

# EPILEPSY, HORMONES, AND ANTIPILEPTIC DRUGS

Doctoral thesis by  
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## **2. LIST OF PAPERS**

**1)** Svalheim S, Taubøll E, Bjørnenak T, Røste LS, Mørland T, Sætre ER, Gjerstad L.

Do women with epilepsy have increased frequency of menstrual disturbances?

Seizure 2003; 12:529-533.

**2)** Svalheim S, Taubøll E, Bjørnenak T, Røste LS, Mørland T, Sætre ER, Gjerstad L.

Onset of epilepsy and menarche--is there any relationship? Seizure 2006; 15:571-575.

**3)** Svalheim S, Taubøll E, Surdova K, Ormel L, Dahl E, Aleksandersen M, McNeilly A, Gjerstad L, Ropstad E. Long-term levetiracetam treatment affects reproductive endocrine function in female Wistar rats. Seizure 2008; 17:203-209.

**4)** Svalheim S, Taubøll E, Luef G, Lossius A, Rauchenzauner M, Sandvand F, Bertelsen M, Mørkrid L, Gjerstad L. Differential effects of levetiracetam, carbamazepine and lamotrigine on reproductive endocrine function in adults. Epilepsy & Behavior 16 (2009) 281-287.

### 3. ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
ADH	Antidiuretic hormone
AED	Antiepileptic drug
ASEX	Arizona Sexual Experience Scale
cAMP	Cyclic adenosine monophosphate
CBG	Corticosteroid-binding globulin
CBZ	Carbamazepine
DHAES	Dehydroepiandrosteronsulphate
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic acid
EEG	Electroencephalogram
EIAED	Enzyme-inducing AED
FAI	Free androgen index
FSH	Follicle-stimulating hormone
GABA	Gamma-aminobutyric acid
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
hCG	Human chorionic gonadotropin
HPG	Hypothalamic-pituitary-gonadal
ICD	International classification of diseases
IGF-1	Insulin growth factor 1
IGFBP-1	Insulin growth factor binding protein 1
ILAE	International League Against Epilepsy
LEV	Levetiracetam
LH	Luteinizing hormone
LRE	Localization-related epilepsy
LTG	Lamotrigine
LTLE	Left temporal lobe epilepsy
mRNA	Messenger ribonucleic acid
PB	Phenobarbital
PCO	Polycystic ovaries
PCOS	Polycystic ovary syndrome
PGE	Primary generalized epilepsy
PHT	Phenytion
PTZ	Pentylentetrazol
RTLE	Right temporal lobe epilepsy
SHBG	Sex hormone binding globulin
SV2A	Synaptic vesicle protein 2
TSH	Thyroid-stimulating hormone
VPA	Valproate

## 4. INTRODUCTION

### 4.1 EPILEPSY

Epilepsy is one of the most common brain disorders. The term “epilepsy” has been used since 500 BC and translates literally to ‘attack’ or ‘assault’. Throughout history, persons suffering from epilepsy have been regarded as being possessed by evil spirits, and the disease has also been termed “the sacred disease”. Theories on the causes of epilepsy have varied from invasion by demons, to shifting lunar phases, to sexual obsession or masturbation. Thus associations between cyclicity and seizures, and between gender and epilepsy, have been suspected since ancient times, but on a very different basis from our understanding today.

In developed countries, the prevalence of active epilepsy is 0.7 % (Hauser et al., 1996; Keränen and Riekkinen, 1989), while the incidence is about 50 per 100,000 persons (Hauser et al., 1996; Olafsen et al., 2005). Extrapolated to Norwegian conditions, this is approximately 2,000 patients receiving an initial diagnosis of epilepsy in Norway annually and a total of 30,000 of the Norwegian population suffering from epilepsy. The disease probably has an approximately equal distribution amongst men and women in Norway, as found in Iceland (Olafsen et al., 2005) and Sweden (Brorson, 1970), or there might be a slight predominance of males (Forsgren, 1992; Keränen et al., 1989).

Epilepsy is a chronic condition, characterized by recurrent, unprovoked epileptic seizures (ILAE (International League Against Epilepsy) commission report, Fisher et al., 2005). An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain (Fisher et al., 2005). Epilepsy usually has neurobiological, cognitive, psychological, and social consequences (Fisher et al., 2005).

Epilepsy affects people in all age groups, with the highest incidence in the first year of life, and in the elderly above 65 years (Hauser et al., 1993; Olafsson et al., 2005). Thus the disease affects numerous men and women of fertile age and several aspects of the condition, particularly regarding fertility, pregnancy, and sexual life, are of great importance to the patients.

Epileptic seizures can differ widely in presentation, and in order to optimise effective treatment, systematic classification systems are necessary. The currently valid ILAE ‘*Classification of Epileptic Seizures*’ was devised in 1981 and is based on clinical and EEG manifestations. Seizures are principally divided into partial and generalized seizures, but some seizures are unclassified. In adults, partial seizures are most common, accounting for 60 – 70 % of all seizures (Hauser et al., 1993; Forsgren et al., 1996; Oun et al., 2003).



## 4.2 HORMONES

Hormones are chemical messengers carried by blood or lymph vessels to act on cells or organs distant from their original sites of secretion. When hormones are secreted into the bloodstream, many immediately bind to plasma proteins with high specificity and affinity. Sex hormones (oestrogens, testosterone, and DHT) bind to a protein called SHBG (sex hormone binding globulin) and also to albumin, but with a much lower affinity. Prolactin is transported in plasma by the protein transcortin or corticosteroid binding protein (CBG). Equilibrium is established between free and bound hormone; the greatest proportion of hormones are carried and bound, and only the free, unbound portion is physiologically active. Protein binding of hormones is of importance for drug interactions, because some drugs displace hormones from their binding site.

Hormones can also either bind directly, or through secondary messengers, to DNA (chromatin) thereby affecting gene transcription and the production of the respective mRNAs. This alters the quantities of specific proteins and therefore impacts on metabolic processes.

### 4.2.1 The hypothalamus and the pituitary gland

The pituitary gland consists of two major components: the adenohypophysis and the neurohypophysis. The adenohypophysis consists of the anterior lobe and the rudimentary intermediate lobe. The anterior pituitary secretes a number of hormones that regulate the growth and function of other endocrine glands or influence metabolic functions in target tissues. These include growth hormone (GH), thyroid-stimulating hormone (TSH), prolactin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and adrenocorticotrophic hormone (ACTH). The release of hormones from the anterior pituitary gland is controlled by releasing hormones from the hypothalamus. Gonadotropin-releasing hormone (GnRH) is produced in the hypothalamus and controls the secretion of LH and FSH from the pituitary gland. Hypothalamic hormones are released from the hypothalamic nerve fibre endings around the capillaries of the hypothalamic-hypophyseal system in the pituitary stalk, and reach the anterior lobe through a special portal system that connects the hypothalamus and the anterior lobe (Figures 1 and 2).

The neurohypophysis consists of the infundibular process, or posterior lobe. The posterior pituitary contains two active hormones, antidiuretic hormone (ADH) and oxytocin. Both hormones are produced in the hypothalamus and transported by axoplasmic flow to nerve

endings in the posterior pituitary, where they are released into circulation via appropriate stimulation.

Regions of the hypothalamus that are involved in the regulation, production, and secretion of GnRH receive extensive direct connections from the cerebral hemispheres, especially from the temporolimbic structures that are commonly involved in epilepsy, and most notably from the amygdala.

**The gonadotropins (FSH, LH, and hCG):** These hormones are responsible for gametogenesis and steroidogenesis in the gonads. Human chorionic gonadotropin (hCG) is a pregnancy hormone which is synthesized in the placenta.

**FSH** binds to specific receptors on the plasma membrane of the follicular cells in the ovaries and the Sertoli cells in the testes. Adenylate cyclase acts as the intracellular messenger. FSH promotes follicular growth and prepares the follicles for the ovulation-inducing action of LH, and enhances the LH-induced release of oestrogens. In the male, FSH binds to the Sertoli cells, where it induces the synthesis of an androgen-binding protein involved in the transport of testosterone to the seminiferous tubuli and epididymis. This is important for achieving the high local levels of testosterone required for spermatogenesis. FSH stimulates seminiferous tube and testicular growth and is important in initiating spermatogenesis.

**LH** binds to specific plasma membrane receptors and stimulates the production of progesterone by the corpus luteum and testosterone by the Leydig cells. Cyclic adenosine monophosphate (cAMP) acts as an intracellular messenger for LH. The LH spike in the middle of the menstrual cycle triggers ovulation. LH can stimulate interstitial cells in the ovaries to produce a number of androgens and androgen precursors (androstenedione, dehydroepiandrosterone, and testosterone). Women with polycystic ovary syndrome (PCOS) have elevated LH concentrations and increased androgen production. In males, LH stimulates testosterone production.

**Regulation of FSH and LH:** Secretion of LH and FSH is regulated by a classic feedback loop, regulated by the gonadal steroid hormones. Sex hormones administered for a prolonged period will inhibit the secretion of LH and FSH. Positive feedback control is also involved, since oestradiol is responsible for the ovulatory burst of LH. The secretion of LH and FSH is regulated by a single hypothalamic releasing factor, GnRH. GnRH release is inhibited by the target organ hormones, testosterone and oestradiol, and by endorphins. GnRH release is

stimulated by dopamine, alpha adrenergic agents, and prostaglandins. Although separate releasing factors have not been found, plasma FSH and LH do not always change concordantly. In the testicles another factor, inhibin, inhibits FSH release.

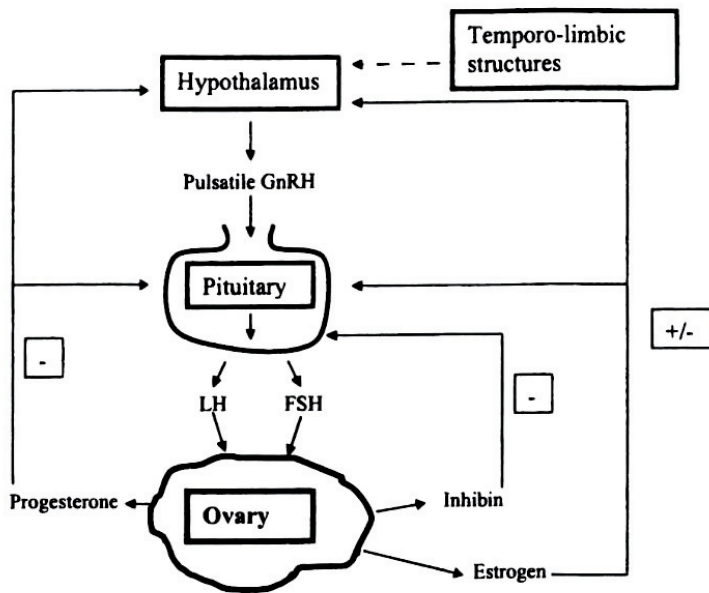
**Prolactin:** Prolactin is involved in initiation and maintenance of lactation. Excessive prolactin secretion can cause amenorrhoea and galactorrhoea. In men, excessive prolactin is associated with gynecomastia and impotence.

#### 4.2.2 The gonadal hormones

**OVARY: Oestrogens:** oestrogens are a family of hormones synthesized in the ovaries or in extra ovarian tissues.  $17\beta$ -oestradiol is the primary oestrogen of ovarian origin. In pregnancy relatively more oestriol is produced (extra ovarian). In and after menopause, oestrone is the main oestrogen in women. Oestradiol is mainly produced in granulosa cells in the ovaries. Oestrogens are formed by aromatization of androgens. Increased activity of these enzymes results in "oestrogenization". Oestrogens stimulate the development of tissues involved in reproduction. Under oestrogen stimulation, the vaginal epithelium and uterine endometrium proliferates, the myometrium matures, and breast ducts proliferate. Oestrogens also have an anabolic effect on bone and cartilage, promote growth, and are responsible for most secondary sexual characteristics in the female.

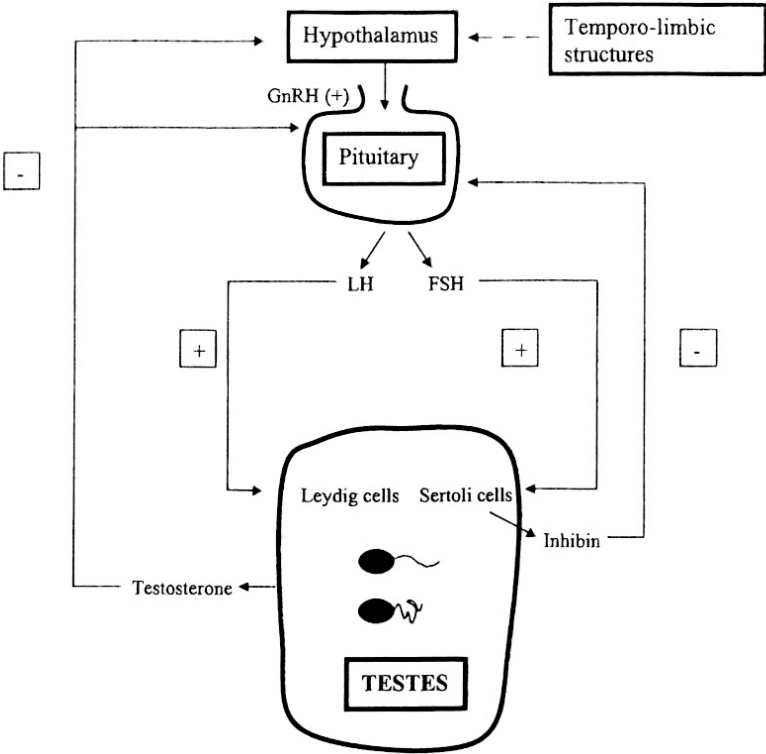
**Progesterone:** Progesterone is synthesized and secreted by the corpora lutea. As with oestrogens and testosterone, progesterone is derived from cholesterol (figure 3), but the enzyme aromatase is not in the synthesis pathway. Progesterone is metabolized in the liver. Progesterone requires a previous or concurrent action of oestradiol to have an effect. Progesterone reduces the proliferation and growth of vaginal epithelium and converts the uterine epithelium from proliferative to secretory. Progesterone enhances development of the acinar portion of breastglands after ductal development has been stimulated by oestrogens.

**Figure 1. Female sexual hormones**

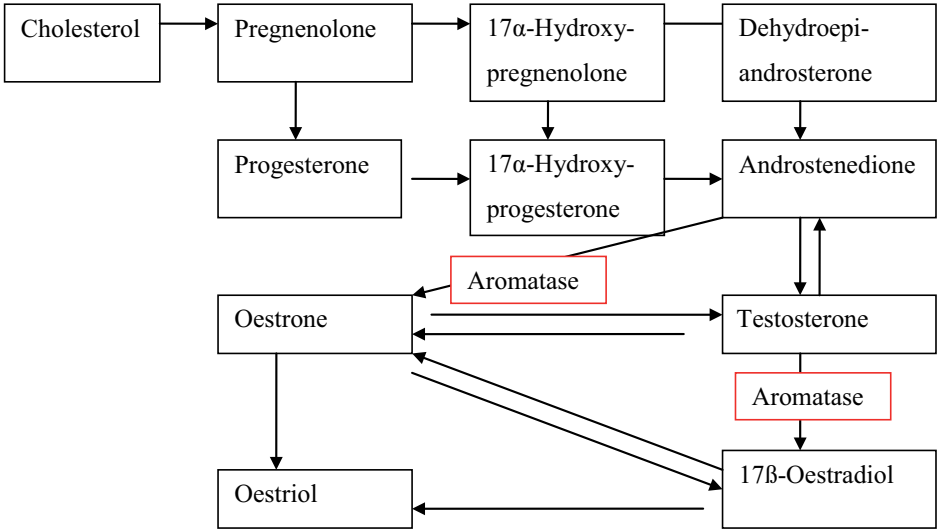


**TESTES: Androgens:** Testosterone is synthesized in the Leydig cells in the testicles, under the influence of LH. Testosterone is the major hormone produced in the foetus, but testes only produce androsterone after birth. The ability to produce testosterone is restored at puberty and continues throughout life. The androgens (principally testosterone and 5-alpha-DHT) are involved in sexual differentiation, spermatogenesis, development of secondary sexual characteristics, anabolic metabolism, and in masculine behaviour patterns. Testosterone is important for sexual fantasizing and desire, but is not involved in achieving an erection.

**Figure 2. Male sexual hormones**



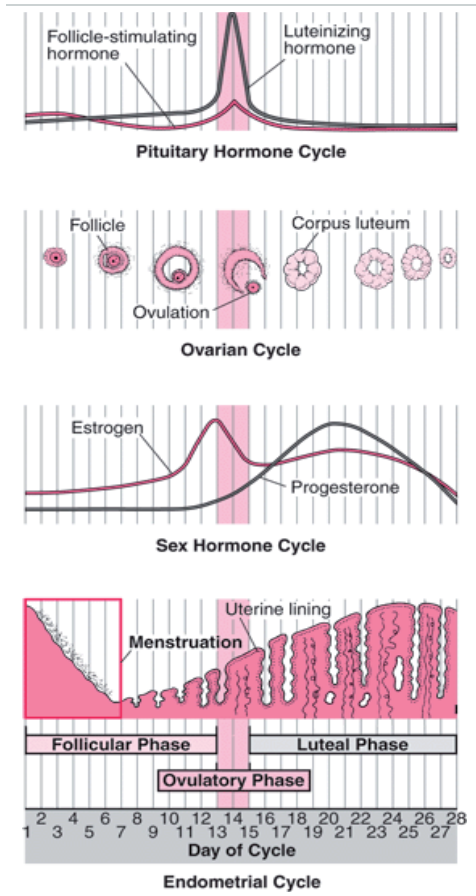
**Figure 3. Biosynthesis of oestrogens**



**Puberty:** In females, puberty is initiated by a gradual increase in gonadotropic hormones from the pituitary gland, beginning in about the eighth year of life. This stimulates a gradual increase in oestrogen production, culminating in the first menstruation (menarche) usually between 11 and 16 years of age. In males, testosterone is produced in the testes from early embryonic life and is responsible for the development of male sexual organs. The serum concentration of testosterone is low at birth, but increases during the first year of life. From the second year of life until onset of puberty, testosterone concentrations are very low, but from onset of puberty at ages 12-13 years, until about age 20 years, plasma testosterone increases. Testosterone concentration then remains stable until old age, when it declines.

**Menstrual cycle:** After menarche, menstruation follows a cyclical, approximately monthly, pattern. GnRH from the hypothalamus is secreted in 5 to 25 minute pulses, at 1 to 2 hours intervals. This causes the anterior pituitary to secrete LH and FSH. The menstrual cycle is conventionally described as being 28 days, but may range from 21 to 35 days. On day one, the menstrual bleeding starts, and continues for approximately 5 days. At the same time the follicular phase starts and the endometrium is built up, preparing to receive a fertilized ovum. Under the influence of predominantly FSH, a follicle matures. Ovarian blood flow increases and the maturing follicle produces increasing amounts of oestradiol. On day 13, oestradiol concentrations peak and, by a positive feedback effect, the surge of GnRH and LH is released. This results in a peak in LH, which is necessary for ovulation to occur. At this time, body temperature increases by approximately 0.5°C. Days 14 to 28 is called the luteal phase and is characterized by formation of the corpus luteum. The corpus luteum produces mainly progesterone, but also some oestradiol. If pregnancy does not occur, the corpus luteum gradually involutes. At days 27 and 28 there is a sudden decline in oestradiol and progesterone secretion, followed by constriction of the spiral arterioles and development of endometrial ischaemia and desquamation; i.e menstruation. (Figure 4).

**Figure 4. Changes during the menstrual cycle**



From “The Merck Manuals online medical library”

### **4.3 CLINICAL OBSERVATIONS IN MEN AND WOMEN WITH EPILEPSY**

#### **4.3.1. Puberty**

Onset of puberty can have an effect on epilepsy. Some childhood epilepsies, such as benign childhood focal seizures, tend to remit, but puberty may trigger the onset of some other epilepsies. Juvenile myoclonic epilepsy typically has its debut around puberty. Several factors which are known to influence seizure threshold may alter during the pubertal years. Alcohol may be introduced and sleeping habits often change. Life, in general, is often more irregular than it was in childhood and reverts to later in adult life. Our knowledge about hormonal influences on brain excitability has resulted in the development of a hypothesis that puberty or menarche may be important triggers for epilepsy, and associations between onset of epilepsy and menarche have been reported for up to 20 - 30 % of female patients (Klein et al., 2003; Morrell et al., 1998).

#### **4.3.2 Menstrual disturbances**

Menstrual disturbances are common among healthy women. A study performed many years ago reported menstrual disturbances in up to 27 % of women without epilepsy (Aray, 1939). In another study of women of reproductive age, 18 % had irregular cycles (Polson et al., 1988). When comparing studies on menstrual disturbances it is important to consider how menstrual disturbances are defined in the individual studies. A common definition used in many studies, and defined by Polson in 1988, is a menstrual cycle of less than 22 days or of more than 35 days, or duration of bleeding of more than 8 days, or a variation of more than 4 days in two consecutive periods. Women with epilepsy have been shown to have more menstrual disturbances than women without epilepsy (Herzog et al., 1986; Bilo et al., 1988, 2001; Murialdo et al., 1997; Hamed et al., 2007) and also often have amenorrhoea or oligomenorrhoea (Herzog et al., 2003; Hamed et al., 2007). Menstrual disturbances are often seen in women with polycystic ovaries (PCO), which occur frequently in women with epilepsy (Herzog et al., 1984, 1986), and are also associated with the use of valproate (VPA). PCOS occurs in between 4 and 6 % of women in the general population (Knochenhauser et al., 1998; Clayton et al., 1992) and in between 10 and 25 % of women with epilepsy, even if they are not receiving antiepileptic drugs (AEDs) (Herzog et al., 1984, 1986; Hamed et al., 2007). The reasons for these disturbances have been debated in depth by doctors and other professionals in this field. Are they caused by the epilepsy itself? The



medication? Or is the underlying disease or psychosocial factors responsible? Probably all these factors play a role, with their contributions varying depending on the individual cases.

The hormonal fluctuations during the menstrual cycle have been regarded as relevant for the cyclical pattern of seizures in women with epilepsy. Herzog et al. (1997) described three different patterns of epilepsy, associated with different phases in the menstrual cycle:

- 1) Perimenstrual (Days -3 to 3, when start of menstruation is day 0);
- 2) Ovulatory (Days 10 to -13);
- 3) Anovulatory cycles: an increased frequency of seizures in the perimenstrual, luteal (days -12 to -4), and ovulatory phases, rather than in the follicular phase (days 4 to 9).

This description of catamenial epilepsy has been widely accepted.

### **4.3.3 Fertility**

People with epilepsy frequently have lower birth rates than the general population. In the Rochester study from 1986, fertility in males was reduced to 80 % and to 85 % in women (Webber et al., 1986). Several later studies have confirmed this finding (Schupf and Ottman, 1996; Dansky et al., 1980; Artama et al., 2004, 2006). However, in a population-based study from Iceland, no difference in birth rate was detected between female epilepsy patients and the reference group (Olafson et al., 1998). Alterations in sperm motility and sperm count have been found in males with epilepsy; Taneja et al. (1994) showed that both phenytoin (PHT)-treated and untreated males with epilepsy had reduced sperm motility, and lower seminal fluid volumes and spermatozoa concentrations than controls. VPA-treatment has also found to be associated with fertility problems and abnormal sperm (Curtis et al. 1994; Yerby and Mc Coy, 1999). Røste et al. (2003) found alterations in semen quality in males treated with either VPA or carbamazepine (CBZ) compared with controls.

Fertility rate is a complex issue that is affected not only by the epilepsy itself and the associated medication, but is also influenced by the patient's social and psychological situation. The frequency of marriage is lower among epilepsy patients than in the general population, but birth rate is also reduced among married patients (Schrumpf et al., 1996; Dansky et al., 1980). Because epilepsy is a symptom of an underlying brain disease in many patients, it can be the underlying disease (inherited diseases, cancer, cerebro-vascular diseases etc) that causes the reduced fertility, rather than the associated epilepsy. The burden of suffering from epileptic seizures and the increased risk of malformations in offspring due to

AEDs can influence decisions on having babies. Real infertility only exists if pregnancy does not occur despite persistent attempts; this aspect has been studied very infrequently in humans.

#### **4.3.4 Sexuality**

According to the diagnostic classification system ICD-10, the definition of sexual dysfunction includes “the various ways in which an individual is unable to participate in a sexual relationship as he or she would wish”. In a study by Morrell and Guldner (1996), patients with localization-related epilepsy (LRE) and primary generalized epilepsy (PGE) reported reduced sexual arousability. Furthermore, women with LRE reported greater sexual anxiety, more dyspareunia and vaginismus, and sexual dissatisfaction, and women with PGE experienced dysorgasmia and sexual dissatisfaction. Bergen et al. (1992) showed that sexual desire was significantly lower in a group of female epilepsy patients, without any correlation with type of AED used, duration of epilepsy, or seizure type. Diminished sexual interest and activity is found in both men and women with temporal lobe epilepsy, and in men this also occurs in conjunction with a reduced potency. Comparison of patients with right temporal lobe epilepsy (RTLE) and left temporal lobe epilepsy (LTLE) by Herzog et al. (2003b), demonstrated that women with RTLE had poorer scores in the Arizona Sexual Experience Scale (ASEX) questionnaire for measuring sexual function.

Three different mechanisms have been put forward as explanations for these findings. These are:

- 1) Ictal or interictal activity of epileptic foci located in medial temporal limbic structures may disrupt hypothalamic regulation of pituitary gonadotropins or prolactin function (Herzog et al., 1982, 1986a, b; Pritchard et al., 1983; Spark et al., 1984).
- 2) Various effects of AEDs on sexual functions (Barragry et al., 1978; Mattson et al., 1985; Herzog, 1991; Morrell et al., 2005).
- 3) The psychosocial factors associated with epilepsy (Levin et al., 1988).

In studies of healthy volunteers, sexual dysfunction has been correlated with depression, negative experiences in sexual relationships, and reduction in overall well-being (Laumann et al., 1999). Women with epilepsy are often overrepresented in these groups, and it is well known that depression is a common problem among epilepsy patients (Morrell et al., 2005). However, sexual problems are common in most chronic diseases (Schover and Jensen, 1988; Spector and Carey, 1990) and also occur frequently in healthy persons.

Cultural aspects may also be of importance and should be considered when evaluating the results of such studies. In a study of out-patients from Egypt (Demerdash et al., 1991), it was observed that sexual dysfunction was less common in women with epilepsy than in healthy controls in USA, where most such studies are performed.

#### **4.4 INFLUENCES OF EPILEPSY ON HORMONES**

Epilepsy itself can affect reproductive endocrine function. Both animal and human studies have demonstrated that hormone levels and reproductive function are altered after temperolimbic seizures (Edwards et al., 1999a, b; Feeny et al., 1998). In female rodents, amygdala seizures can cause anovulation, elevated serum oestradiol, and accumulation of ovarian follicular cysts (Edwards et al., 1999a). In male rats, amygdala kindled seizures and generalized seizures have been shown to elevate serum testosterone, oestradiol, and prolactin, and also to disrupt normal gonadal structure (Edwards et al., 1999b). In male cats, amygdala kindling has been shown to result in hypo-sexuality and fewer offspring (Feeny et al., 1998). Unilateral amygdaloidal seizures in rats have been shown to activate specific hypothalamic nuclei involved in reproductive endocrine regulation (Silveira et al., 2000), with greatest effect ipsilateral to the electric stimulation. This study supports the hypothesis that seizures originating from left or right amygdala can result in different reproductive endocrine disorders. There is also clinical evidence that epileptiform discharges disrupt the temporolimbic modulation of hypothalamo-pituitary function (Sperling et al., 1986; Herzog, 1993).

LTLE in women is associated with significantly higher pulse frequencies of GnRH secretion (Herzog et al., 2003a), which, in turn, are associated with higher LH/FSH ratios and higher testosterone levels (Herzog et al., 2003a). This endocrine change is characteristic of PCOS, and has been associated with the increase in PCOS seen in epilepsy patients. In male rats, testicular steroidogenesis was suppressed, and serum testosterone concentrations significantly decreased, following brain cuts above the amygdala on the left side (Banczerowski et al., 2003a).

RTLE is associated with lower GnRH pulse frequencies (Herzog et al., 1986, 2003a), which, in turn, are associated with decreased LH and oestradiol levels. These reductions are characteristic of hypothalamic amenorrhoea (Herzog et al., 1986, 2003a; Kalinin and Zheleznova, 2007). Prolactin elevation commonly follows right lateral discharges (Sperling et al., 1986) and generalized seizures (Abbott et al., 1980; Rao et al., 1989). In male rats, a brain

cut above the amygdala on the right side induced a rise in basal testosterone secretion from both testes (Banczerowski et al., 2003a), while kainic acid injection in the amygdala resulted in a decrease in testosterone (Banczerowski et al., 2003b).

#### **4.5 INFLUENCES OF HORMONES ON EPILEPSY**

The sex hormones have been demonstrated to have differing effects on seizure susceptibility. The effects of the different sex hormones are described individually in the following sections.

##### **4.5.1 Oestradiol**

Studies from the 1960s by Logothetis and Harner (1960) and Woolley and Temiras (1962) showed that oestradiol administration to ovary-ectomized rats resulted in proconvulsant effects. Early studies have also shown that application of oestrogens directly to the cerebral cortex caused rapid and potent epileptogenic effects (Logothetis et al., 1959; Logothetis and Harner, 1960; Marcus and Watson, 1966; Hardy, 1970). Oestradiol has been shown to promote kindling in animals (Hom and Buterbaugh, 1986; Buterbaugh, 1989; Edwards et al., 1999c). Oestradiol also potentiates seizures induced by pentyletetrazol (PTZ) (Nicoletti et al., 1985; Woolley, 2000). The excitatory effects of oestrogens can be partially explained by their ability to enhance glutamate-receptor excitatory neurotransmission (Smith et al., 1987, 1988; Wong and Moss, 1994) and decrease gamma-aminobutyric acid (GABA)-ergic inhibition (Murphy et al., 1998). Oestradiol acts on neurons in the limbic system, cerebral cortex, and other regions important for seizure susceptibility. This pro-convulsive effect of oestrogens has been proposed to be of importance in the variation in seizure susceptibility in different phases of a woman's life.

##### **4.5.2 Progesterone**

The first report on progesterone's anticonvulsant effect in the PTZ test was published by Hans Selye (1942). More reports have gradually been published on the anti-seizure effects of progesterone, from both animal and human studies (Craig and Deason, 1968; Landgren et al., 1978; Herzog, 1995, 1999a, b; Lonsdale and Burnham, 2007; Taubøll et al., 1993a, c, 1994; Taubøll and Gjerstad, 1993b). Progesterone and its metabolites directly influence brain excitability through a steroid site on or near the GABA/benzodiazepine receptor-chloride ionophore complex (Majewska et al., 1986; Harrison et al., 1987; Gee et al., 1987, 1988;

Peters et al., 1988; Turner et al., 1989). The effect of progesterone and its metabolites is similar to that of barbiturates, but they do not share a common site of action on the GABA<sub>A</sub> receptor complex. The steroids have been shown to prolong the effective open time of the chloride channels, thereby inhibiting excitation of neurons (Barker et al., 1986). This contrasts with the findings for benzodiazepines and barbiturates, which mainly act by increasing the frequency of GABA-channel openings (Study and Barker, 1981). In addition to the GABA effect, progesterone may also affect excitatory mechanisms. In studies performed by Smith et al. (1987) it was shown that after systemic or topical application of the steroid in Purkinje cells from cat, both progesterone and several metabolites decreased glutamate responsiveness. Progesterone therapy has been beneficial in the treatment of catamenial epilepsy (Mattson et al., 1984; Herzog 1995, 1999 a, b).

#### **4.5.3 Androgens**

The effect of androgens on seizure susceptibility is more complex. Whilst testosterone lowers the seizure threshold, increases PTZ seizures, and enhances the development of amygdala-kindled seizures (Edwards et al., 1999; Reddy, 2004a), androgens also show an anticonvulsant effect, probably mediated by their interactions with the GABA<sub>A</sub> receptor (Frye and Reed, 1998; Reddy 2004b).

### **4.6 MEDICATION AND GENDER-SPECIFIC ISSUES**

#### **4.6.1 Enzyme-inducing drugs**

Phenobarbital (PB), PHT, and CBZ are all inducers of hepatic microsomal enzymes, and thus accelerate the metabolism of other drugs, and also the breakdown and production of SHBG. This increases SHBG, and reduces free, circulating androgens and oestrogens, resulting in a decreased level of biologically active sex hormones (Macphee et al., 1988; Isojärvi et al., 1988, 1990a, b, 1991; Rättyä et al., 2001; Murialdo et al., 1994, 1998). In a randomized study by Mattson et al. (1985), impotence and decreased libido was reported in 16 % of epilepsy patients starting monotherapy with PB, 12 % after starting PHT, and 13 % after starting CBZ. Elevated oestradiol has also been found in men with epilepsy taking PHT (Isojärvi et al., 1990a; Herzog, 1991; Murialdo et al., 1994). Associations between high oestradiol levels and sexual dysfunction have been found in some men (Herzog, 1991). CBZ has not been found to elevate serum total testosterone or oestradiol, but an increase in SHBG decreases the level of

free androgens (FAI = free androgen index) (Mcphee et al., 1988; Isojärvi et al., 1988, 1990a, 1991, 1995a, b; Isojärvi, 1990b; Rättyä et al., 2001). In some men, sexual dysfunction arises after long-term CBZ treatment. In women taking CBZ, a similar pattern occurs as in men, with a progressive increase in SHBG. This results in a decreased amount of bioactive oestradiol, which can be associated with menstrual disturbances after long-term treatment. Low serum dehydroepiandrosteronsulphate (DHAES) has also been reported in men and women taking PHT and CBZ (Macphee et al., 1988; Isojärvi, 1990b; Levesque et al., 1986). DHAES is a weak androgen, secreted from the adrenal cortex, and the clinical implication of low DHAES is unknown.

Both CBZ and PHT have been associated with changes in semen quality (Chen et al., 1992), and two relatively recent studies have shown that CBZ is associated with reduced sperm motility and increased frequency of morphologically abnormal sperm (Røste et al., 2003; Isojärvi et al., 2004).

Despite these findings there is no obvious significant reduction in fertility in patients using enzyme-inducing AEDs (EIAEDs) (Artama et al., 2006).

#### **4.6.2 Valproate**

Although VPA was introduced in the early 1960s, the first systematic report on VPA suggesting reproductive endocrine disturbances was not published until 1993 (Isojärvi et al., 1993). Since then numerous studies have been published, showing a high incidence of menstrual disturbances, linked to obesity, hyperandrogenism, and PCO (Isojärvi et al., 2001, 2005; Murialdo et al., 1998; Vainionpää et al., 1999; Morrell et al., 2003; Luef et al., 2002). Development of PCO seems to be especially common when VPA is started before 20 years of age (Isojärvi et al., 1993; Vainionpää et al., 1999), but also occurs when VPA treatment is started later in life. It has been suggested that this reproductive disorder could be secondary to obesity, but PCO also occur in lean women (Isojärvi et al., 1996, 2001). In obese women, hyperinsulinemia and low insulin growth factor binding protein1 (IGFBP-1) have been found. Insulin and insulin growth factor 1 (IGF-1) are well recognised as stimulants of ovarian androgen synthesis (Giudice, 1992; Conover et al., 1992). Testosterone levels are elevated in both lean and obese women using VPA (Isojärvi et al., 1996, 2001), but insulin and IGFBP-1 are only elevated in obese women. The endocrine effects caused by VPA are at least partially reversible (Isojärvi et al., 1998). In the 1990s, the endocrine side-effects of VPA were heavily debated, especially the issue of VPA's association with PCOS (Genton et al., 2001; Isojärvi et

al., 2001; Bauer et al., 2000). As VPA is not associated with elevated LH levels (Isojärvi et al., 1993, 2001; Murialdo et al., 1998; Rättyä et al., 2001), it is unlikely that it stimulates ovarian androgen synthesis via LH secretion. The endocrine effect of VPA occurs in the ovary, where it acts as an enzyme inhibitor, inhibiting the conversion of testosterone to oestradiol (Peruccae et al., 1984), and this hypothesis is supported by results from various animal studies (Gregoraszczuk et al., 2000; Taubøll et al., 2002, 2003; Røste et al., 2002). High levels of testosterone in the ovary will arrest follicular maturation and promote the development of PCO (Hsueh et al., 1984). Persons using VPA for reasons other than epilepsy also have more menstrual disturbances and PCO than controls (O'Donovan et al., 2002). In total, these data suggest that the epilepsy *per se*, is unlikely to be the reason for the endocrine changes seen in women taking VPA.

In men, VPA reduces serum levels of gonadotropins and androstenedione (Rättyä et al., 2001; Isojärvi et al., 2004). Cases of infertility have been noted in males using VPA. VPA has been shown to affect sperm motility and is associated with an increase in frequency of morphologically abnormal sperms (Isojärvi et al., 2004; Røste et al., 2003).

#### **4.6.3 Lamotrigine**

Studies of lamotrigine (LTG) in both animals and humans (men and women) have not detected any endocrine side-effects (Herzog et al., 2004; Isojärvi et al., 1998; Biton et al., 2001; Røste et al., 2001). LTG is therefore generally regarded as safe with respect to endocrine side-effects. However, LTG has recently been found to interact with oestrogens, such that LTG concentrations are reduced when oestrogens levels are high, due to use of birth control pills (Reimers et al., 2005; Christensen et al., 2007).

#### **4.6.4 Levetiracetam**

Levetiracetam (LEV) is a relatively new, broad-spectrum AED with positive reports regarding both effects and side-effects (Ben-Menachem, 2003). Recent studies have shown a promising effect of LEV on generalized epilepsies like tonic-clonic, absence, and myoclonic epilepsy, for which VPA has previously often been the drug of choice (Krauss et al., 2003; Di Bonaventura et al., 2005; Labate et al., 2006). Safety data indicate that LEV is well tolerated by the majority of patients, and endocrine side-effects have not been reported in humans to date (Ben-Menachem, 2003; Harden, 2001; Briggs and French, 2004). However, a recent study, utilizing ovarian follicular cells from prepubertal pigs, demonstrated that LEV affects

basal steroid hormone secretion (Taubøll et al., 2006) indicating an effect of the drug on steroidogenesis. LEV does not seem to act by any of the main mechanisms currently accepted for AED anti-seizure action (GABAergic facilitation, inhibition of Na<sup>+</sup> channels, or modulation of low-voltage activated Ca<sup>2+</sup> current) (Lynch et al., 2004). LEV binds to a synaptic vesicle protein, SV2A (Lynch et al., 2004), which is widely distributed in most endocrine tissue (Buckley and Kelly, 1985; Portela-Gomez et al., 2000; Bajjalieh et al., 1994), including male gonads (Bajjalieh, personal communication). Specific information regarding the ovary is currently unavailable. However, these data suggest a possible means by which LEV may influence endocrine function.

## **5. AIMS OF THESIS**

- 1) To investigate whether menstrual disturbances are more frequent among women with epilepsy than in the general population.
- 2) To investigate whether menstrual disturbances in women with epilepsy are associated with epilepsy type, seizure severity, type of AED used, and use of mono- or polytherapy.
- 3) To investigate whether epilepsy affects age of menarche, and whether the natural endocrine changes during puberty can result in debut of epilepsy.
- 4) To use a female animal model to investigate the occurrence of endocrine side-effects associated with use of the new drug LEV.
- 5) To use a human study to investigate whether the results of the animal study (pt. 4) are of clinical relevance.



## **6. SUMMARY OF PAPERS**

### **6.1 PAPER 1**

The aim of this study was to examine the frequency of menstrual disturbances in a large population of epilepsy patients, which had not been specifically selected. Most previous investigations of menstrual disturbances in epilepsy patients have been small and based on a limited patient group, often focussing upon poorly functioning patients from tertiary care centres.

A retrospective questionnaire of a cohort of female outpatients, aged between 18 and 45 years, was conducted. In order to optimise controls, regarding age and lifestyle, each patient chose a close female friend who agreed to serve as a control.

Completed questionnaires were received from 265 patients and 142 controls. Menstrual disturbances were more frequent in patients with epilepsy (48.0 %) than in controls (30.7 %) ( $P = 0.004$ ). Menstrual disturbances were more frequent in patients on polytherapy than on monotherapy ( $P = 0.049$ ), and more frequent in patients with high seizure frequency ( $> 5$  seizures/year) than in patients with a lower seizure frequency or free of seizures ( $P = 0.006$ ). The frequency of menstrual disturbances was higher in patients using VPA than CBZ monotherapy ( $P = 0.045$ ).

The investigation confirmed that women with epilepsy have an increased frequency of menstrual disturbances compared with women without epilepsy. In women with high seizure frequency and/or using polytherapy, the frequencies of menstrual disturbances are increased yet further. The highest frequency of menstrual disturbances occurred in women using the AED, VPA.

### **6.2 PAPER 2**

In this study we wanted to investigate the relationship between debut of epilepsy and age of menarche in a large population of female epilepsy patients. We were interested in discovering whether sexual maturation in puberty could induce the debut of seizures, as has been previously indicated in some studies. We also wanted to explore the possibility that epileptic activity could induce alterations in pubertal endocrine maturation and thereby age of menarche.

A retrospective, questionnaire of a cohort of 265 female outpatients from three Norwegian hospitals, and 142 controls, aged between 18 and 45 years, was conducted. Parameters regarding epilepsy and reproductive health issues were registered. Perimenarche was defined as the periods 2 years before and 2 years after the year of menarche.

We found a significantly higher frequency of patients with epilepsy debut between 10 and 18 years compared with a younger age-group (0 - 9 years;  $p < 0.01$ ). However, there was no significant difference in occurrence of epilepsy debut in the perimenarche period compared with the 5 year periods before and after perimenarche, and no significant difference in epilepsy debut in the year of menarche compared with the 5 years before and after menarche. Age at menarche did not differ significantly in those with epilepsy debut before menarche from those with debut after menarche. Epilepsy type (idiopathic generalized or partial) did not influence the age of menarche.

This study did not confirm former observations of clustering of epilepsy debut at menarche nor in the perimenarche period, or alterations in age of menarche in girls with epilepsy. However, onset of epilepsy was more frequent in the adolescent age group than in childhood.

### **6.3 PAPER 3**

The aim of this study was to investigate possible effects of LEV on endocrine function and ovarian morphology in non-epileptic rats. LEV is one of the newer AEDs and endocrine side-effects have not previously been reported in humans. However, a study on ovarian follicular cells from prepubertal pigs showed that LEV affected basal steroid hormone secretion.

Thirty female Wistar rats were fed per-orally with either 50 mg/kg LEV ( $n = 15$ ) or 150 mg/kg LEV ( $n = 15$ ) twice daily for 90 - 95 days. Twenty rats received a control solution.

There was a significant, dose-dependent increase in mean ovarian weight after LEV treatment. Mean numbers of ovarian follicular cysts were unchanged, but there were significantly more corpora lutea and secondary follicles in the treated animals. Serum testosterone was significantly increased in treated animals (0.50 nmol/l versus 0.16 nmol/l in controls,  $p < 0.05$ ), while oestradiol was reduced (67.4 compared with 257.5 pmol/l in controls,  $p < 0.05$ ). The low-dose group had significantly higher serum progesterone

concentrations than the control group (56.8 nmol/l versus 34.7 nmol/l, respectively,  $p < 0.05$ ). FSH was lower in the treated animals (3.3 ng/ml versus 5.5 ng/ml,  $p < 0.05$ ) while LH was unaffected.

Our findings indicate a possible effect of LEV on the hypothalamic-pituitary-gonadal (HPG) axis and ovarian morphology in non-epileptic rats. These effects have not previously been described for other AEDs.

#### **6.4 PAPER 4**

The aim of this study was to investigate whether men and women with epilepsy and treated with LEV exhibited differences in serum hormones, compared with epilepsy patients using CBZ or LTG in monotherapy for epilepsy, and compared with healthy controls. Sexual function was also assessed and compared.

A total of 306 subjects, aged between 18 and 45 years, participated in the study. The epilepsy patients had been using LEV (30 men, 26 women), LTG (37 men, 40 women), or CBZ (63 men, 30 women) monotherapy for at least 6 months. 36 men and 44 women served as healthy controls. Blood samples for hormone analyses were collected, and questionnaires completed for assessing sexual function (ASEX) and other health-related factors. No endocrine changes were found in women using LEV, but SHBG was higher and progesterone lower in women using CBZ. In women using LTG, concentrations of DHEAS were higher and androstenedione lower. ASEX scores were significantly lower in female patients using LTG or LEV than in patients using CBZ and than in control subjects, indicating that LTG and LEV use were associated with improved sexual function in women.

No specific hormonal patterns were identified in the men treated with LEV. Male patients in all treatment groups had lower androstenedione and free testosterone concentrations than control subjects. Men using CBZ had a lower FAI and DHEAS concentration, and higher SHBG, FSH, and LH concentrations. ASEX scores for men were similar in all groups.

We found no drug specific hormonal effect of LEV in either men or women. Women treated with LTG or LEV were more satisfied with their sexual function than patients treated with CBZ and than healthy controls, while sexual function in males appeared to be unaffected by treatment. The results of this study did not confirm our results from the animal study regarding LEV.

## 7. DISCUSSION

In paper 1 we focus on menstrual disturbances in women with epilepsy. Several previous studies have shown an increased frequency of menstrual disturbances in women with epilepsy (Bilo et al., 1988; Herzog et al., 2003a; Morrell et al., 2002), but one problem with some of these studies is that the controls are poorly matched to the patient group. The optimal situation is that the control group consists of persons that differ only from the patient group with respect to the diagnosis and its treatment. Socioeconomic environment and health status (regarding non-epilepsy diseases) in the two groups should be as similar as possible. In order to optimise the control group in our study, the epilepsy patients were asked to choose one close friend for the control group questionnaire. We believe that this matching procedure for choice of control group was good, because the friends chosen were usually about the same age and weight as the patients and probably from the same social environment. The intention was to analyse patients and controls as individual pairs, but as we received completed questionnaires from 265 patients but only 142 controls this approach was not possible. Nevertheless there were sufficient controls for statistical comparison between groups.

Another problem with former studies has been that the participating epilepsy patients often have a severe epilepsy burden (many seizures, polytherapy etc.), and are therefore probably not a good representation of women with epilepsy in the general population. The aim of our investigation was to study an unselected group of patients, representative of the whole epilepsy group, except for those with major mental deficits. We also wanted to focus upon a relatively healthy group of epilepsy patients in order to minimize ambient factors which may have impacted on the results. By examination of patient registries at the participating hospitals, we were able to include all women in the right age group (between 18 and 45 years), excluding those with major intellectual deficits who would not be capable of answering the questionnaires.

The response rate achieved in this study was 53 % (265/500). In order to determine whether the non-responders differed from the responders, we examined medical records from 45 of the non-responders. Analysis of age, epilepsy debut age, seizure type, and medication demonstrated that the only parameter that differed between the responder and non-responder

groups was age of epilepsy debut, which was about three years lower in the non-responder group.

In this large study of 265 epilepsy patients we found a clear elevation in menstrual disturbances among epilepsy patients (48 %), compared with women without epilepsy (30.7 %) ( $p = 0.004$ ), although menstrual disturbances were common in both groups. However, as the exact value within each group is dependent on the definition of a menstrual disturbance, and this definition often varies between different studies, it is the difference between the groups that is of greatest interest, rather than the exact percentages. In this study a menstrual disturbance was defined by the examiner (according to Polsen et al., 1988), rather than by the patient, on the basis of answers provided to ten questions about the regularity and duration of menstrual periods. This is probably a relatively sensitive method for detection of menstrual disturbances, and obviously both patients and the controls answer exactly the same questions. One problem could have been the retrospective design of the study, which can always be influenced by recall-bias, but this problem would have affected both groups equally.

Following the detection of an increased frequency of menstrual disturbances in women with epilepsy, the next step was to explore the aetiology of the menstrual disturbances. Which factors were likely to be of importance: the drug treatment? the location of epileptic focus? the EEG pathology? the psychosocial burden of having a chronic disease? In this study we were able to address some these questions. We found more menstrual disturbances among women using VPA monotherapy than CBZ monotherapy. This result can probably only be explained by the use of the different AEDs. The blinded design of this study made a sub-classification of seizures due to localisation impossible, but primary generalized seizures could be separated from partial seizures and we were unable to detect any difference in frequency of menstrual disturbances between these two groups. Only the CBZ and VPA monotherapy groups were large enough for meaningful comparisons between epilepsy types. We did not find significantly more menstrual disturbances in the group of patients using CBZ monotherapy for generalized seizures (45.5 %,  $n = 22$ ) than in the group using CBZ for partial seizures (36.4 %,  $n = 33$ ) ( $p = 0.22$ ). In patients using VPA in monotherapy for generalized seizures the number of menstrual disturbances was not significantly higher than in the group using VPA for partial seizures (63.6 % and 55.5 %, respectively). However, there was a higher percentage of menstrual disturbances in women with generalized seizures, in both the CBZ group and the VPA group, but the differences were not significant. These results

demonstrate that menstrual disturbances in the study were independent of seizure type (primary generalized vs. partial onset), but the possible endocrine effects of the epileptic focus occurring in specific areas of the brain were not explored.

The occurrence of more than five seizures during the previous 12 months was associated with more menstrual disturbances. Patients with more seizures may have more epileptic activity in their EEG, so it is possible that the epileptic activity contributes to the menstrual disturbances. However, exploration of this hypothesis is complicated by the fact that patients in this group use more AEDs than patients with fewer seizures, and it is very difficult to investigate these two factors separately in human clinical studies.

Although experiencing irregular menses is not necessarily a problem *per se*, it can be an indicator of hormonal irregularities that can cause alterations in fertility and sexual life, as well as impacting upon more cosmetic alterations in fat distribution and body-hair pattern.

Infertility is the most serious problem caused by hormonal changes. Although this study was not intended or designed to investigate infertility induced by epilepsy or its treatment, we nevertheless noted that the women in the control group had more children per women (1.1 children per woman) than the patients (0.8 children per woman). The reason for this small difference can be multifactorial and is not necessarily caused by infertility. Infertility caused by epilepsy or AEDs in a subset of patients could be more usefully investigated in a prospective study.

We wanted to use this large population of 265 epilepsy patient and 142 controls to study other relevant endocrine issues. Several human and animal studies have demonstrated that oestrogens facilitate seizures by lowering the seizure threshold. In the period of 2 to 4 years before menarche, oestrogen production in girls starts to increase. Secretion of progesterone, which has the opposite effect on seizure threshold, starts after the first menstruation. As some studies have shown clustering of epilepsy debuts in the years around menarche (Klein et al., 2003; Morrell et al., 1998) we were interested in exploring this possibility in our study population. Thirty patients were excluded from this arm of the study because their epilepsy was secondary to neurodegenerative disorders, vascular diseases, multiple sclerosis etc., and therefore probably less likely to be influenced by hormones, and we included only those patients with epilepsy debut between the ages of 0 and 18 years old. This enabled us to exclude those individuals for whom other causes of epilepsy were more likely. Although we

found a higher number of patients with debut of epilepsy in the age group between 10 and 18 years than in the age range between 0 and 9 years, we did not reproduce the findings of Klein et al. (2003) which showed a clustering of epilepsy debut in the 2 years before and after the year of menarche. As oestrogen increase can start more than 4 years before menarche, it is possible that only investigating two years before menarche was too short a period to demonstrate a clustering effect in our patient group. Other possible explanations for the increase in epilepsy debut between the ages 10 and 18 years can be multifactorial; important lifestyle changes often occur during adolescence including decreased sleep, introduction of alcohol, and disruption of routines. These factors are all known to provoke seizures. Some common epilepsy syndromes, such as juvenile myoclonic epilepsy, also tend to start between 10 and 18 years of age. One weakness in our study is that we did not follow the entire female population. The participants were between 18 and 45 years, and therefore individuals who developed epilepsy in childhood, but recovered before the age of 18, were not included. This exclusion might have affected our results.

The final issue we wanted to investigate in this population was whether epilepsy was associated with delaying or hastening menarche. We compared menarche age in patients with debut of epilepsy prior to menarche with those whose epilepsy debut had occurred after menarche. Mean ages of menarche for each group were 13.2 and 12.7 years, respectively, which is not significantly different ( $p = 0.071$ ). Additionally, no difference in menarche age could be detected between those suffering from partial or generalized epilepsy, indicating that for large groups of women with epilepsy, their epileptic discharges probably do not affect important hormonal systems like puberty and menarche. It would have been interesting to explore whether having many seizures and much epileptic activity in EEG was associated with timing of menarche, but detailed EEG information on the patients was unavailable.

One reason why associations between menarche/puberty and epilepsy debut/exacerbation of seizures are interesting, is because they provide an indication of the extent to which hormones influences epileptic seizures in humans. The influences of oestrogens and progesterone on seizures have been studied closely in *in vitro* and animal models, but although several studies have investigated menstrual cycle related seizures (catamenial) and some have explored associations between menopause and seizures, in this phase the results are more complex and difficult to interpret. Knowledge on this subject is likely to be important for optimising treatment of epilepsy during different life stages and might also give further insights regarding the normal fluctuation of the disease.

In paper 1, we found that menstrual disturbances were more frequent in women using VPA and polytherapy than in other women with epilepsy, supporting the need to improve our knowledge regarding the endocrine potential of different AEDs. Many patients start using AEDs as children and continue to use them for several years, often throughout life. LEV is a relatively new drug, with a mode of action which differs from that of all well-known and established AEDs. Our knowledge about binding to a synaptic vesicle receptor, SV2A, is relatively recent, but it is thought that the effect of LEV is mediated through this binding. The SV2A receptor occurs in several places in the central nervous system and in almost all endocrine tissue, and therefore having a complete understanding of LEV's effect on the endocrine system is especially interesting. The field of indications for use of LEV is continuously increasing, and therefore more and more patients are using this AED. Thus, we were particularly interested in exploring the possibility of endocrine side-effects associated with use of LEV.

Our initial studies were based on a rat model previously used to study endocrine side-effects of VPA. In order to mimic long-term treatment in humans, the rats received LEV treatment for between 90 and 95 days. During this period the rats would have experienced approximately 20 menstrual cycles, the equivalent of which would normally take about 1.5 - 2 years in women.

Choice of drug dose in such studies can be difficult, because rats and humans do not necessarily metabolize drugs by the same pathways. The recommended serum concentration for LEV is broad and has altered over time as more experience of the drug has been acquired. A publication from 2008 (Patsalos et al., 2008) recommends a reference range from 70.5 - 270  $\mu\text{mol/l}$ , but when our studies were planned the recommended range was 40 - 130  $\mu\text{mol/l}$ . In order to find the most suitable dose for our study, three different doses (50 mg/kg, 100 mg/kg and 150 mg/kg) were tried initially; after monitoring serum concentrations, we decided to continue with the highest and lowest of these doses. The serum concentrations of LEV fluctuated over the course of the day, being high during the first few hours after feeding and then gradually declining. In humans, serum concentrations are usually measured as fasting values, about 12 hours after the last dose. The serum concentrations that are reported in our paper (high dose animals, 277  $\mu\text{mol/l}$ ; low dose animals, 122  $\mu\text{mol/l}$ ) are those measured at euthanasia, about 4 hours after the last dose of LEV.



Several differences between the treatment groups were found, regarding both ovary morphology and serum hormones. Ovary weight was higher in the treated rats, and the weight increase was dose dependent. More secondary cysts and corpora lutea were found in the treated animals. Although few cysts were found in any of the animals, regardless of treatment, the low dose treated animals had the least. As an isolated finding, these findings could indicate a drug-induced effect, resulting in bigger and heavier ovaries due to higher ovarian activity with production of more ova (and therefore more follicles and corpora lutea). This could, *per se*, be considered a positive effect, resulting in increased fertility. These morphological findings differ from those found in rats treated with VPA (Røste et al, 2002), in which VPA treated rats had more ovarian cysts, fewer corpora lutea, and reduced ovary weight compared with controls. Thus the effects found with LEV appear to be drug-specific, rather than nonspecific AED-treatment effects.

The hormone measurements showed higher testosterone and lower oestradiol in the treated animals, whilst FSH was lower. The reason for these findings is not obvious and several possible explanations are discussed in the paper. A suppression of FSH could affect both follicular development and disturb ovarian steroid secretion. Testosterone is the principal ovarian secretory product in follicles deprived of FSH, but this does not explain the high number of corpora lutea nor the elevated progesterone concentrations. Induction of a pseudo-pregnancy-like state, in which corpora lutea are activated and secrete prolactin and persist for much longer than normal, can also induce follicle growth and reduce oestradiol levels, and such a scenario is a plausible alternative explanation. Pseudo-pregnancy is readily induced in rats by taking vaginal smears (Smith et al., 1975). Nevertheless we consider our method useful; all animals were handled identically, regardless of treatment group, and were selected randomly with respect to time of vaginal smear and also euthanasia. Thus the handling procedures would affect the animals in all groups equally. The same method was used in a previous experiment on VPA and hormonal side-effects (Røste et al., 2001), and the findings from that study were comparable with results for other research, and also transferable to humans. Therefore we believe that our results are of importance and worthy of follow-up in human clinical studies.

A study on ovary cells in prepubertal pigs (Taubøll et al 2006) concluded that LEV affected the conversion of testosterone to oestradiol after FSH stimulation of the ovary cells, but only at high drug levels. In basal conditions (without gonadotropins present) testosterone was increased and oestrogen reduced (only at high LEV concentrations). This study supported

some of the results from our study, and thus increased our interest in proceeding further to human, clinical studies on LEV.

Our animal model study was one of the first to explore possible endocrine side-effects from LEV, and as pronounced effects were identified, which differed from those found with other AEDs, we considered it essential to continue our investigations and discover whether these animal model results were transferable to humans.

In paper 4 we investigated possible endocrine side-effects of LEV in human subjects. We wanted to perform a cross-sectional study in order to maximise patient numbers. We also wanted to include only persons on LEV monotherapy in order to reduce the effects of concomitant factors on our results. However, at the time of the study LEV was quite new in Norway and only supplied as an add-on to other drugs. Therefore, as insufficient patients were available in Norway for this study design, we chose to cooperate with a centre in Innsbruck, Austria. Cross-border cooperation provides new challenges and it is important to ensure that the same inclusion and exclusion criteria are followed. In order to address some potential problems, all the blood samples were frozen and all the hormone analyses were conducted in the same laboratory in as few batches as possible. As cultural differences may also exert an effect e.g. regarding answers to the sexual-function questionnaire, despite both Norway and Austria being highly-developed European countries, we included a further 30 control subjects from Austria (15 male and 15 female) in the questionnaire section of the study. In order to find specific gender-related effects, we wanted to include both male and female participants in this study and we also wanted to compare the effects of LEV on particular parameters, not only with those in healthy controls, but also in epilepsy patients of the same age groups but using older drugs with well known endocrine potentials. Thus, epilepsy patients using CBZ or LTG were also included in the study.

Endocrine changes were not detected in either men or women using LEV. Hormone levels were not statistically different from those in controls, and the women using LEV did not report more menstrual disturbances. Whilst these results are clearly encouraging for the patients and their doctors, the discrepancies in results from the human and animal studies need to be considered.

One reason for the discrepancy could be that animals and humans react differently to LEV exposure. We consider this to be rather unlikely because when the same model was used for investigating VPA treatment, studies from rats and humans provided similar results. However, LEV is a different drug from VPA with a different mode of action, and therefore

there may be differences in effects between species. If this is not the explanation, we must explore whether the dissimilar results might be explained by other factors, such as exposure time to the drug, age of participants etc. In the rat study, the exposure period was 90 days or approximately 20 cycles, which is longer than in the human study, in which the shortest exposure time was 6 months (although most patients had been using the drug for a longer period). In the human study, the age of the participants was between 18 and 45 years, whilst in the animal model the rats were 120 days old when they started LEV treatment. Compared with humans, this resembles a young population of fertile females. In the study of Taubøll et al. (2006) which investigated the effect of LEV on ovarian cells, the cells were taken from prepubertal rats. Therefore, an effect of LEV on younger females than those included in paper 4, where the mean age was 29.2 years (males: 30.2 years), cannot be excluded.

It is also important to remember that the study in paper 4 was cross-sectional and therefore effects in a subgroup of patients could have been masked due to the study design. In a prospective study, all the participants could have been followed regularly, and an effect in individual patients would have been readily observed. In a recent abstract (Harden et al., 2008) eleven male patients were followed for four weeks after commencing LEV treatment and total testosterone and free testosterone concentrations were found to increase in seven and eight patients, respectively. Our study cannot rule out an effect in sub-groups of individual patients.

As a further measure of endocrine function, a questionnaire on sexual function was included in our study. Female epilepsy patients on LEV and LTG reported better sexual functions than both the controls and the patients being treated with CBZ. Men in all groups reported a high degree of sexual contentment and there were no differences in sexual function between groups. The difference between female controls and patients treated with LEV and LTG was surprising and interesting. One possible explanation could be the psychotropic effects of the drugs; LTG has been used for treatment of depression and bipolar disorders for years, and has beneficial effects on quality of life (Zarzar et al., 2007) and mood stabilisation (Miller et al., 2008). LEV is not approved for use in psychiatric conditions, but some studies have indicated that it may have a beneficial effect on mood disorders (Ciesielski et al., 2006; Muralidharan and Bhagwagar, 2006; Mazza et al., 2008). Another explanation could be that our control group had higher scores than expected in the population. One of the study exclusion criteria was use of hormonal contraception, and thus it is tempting to speculate that perhaps the most sexually active women were excluded from the study due to their more

frequent use of contraceptive pills, hormonal IUD etc. It should be noted that women with epilepsy are often advised against the use of hormonal contraception because of the potential for interactions with AEDs.

The intention in our study was to have an unselected out-patient group, from secondary care hospitals, as the study subjects. Many studies in which a marked sexual dysfunction has been reported have recruited their study patients from tertiary care centres. Such patients often have more seizures, greater comorbidity, and use more AEDs. Studies with patients similar to those in our study have provided results which support those that we found (Jensen et al., 1990; Gil Nagel et al., 1990). A relatively new study on adolescents (deVincentiis et al., 2008) concluded that adolescents with epilepsy usually have normal sexual functions. Thus, we can conclude that in this patient group the risk of sexual life being severely affected by epilepsy and medication with LEV, LTG, or CBZ in monotherapy, is low.

## 8. CONCLUSIONS

1. Women with epilepsy have an increased frequency of menstrual disturbances.
2. Menstrual disturbances are more frequent in women with high seizure frequency, using VPA, and using polytherapy.
3. Debut of epilepsy seems to be more frequent in the adolescent age group (between 10 and 18 years), than in childhood (between 0 and 9 years), without a clustering in the perimenarche; epilepsy does not influence the time of menarche.
4. Long-term treatment with LEV in female rats changes ovarian morphology, resulting in more secondary follicles and corpora lutea, and increased serum testosterone and reduced oestradiol.
5. In men and women aged between 18 and 45 years, treatment with LEV does not alter sex hormones or sexual function.

## 9. FUTURE CHALLENGES

As this research has emphasised, there are several close associations between epilepsy, hormones, and AEDs. Epilepsy is common in childhood and in the fertile years, and therefore it is very important that our knowledge on the endocrine potential of all AEDs is as broad and as detailed as possible.

Our human clinical study provided promising results regarding the use of LEV, but further endocrinological questions remain to be answered. Studies of porcine cell cultures (Taubøll et al., 2006) indicated that LEV may have effects on ovary cells deprived of gonadotropins, as is the situation in pre-pubertal children. Endocrine studies should therefore be conducted in children and teenagers. Additionally, our lack of data on the possibility of endocrine effects developing after many years of LEV treatment indicates that prospective, long-term studies in adults should also be performed.

All new AEDs should be investigated for possible endocrine effects before they are approved for common use. We recommend that a useful approach would be to use a battery of experiments, starting with experimental endocrine cell models (ovary and testes cells), and followed by studies in whole animals, of both sexes and at different ages. As drugs can have gender-specific effects and the effects can vary between different developmental ages, it is important that these aspects are not neglected. Finally, tests for hormonal side-effects should be a mandatory part of the human testing programme prior to approval.

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