the complex interplay between epilepsy, hormones, and AEDs can be explored. Animal experiments should be an integral tool in the study of reproductive endocrine disorders in epilepsy.

Keywords

Epilepsy · Antiepileptic drugs · Hormones · Animal models · Gender issues

Experimentelle Modelle zum Studium hormoneller Veränderungen bei Epilepsie

Zusammenfassung

Reproduktive endokrine Dysfunktion ist sowohl bei Männern als auch bei Frauen mit Epilepsie verbreitet. Die Gründe dafür sind vielschichtig und wechselseitig: Epilepsie kann Hormone beeinflussen, Hormone können epileptische Anfälle begünstigen. Darüber hinaus können etliche antiepileptische Medikamente (AEDs) endokrine Nebenwirkungen haben, und psychosoziale Faktoren sowie Komorbidität steigern noch die Komplexität des Problems. Tiermodelle oder experimentelle Modelle mit menschlichem Gewebe oder Zelllinien ermöglichen neue Ansätze zur Erforschung der unabhängigen Auswirkungen der Epilepsie selbst, der Hormone sowie der AEDs, jeweils eigenständig und ohne Störfaktoren. Dieser Beitrag gibt einen Überblick über die Literatur zu Tiermodellen und ausgewählten Experimenten mit menschlichen Zelllinien in Verbindung mit reproduktiver endokriner Dysfunktion bei Epilepsie. Im Vergleich der Ergebnisse von klinischen und experimentellen Studien sowie durch die Entwicklung entsprechender Tiermodelle können etliche mechanistische Fragestellungen zu dem komplexen Zusammenspiel zwischen Epilepsie, Hormonen und AEDs sondiert werden. Tierstudien sollten ein wesentliches Hilfsmittel für die Erforschung von reproduktiven endokrinen Dysfunktionen bei Epilepsie sein.

Schlüsselwörter

Epilepsie · Antiepileptika · Hormone · Tiermodelle · Geschlechterfragen

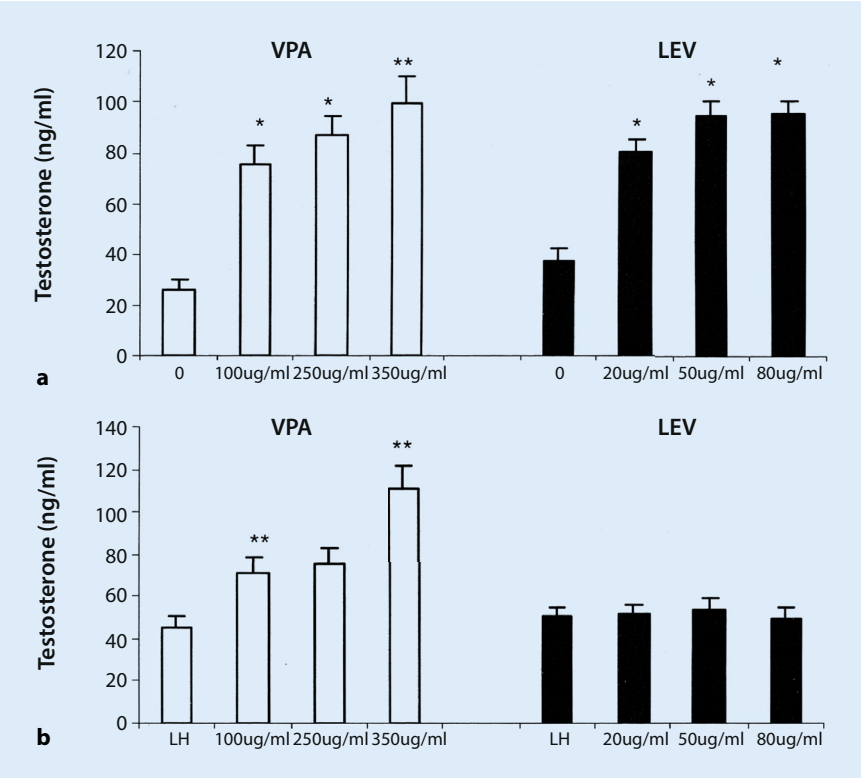

Fig. 2 ▲ The effect of levetiracetam (LEV) and valproate (VPA) on (a) basal and (b) LH-stimulated testosterone secretion. Significant differences compared with control. *p < 0.05; **p < 0.01. (Copyright John Wiley & Sons Inc. [67])

fers from that of both barbiturates and benzodiazepines (BZ).

Progesterone and its metabolites may also affect excitatory mechanisms. A series of studies on Purkinje cells from a rat cerebellum [48–50], demonstrated that both progesterone itself and several of its metabolites, including 3α-5α-THP, decreased glutamate responsiveness after either systemic or topical application.

Seizure susceptibility may also be altered by changes in the subunit composition of the GABA-A receptor. The most striking finding following progesterone withdrawal, a model for catamenial epilepsy, is the marked up-regulation of the α-4 subunit of the GABA receptor [23, 51–54], which is insensitive to BZ. The increase in α-4 expression also leads to a decrease in inhibition gated by the GABA-A receptor (see [26]). Dynamic changes in the GABA receptor subunit composition in situations with progesterone withdrawal, which occurs premenstrually, also alter the seizure threshold and sensitivity to AED treatment on a cyclic basis. In this context, it is of relevance that some neurosteroids may be able to modulate all isoforms of GABA-A receptors, including those containing the α-4 subunit. This provides possibilities for specific treatment of women with catamenial epilepsy with new drugs, such as Ganaxalone – in essence a neurosteroid, which also acts at the α-4 subunit.

Although the effects on non-classical mechanisms are by far the most important for the role of progesterone and its metabolites as anticonvulsants, a possible effect on intracellular, classical progesterone receptors cannot be completely ruled out. A series of experiments, mainly on PTZ-induced seizures in ovariectomized rats, suggested a possible role for classical progesterone receptors as being relevant for the anticonvulsant effect [36].

Androgens

Although a general anti-seizure effect is most commonly observed, androgens have more varied effects [55]. Administration of androgens directly to the hippocampus of castrated rats reduces PTZ-induced seizures, while testosterone increases the electroconvulsive threshold in males at low dose, but in both sexes at higher doses (see [56]). The variable actions of testosterone may partly be due to its metabolism to 17β-estradiol, which is generally excitatory, but also to androstanediol and dihydrotestosterone, which exert potent antiepileptic effects (see [55]).

Regarding their role in epilepsy, androgens act primarily at non-classical, membrane receptors [57–58], as powerful and positive modulators of the GABA-A receptor. For instance, Reddy and Jian [58] showed how androstanediol produced a concentration-dependent enhancement of GABA-activated currents. Systemic doses of androstanediol (5–100 mg/kg) resulted in a dose-dependent suppression of seizures in a mouse hippocampal kindling model, which is a model of temporal lobe epilepsy, with high doses providing complete seizure protection.

Androgens also have effects on neuronal structure, increasing the number of spine synapses in the stratum radiatum of area CA1 in the rat hippocampus [59]. Androgens may also affect spine synapse density in the hippocampus in female rats and contribute to plastic changes over the course of the menstrual cycle [60].

Similar to the other sex steroids, androgens also act at classical, intracellular receptors, as demonstrated by the proconvulsant effect of flutamide, an antagonist of the intracellular androgen receptor. Further, it has been shown that testicular feminized mice, which are totally insensitive to androgens due to mutations in the intracellular androgen receptor, do not exhibit the anti-seizure effects from exposure to androgens that are observed in wildtype animals with intact intracellular androgen receptors (see [55]).

Endocrine effects of AEDs on sex steroid hormones

Several AEDs exert a direct effect on the production of sex steroid hormones. The first study to address this question in

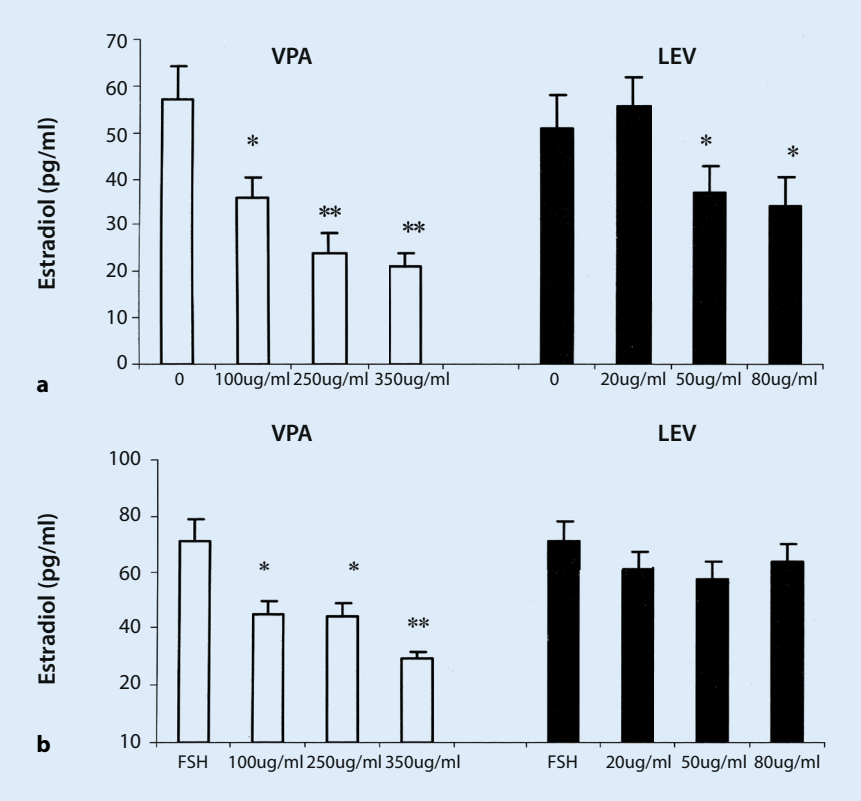

Fig. 3 ▲ The effect of levetiracetam (LEV) and valproate (VPA) on (a) basal and (b) FSH-stimulated estradiol secretion. Significant differences compared with control. *p < 0.05; **p < 0.01. (Copyright John Wiley & Sons Inc. [67])

animals was published in 1990 and investigated the effects of valproate (VPA), carbamazepine (CBZ), and phenytoin on different steps of testosterone biosynthesis in isolated rat Leydig cells [61]. Using submaximally stimulating concentrations of human chorionic gonadotropin (hCG), leading to physiological testosterone secretion rates, half-maximal inhibition of testosterone formation occurred in the presence of 15 µM CBZ, 180 µM phenytoin, or 900 µM VPA. Only the values for CBZ were in the clinically therapeutic range.

Some, but not all, BZ may alter androgen production, perhaps due to the selective effects of different BZ on various types of BZ receptors. Peripheral-type BZ receptors have been characterized in various tissues like the ovary and testis. Clonazepam, which acts only on the central-type BZ receptors, did not affect androgen production, whereas diazepam, which binds to both central and peripheral BZ receptors, induced a significant increment of basal and hCG stimulated testosterone production [62]. However, other studies found that chronic treatment of male rats with diazepam is associated with lowered serum testosterone levels [63]. As there was no difference in serum the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH), nor in the hypothalamic luteinizing hormone-releasing hormone (LHRH) content, it was suggested that diazepam could act directly on the testicular interstitial cells to reduce testosterone production [63]. This is supported by data that shows that the peripheral-type BZ receptor agonist Ro 5-4864 affects androgen production from suspensions of isolated rat interstitial cells, suggesting that BZ acting on peripheral BZ receptors have a direct effect on Leydig cells [62].

Long-term AED treatment may have profound effects on reproductive endocrine function. Due to the heated debate on the possible effects of VPA, the majority of studies focused on this drug. However, it should be borne in mind that the lack of reported effects on reproductive endocrine function from other drugs does not imply that they have been proven to be *without* such effects.

Long-term VPA treatment in female, non-epileptic rats has been shown to result in a marked increase in the testosterone/estrogen ratio, mainly by decreasing estrogen levels [64]. Regarding the gonadotropins, there was no increase in LH after VPA treatment; indeed, a trend towards reduced LH levels at high VPA doses was observed. No change was seen in FSH levels. Lamotrigine, on the other hand, did not affect any of the hormones studied. Taken together, the effects of long-term VPA treatment, with a pronounced reduction in estrogen concentrations, a notable increase in the testosterone/estrogen ratio, and minor effects, if any, on gonadotropins, suggest that VPA has a direct effect on the production of peripheral sex steroid hormones in the ovary.

In order to investigate the possibility of a direct effect of VPA on follicular steroidogenesis in more detail, the secretion of testosterone and estradiol from isolated porcine ovarian follicles has been studied [65]. In this study, co-cultures of theca and granulosa cells were used. Such co-cultures are thought to provide a better reflection of the *in vivo* situation than models using isolated cell cultures, as theca cells and granulosa cells work closely together *in vivo* as a functional unit. Using concentrations from 600 to 1500 µmol/l VPA, which includes clinically relevant concentrations, it was shown that VPA increased the secretion of testosterone, decreased estradiol secretion, and reduced the conversion of testosterone to estradiol. Two subsequent studies confirmed and extended these findings [66–67]. VPA was demonstrated to cause a significant increase in LH-stimulated testosterone secretion and a decrease in FSH-stimulated estradiol secretion (Fig. 2 and 3). VPA also decreased conversion of testosterone to estradiol in both basal and FSH stimulated cultures. This is of possible clinical importance as gonadotropins are always present in fertile women. These findings have been confirmed in studies using human ovarian follicular cells,

which showed that VPA caused a significant and dose-dependent decrease in basal and FSH-stimulated estradiol secretion. Further, VPA reduced CYP19 aromatase activity in FSH-stimulated cells at higher concentrations [66].

VPA may also alter enzyme activities and gene expression. In long-term cultures of human theca cells treated for 72 h with sodium VPA (30–3000 µmol/l), Nelson-deGrave and co-workers [68] observed an increase in basal and forskolin-stimulated dehydroepiandrosterone (DHEA), androstenedione, and 17alpha-hydroxyprogesterone production. The most pronounced effect of VPA on androgen biosynthesis occurred in the dose range of 300–3000 µmol/l, which includes therapeutic levels for the treatment of epilepsy and bipolar disorder. The study also showed that VPA can increase dehydroepiandrosterone (DHEA) and androstenedione and increase the expression of CYP17 and CYP11A genes, as seen in polycystic ovary syndrome. Thus, VPA may have a direct effect on steroidogenesis by affecting gene expression, converting normal theca cells to a polycystic ovary phenotype. In line with this, Gustavsen et al [69] used a model of human adrenal carcinoma cells that are capable of full steroidogenesis to show that VPA reduced estradiol levels and caused a general down-regulation of expression of genes encoding for enzymes early in steroidogenesis. Using the same cell line, gene analyses suggested that VPA affects NR0BI expression [70]. NR0BI inhibits promoters of other genes involved in steroidogenesis, and the altered expression of NR0BI might explain the observed down-regulation in hormone production. In the same study, expression of CYP19 was reduced following exposure to 900 µmol/l of VPA, which is a clinically relevant concentration. The CYP19 gene codes for aromatase catalyse androgens to estrogens, which is in line with the reduced conversion of testosterone to estradiol [67].

A direct effect of VPA is further supported by the findings of Hattori et al. [71], who demonstrated the presence of the enzyme microsomal epoxide hydrolase (mEH) in human ovaries. mEH is important in detoxification of various substances, and several studies have shown that VPA inhibits mEH activity [72]. Hattori et al. [71] showed that human granulosa cells expressed mEH, and that inhibition of mEH suppressed conversion of testosterone to estradiol. The action of VPA as an inhibitor of mEH may represent a route by which VPA reduces estrogen levels, thereby increasing the testosterone/estrogen ratio. This will lead to an androgen-dominant microenvironment in the ovary, and thereby possibly to polycystic changes, without an increase in LH levels [71].

However, a study in Rhesus monkeys [73] indicates a lack of endocrine effects after long-term treatment with VPA. Only 7 animals were studied, and this study also demonstrated a trend towards an increase in testosterone/estrogen ratio, lower estrogen levels, and an elevated LH: FSH ratio. In addition, body weight was significantly increased.

It is important that the potential direct effects of drugs other than VPA should also be investigated in more detail, especially "newer" AEDs. Levetiracetam (LEV) is of particular interest as it binds to the synaptic vesicle protein, SV2A [74]. SV2A is widely distributed in the nervous system and also in endocrine tissue. LEV may exert its effect both at the central and peripheral level as SV2A is expressed in the pituitary gland and the hypothalamus, but also in the ovary. In a primary investigation on the possible endocrine effect, we showed that LEV affected only basal, but not gonadotropin-stimulated, testosterone and estrogen secretion from porcine ovarian follicular cells [67]. This suggests that LEV could be an alternative drug for women of fertile age, as their gonadotropin status resembles the situation of gonadotropin stimulation. However, shortly after this first report was published, Svalheim et al [75] showed that long-term treatment of healthy female rats with LEV resulted in endocrine changes, with increased testosterone and reduced estradiol levels. In addition, there was a dose-dependent increase in ovary weight and an increase in the number of corpora lutea and secondary follicles, but no effect on number and dimension of ovarian cysts. These changes were observed at therapeutically relevant drug concentrations. It is also important to note that the changes differed from those associated with exposure to VPA, and thus represent a drug-specific pattern. Nevertheless, three more recent studies did not confirm any effect of LEV on hormone production and expression of genes related to steroidogenesis in both human adrenal cells (H295R cells) [69–70] and in human ovarian follicular cells [66]. In sum, at present LEV may be considered to have minor, if any, endocrine effects.

Topiramate is another drug that has been studied briefly with regard to endocrine effects, and was found to reduce fertility and ovarian weight in female rats that were exposed (100 mg/kg) for 12 weeks [76]. In male rats given the same dose for 8 weeks led to decreased spermatogenesis, sperm motility, and weight of reproductive organs [77]. However, although the serum concentrations were not measured and the clinical relevance of the observations is uncertain, these results underline the importance of studying all AEDs more closely with regard to possible endocrine effects.

Sex steroid hormones may also be influenced by AEDs via a centrally mediated effect by altering gonadotropin secretion. A study in male rats [64] demonstrated a dose-dependent increase in LH after long-term VPA treatment and also an increase in FSH at the highest dose. While this finding considered in isolation might imply a direct effect of the drug on the central nervous system, the increased gonadotropin levels may also be considered as compensatory mechanisms that are related to the marked peripherally induced effects observed in the animals. The lack of a centrally mediated effect is supported by the finding of unchanged FSH and prolactin levels in mice after 8 weeks of VPA treatment at doses that led to a reduction in pubertal maturation and alterations in ovarian and testicular function [78]. However, long-term VPA treatment has been shown to delay GnRH cell morphological maturation within the hypothalamus in young mice, although not in adults [79]. Long-term, low-dose, VPA or CBZ treatment in rats has also been shown to increase prolactin concentrations and reduce FSH and LH

levels in male rats [80]. Furthermore, it should be remembered that GABA-ergic mechanisms are involved in the secretion of GnRH at the hypothalamic level, and several of our most commonly used AEDs exert their effects through actions on the GABA receptor. Thus, a centrally mediated effect of AEDs on sex hormone regulation cannot be ruled out and should be explored in further studies.

In conclusion, there is an intricate multidirectional interplay between epilepsy, sex steroid hormones, and AEDs. The complexity of interactions precludes investigation of the impact of each individual factor in clinical studies. Psychosocial aspects, comorbidity and use of drugs for other indications than epilepsy are further confounding factors. Animal models and experimental models using human tissue allow us to investigate the independent effects of the various factors. By comparing results from clinical and experimental studies several mechanistic questions regarding the complex interplay between epilepsy, hormones, and AEDs can be explored. Animal experiments should, therefore, be an integral tool in the study of reproductive endocrine disorders in epilepsy.

Corresponding address

E. Taubøll
Department of Neurology, Oslo University Hospital – Rikshospitalet
4950 Nydalen, 0424 Oslo, Norway
erik.tauboll@medisin.uio.no

Compliance with ethical guidelines

Conflict of interest. E. Taubøll, K. Heuser, L. Sveberg Røste, and S. Svalheim state that there is no conflict of interest.

All national guidelines on keeping and handling of laboratory animals were followed and the necessary approval of the appropriate authorities was obtained.

References

1. Herzog AG (1997) Disorders of reproduction and fertility. In: Engel J Jr, Pedley TA (Hrsg) Epilepsy: a Comprehensive Textbook. Lippincott-Raven Publishers, Philadelphia, S 2013–2019
2. Isojärvi JIT, Taubøll E, Herzog AG (2005) Effect of antiepileptic drugs on reproductive endocrine function in individuals with epilepsy. CNS Drugs 19:207–223
3. Røste LS, Taubøll E (2007) Women and epilepsy: review and practical recommendations. Expert Rev Neurother 7:289–300
4. Isojärvi JIT, Löfgren E, Juntunen KS, Pakarinen AJ, Paivansalo M, Rautakorpi I, Tuomivaara L (2004) Effect of epilepsy and antiepileptic drugs on male reproductive health. Neurology 27:247–253
5. Røste LS, Taubøll E, Haugen TB, Bjørnenak T, Sætre ER, Gjerstad L (2003) Alterations in semen parameters in men with epilepsy treated with valproate or carbamazepine monotherapy. Eur J Neurol 10:501–506
6. Harden CL (2006) Sexuality in men and women with epilepsy. CNS Spectr 11(8, suppl 9):13–18
7. Borowicz KK (2009a) Hormones and seizures. In: Schwartzkroin PA (Hrsg) Encyclopedia of Basic Epilepsy Research. Academic Press/Elsevier, UK, S 502–506
8. Herzog AG, Coleman AE, Jacobs AR, Klein P, Friedman MN, Drislane FW, Ransil BJ, Schomer DL (2003) Interictal EEG discharges, reproductive hormones, and menstrual disorders in epilepsy. Ann Neurol 54:625–637
9. Herzog AG (1993) A relationship between particular reproductive endocrine disorders and the laterality of epileptiform discharges in women with epilepsy. Neurology 43:1907–1910
10. Feeney DM, Gullotta FP, Gilmore W (1998) Hyposexuality produced by temporal lobe epilepsy in the cat. Epilepsia 39:140–149
11. Mellanby J, Dwyer J, Hawkins CA, Hitchen C (1993) Effect of experimental limbic epilepsy on the estrus cycle and reproductive success in rats. Epilepsia 34:220–227
12. Koikegami H, Yamada T, Usui K (1954) Stimulation of the amygdaloid nuclei and periamygdaloid cortex with special reference to its effects on uterine movements and ovulation. Folia Psychiat Neurol Jpn 8:7–31
13. Velasco ME, Taleisnik S (1960) Release of gonadotropins induced by amygdaloid stimulation in the rat. Endocrinology 84:132–139
14. Yamada T, Greer MA (1960) The effect of bilateral ablation of the amygdala on endocrine function in the rat. Endocrinology 66:565–574
15. Zolovick AJ (1972) Effects of lesions and electrical stimulation of the amygdale on hypothalamic-hypophyseal regulation. In: Eleftheriou BE (Hrsg) The Neurobiology of the Amygdala. Plenum Press, New York, S 745–762
16. Erickson LB, Wada JA (1970) Effects of lesions in the temporal lobe and rhinencephalon on reproductive function in adult female rhesus monkeys. Fertil Steril 21:434–454
17. Edwards HE, Burnham WM, MacLusky NJ (1999a) Epilepsia 40:1490–1498
18. Edwards HE, Burnham WM, Ng MM, Asa S, MacLusky NJ (1999b) Limbic seizures alter reproductive function in the female rat. Epilepsia 40:1370–1377
19. Silveira DC, Klein P, Ransil BJ, Liu Z, Hori A, Holmes GL, LaCalle S, Elmquist J, Herzog AG (2000) Lateral asymmetry in activation of hypothalamic neurons with unilateral amygdaloid seizures. Epilepsia 41:34–41
20. Gerendai I, Halasz B (2001) Asymmetry of the neuroendocrine system. News Physiol Sci 16:92–95
21. Marcus EM, Watson CW, Goldman PL (1966) Effects of steroids on cerebral electrical activity. Arch Neurol 15:521–532
22. Prince DA (1972) Topical convulsant drugs and metabolic antagonists. In: Purpura DP, Penry JK, Tower DB, Woodbory DM, Walter RD (Hrsg) Experimental Models of Epilepsy. Raven Press, New York, S 51–83
23. Reddy DS (2009) The role of neurosteroids in the pathophysiology and treatment of catamenial epilepsy. Epilepsy Res 85:1–30
24. Smith SS, Waterhouse BD, Woodward DJ (1987d) Sex steroid effects on extrahypothalamic CNS. I. Estrogen augments neuronal responsiveness to iontophoretically applied glutamate in the cerebellum. Brain Res 422:40–51
25. Smith SS, Waterhouse BD, Woodward DJ (1988) Locally applied estrogens potentiate glutamate-evoked excitation of cerebellar Purkinje cells. Brain Res 475:272–282
26. Smith SS, Woolley CS (2004) Cellular and molecular effects of steroid hormones on CNS excitability. Cleveland Clin J Med 71(Suppl.2):4–10
27. Taubøll E, Lindström S, Gjerstad L (1994) Acute effects of 17β-estradiol on brain excitability studied in vitro and in vivo. Epilepsy Res 18:107–117
28. Woolley CS (2009) Hormones and epilepsy. In: Schwartzkroin PA (Hrsg) Encyclopedia of Basic Epilepsy Research. Academic Press/Elsevier, UK, S 495–501
29. McGinnis MY, Gordon JH, Gorski RA (1980) Time course and localization of the effects of estrogen on glutamic acid decarboxylase activity. J Neurochem 34:785–792
30. Nicoletti F, Meek JL (1985) Estradiol benzoate decreases nigral GABAergic activity in male rats. Brain Res 332:179–183
31. Wallis CJ, Luttge WG (1980) Influence of estrogen and progesterone on glutamic acid decarboxylase activity in discrete regions of rat brain. J Neurochem 34:609–613
32. O'Connor LH, Nock B, McEwen BS (1988) Regional specificity of gamma-aminobutyric acid receptor regulation by estradiol. Neuroendocrinology 47:473–481
33. Gould E, Woolley C, Frankfurt M, McEwen BS (1990) Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. J Neurosci 10:1286–1291
34. Woolley CS, McEwen BS (1992) Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. J Neurosci 12:2549–2554
35. Woolley CS, McEwen BS (1994) Estradiol regulates hippocampal dendritic spine density via an N-methyl-D-aspartate receptor-dependent mechanism. J Neurosci 14:7680–7687
36. Frye CA, Rhodes ME (2009b) Female sex steroids and neuronal excitability. In: Schwartzkroin PA (ed) Encyclopedia of Basic Epilepsy Research., Academic Press/Elsevier
37. Peters JA, Kirkness EF, Callachan H, Lambert JJ, Turner AJ (1988) Modulation of the GABA-A receptor by depressant barbiturates and pregnane steroids. Br J Pharmacol 94:1257–1269
38. Reddy DS (2013) Neuroendocrine aspects of catamenial epilepsy. Horm Behav 63:254–266

39. McEwen BS, Zigmond RE, Gerlasch JL (1972) Sites of steroid binding and action in the brain. In: Bourne GH (Hrsg) Structure and Function of Nervous Tissue, Bd. 5. Academic Press, New York, S 205–291
40. Morrell JI, Kelley DB, Pfaff DW (1975) Sex steroid binding in the brains of vertebrates: studies with light microscopic autoradiography. In: Knigge K, Scott DS, Kobayashi K, Ishi S (Hrsg) Brain Endocrine Interactions; II. The Ventricular System. Karger, Basel, S 230–256
41. Majewska MD (1987) Steroids and brain activity: essential dialogue between body and mind. Biochem Pharmacol 36:3781–3788
42. Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM (1986) Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232:1004–1007
43. Canonaco M, O'Connor LH, Pfaff DW, McEwen BS (1989) Longer term progesterone treatment induces changes of GABA-A receptor levels in forebrain sites in the female hamster: quantitative autoradiography study. Exp Brain Res 77:407–411
44. Harrison NL, Majewska MD, Harrington JW, Barker JL (1987) Structure-activity relationships for steroid interaction with the gamma-aminobutyric acid-a receptor complex. J Pharmacol Exp Ther 241:346–353
45. Richter JA, Yamamura HI (1985) Effects of pemtobarbital on t-[35S]butylbicyclophosphorithionate and [3H]flunitrazepam binding to membrane-bound and solubilized preparations from rat forebrain. J Pharmacol Exp Ther 233:125–133
46. Barker JL, Harrison NL, Meyers DER, Majewska MD (1986) Clin Neuropharmacol 9(4):392–394
47. Twyman RE, MacDonald RL (1992) Neurosteroid regulation of GABAA receptor single-channel kinetic properties of mouse spinal cord neurons in culture. J Physiol 456:215–245
48. Smith SS, Waterhouse BD, Chapin JK, Woodward DJ (1987a) Progesterone alters GABA and glutamate responsiveness: a possible mechanism for its anxiolytic action. Brain Res 400:353–359
49. Smith SS, Waterhouse BD, Woodward DJ (1987b) Sex steroid effects on extrahypothalamic CNS. II. Progesterone, alone and in combination with estrogen, modulates cerebellar responses to amino acid neurotransmitters. Brain Res 422:52–62
50. Smith SS, Waterhouse BD, Woodward DJ (1987c) Locally applied progesterone metabolites alter neuronal responsiveness in the cerebellum. Brain Res Bull 18:739–747
51. Gulinello M, Gong QH, Li X, Smith SS (2001) Short-term exposure to a neuroactive steroid increases alpha4 GABA(A) receptor subunit levels in association with increased anxiety in the female rat. Brain Res 910:55–66
52. Gulinello M, Gong QH, Smith SS (2002) Progesterone withdrawal increases the alpha4 subunit of the GABA(A) receptor in male rats in association with anxiety and altered pharmacology – a comparison with female rats. Neuropharmacology 43:701–714
53. Smith SS, Gong QH, Hsu FC, Markowitz RS, ffrench-Mullen JM, Li X (1998a) Nature 4(392):926–930
54. Smith SS, Gong QH, Li X, Moran MH, Bitran D, Frye CA, Hsu FC (1998b) Withdrawal from 3alpha-OH-5alpha-pregnan-20-One using a pseudopregnancy model alters the kinetics of hippocampal GABAA-gated current and increases the GABAA receptor alpha4 subunit in association with increased anxiety. J Neurosci 18:5275–5284
55. Frye CA, Rhodes ME (2009a) Male sex steroids and neuronal excitability. In: Schwartzkroin PA (ed) Encyclopedia of Basic Epilepsy Research., Academic Press/Elsevier
56. Borowicz KK (2009b) Sex and seizure sensitivity. In: Schwartzkroin PA (ed) Encyclopedia of Basic Epilepsy Research., Academic Press/Elsevier
57. Reddy DS (2010) Neurosteroids: endogenous role in the human brain and therapeutic potentials. Prog Brain Res 186:113–137
58. Reddy DS, Jian K (2010) The testosterone-derived neurosteroid androstanediol is a positive allosteric modulator of GABAA receptors. J Pharmacol Exp Ther 334:1031–1041
59. Leranth C, Petnehazy O, MacLusky NJ (2003) Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats. J Neurosci 23:1588–1592
60. Leranth C, Hajszan T, MacLusky NJ (2004) Androgens increase spine synapse density in the CA1 hippocampal subfield of ovariectomized female rats. J Neurosci 24:495–499
61. Kühn-Velten W, Herzog AG, Müller MR (1990) Acute effects of anticonvulsant drugs on gonadotropin-stimulated and precursor-supported androgen production in the rat testis. Eur J Pharmacol 181:151–155
62. Ritta MN, Calandra RS (1989) Testicular interstitial cells as a target for peripheral benzodiazepines. Neuroendocrinology 49:262–266
63. Cook PS, Notelovitz M, Kalra PS (1979) Effect of diazepam on serum testosterone and the ventral prostate gland in male rats. Arch Androl 3:31–5
64. Røste LS, Taubøll E, Isojärvi JIT, Pakarinen AJ, Huhtaniemi I, Knip M, Gjerstad L (2002) Effects of chronic valproate treatment on reproductive endocrine function in female and male Wistar rats. Reprod Toxicol 16:767–773
65. Taubøll E, Gregoraszczuk EL, Kolodziej A, Kajta M, Ropstad E (2003) Valproate inhibits the conversion of testosterone to estradiol and acts as an apoptotic agent in growing porcine ovarian follicular cells. Epilepsia 44:1014–1021
66. Taubøll E, Gregoraszczuk EL, Wojtowicz AK, Milewicz T (2009) Effects of levetiracetam and valproate on reproductive endocrine function studied in human ovarian follicular cells. Epilepsia 50:1868–1874
67. Taubøll E, Gregoraszczuk EL, Tworzydło A, Wójtowicz AK, Ropstad E (2006) Comparison of reproductive effects of levetiracetam and valproate studied in prepubertal porcine ovarian follicular cells. Epilepsia 47:1580–1583
68. Nelson-DeGrave VL, Wickenheisser JK, Cockrell JE, Wood JR, Legro RS, Strauss JF 3rd, McAllister JM (2004) Valproate potentiates androgen biosynthesis in human ovarian theca cells. Endocrinology 145:799–808
69. Gustavsen MW, von Krogh K, Taubøll E, Zimmer KE, Dahl E, Olsaker I, Ropstad E, Verhaegen S (2009) Differential effects of antiepileptic drugs on steroidogenesis in a human in vitro cell model. Acta Neurol Scand Suppl 189:14–21
70. von Krogh K, Harjen H, Almås C, Zimmer KE, Dahl E, Olsaker I, Taubøll E, Ropstad E, Verhaegen S (2010) The effect of valproate and levetiracetam on steroidogenesis in forskolin stimulated H295R cells. Epilepsia 51:2280–2288
71. Hattori N, Fujiwara H, Maeda M, Fujii S, Ueda M (2000) Epoxide hydrolase affects estrogen production in the human ovary. Endocrinology 141:3353–3365
72. Kerr BM, Rettie AE, Eddy AC, Loiseau P, Guyot M, Wilensky AJ, Levy RH (1989) Inhibition of human liver microsomal epoxide hydrolase by valproate and valpromide: in vitro/in vivo correlation. Clin Pharmacol Ther 46:82–93
73. Ferin M, Morrell M, Xiao E, Kochan L, Qian F, Wright T, Sauer M (2003) Endocrine and metabolic responses to long-term monotherapy with the antiepileptic drug valproate in the normally cycling Rhesus monkey. J Clin Endocrinol Metab 88:2908–2915
74. Lynch BA, Lambeng N, Nocka K, Kensel-Hammes P, Bajjalieh SM, Matagne A, Fuks B (2004) The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam. Proc Nat Acad Sci USA 101:9861–9866
75. Svalheim S, Taubøll E, Surdova K, Ormel L, Dahl E, Aleksandersen M, McNeilly A, Gjerstad L, Ropstad E (2008) Long-term levetiracetam treatment affects reproductive endocrine function in female Wistar rats. Seizure 17:203–209
76. Khouri NA (2005) Reproductive toxic effects of Topamax ingestion in female Sprague-Dawley rats. Neuroendocrinol Lett 26:843–847
77. Otoom S, Batieneh H, Hassan Z, Daoud A (2004) Effects of long-term use Topiramate on fertility and growth parameter in adult male rats. Neuroendocrinol Lett 25:352–355
78. Snyder PJ, Badura LL (1995) Chronic administration of sodium valproic acid slows pubertal maturation in inbred DBA/2 J mice: Skeletal, histological, and endocrinological evidence. Brain Res 20:203–211
79. Illig AM, Melia K, Snyder PJ, Badura LL (2000) Sodium valproate alters GnRH-GABA interactions during development in seizure-prone mice. Brain Res 885:192–200
80. Soliman GA, Abd AE-M (1999) Effect of antiepileptic drugs carbamazepine and sodium valproate on fertility of male rats. Dtsch Tierärztl Wschr 106:110–113
81. Spiegel E, Wycis H (1945) Anticonvulsant effects of steroids. J Lab Clin Med 36:947–953
82. Taubøll E, Gjerstad L (1993) Comparison of 5α-pregnan-3α-ol-20-one and phenobarbital on cortical synaptic activation and inhibition studied in vitro. Epilepsia 34:228–235