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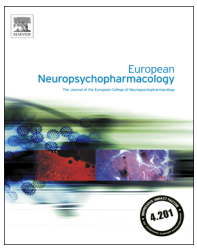

Second generation anti-epileptic drugs adversely affect reproductive functions in young non-epileptic female rats

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Abstract
Reproductive endocrine disturbances are a major health concern in women with epilepsy due to their long term use of antiepileptic drugs (AEDs). Second generation AEDs such as topiramate (TPM) and gabapentin are frequently used for the treatment of epilepsy as well as migraine, bipolar disorder etc. Despite the widespread clinical complications, however the definitive mechanism(s) mediating the side effects of TPM and gabapentin remain obscure. The present study was aimed to evaluate the long term effects of TPM and gabapentin on reproductive functions in young female Wistar rats. Estrous cyclicity, ovarian histology as well as estradiol, LH, leptin and insulin hormones level were studied to elucidate the long-term effect of these AEDs monotherapy on reproductive functions in non-epileptic animals. Further to explore the effects on gonadotropin releasing hormone (GnRH) neuroendocrine plasticity, the expression of GnRH, gamma-amino butyric acid (GABA), glutamic acid decarboxylase (GAD), glial fibrilliary acidic protein (GFAP) and polysialylated form of neural cell adhesion molecule (PSA-NCAM) was studied in median eminence (ME) region of these animals by immunohistochemistry, Western blot hybridization and RT-PCR. Our results demonstrate that TPM and gabapentin treatment for 8 weeks cause reproductive dysfunction as ascertained by disturbed hormonal levels and estrous cyclicity as well as alterations in GABAergic system and GnRH neuronal–glial plasticity. Our findings suggest that treatment with TPM and gabapentin disrupts the complete hypothalamo-hypophyseal-gonadal axis (HPG) through GnRH pulse generator in hypothalamus.

1. Introduction

Although several novel drugs designated as second generation AEDs are increasingly being prescribed for conditions other than epilepsy such as psychiatric disorders, migraine headaches, neuropathic pain, diabetes but their use as adjunct

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therapy and limited sample size of patients are the limiting factors precluding any conclusive determination of their adverse effects (Hill et al., 2010; Shafik, 2012). Women with epilepsy on long term treatments with AEDs often suffer from pathological consequences such as reproductive disorders, obesity, insulin resistance, dyslipidemia, diabetes, atherosclerosis etc. (Luef and Rauchenzauner, 2009; Verrotti et al., 2009). Epileptic women also have lower birth rates and greater risk for the syndromes associated with infertility, such as hypothalamic pituitary axis disruption, polycystic ovary syndrome and menstrual abnormalities, which may be the result of epilepsy itself or treatment with AEDs (Nathan, 2004; Rasgon, 2004).

Few recent reports have addressed the adverse effects of second generation AEDs after their chronic use on fertility and reproductive dysfunctions in animal models (Daoud et al., 2004; Otoom et al., 2004). Reports suggest that ingestion of gabapentin, vigabatrin, lamotrigine and TPM caused reproductive dysfunction as ascertained by significant reduction in spermatogenesis, sperm mortility, body weight and weight of reproductive organs in male rats. Since there are no such reports in the literature on female animal models so the present study may help to understand the mechanisms of action underlying these reproductive disturbances in female animal models taking these novel AEDs.

Leptin is secreted from white adipose tissue and maintains energy balance by affecting synthesis and secretion of neuropeptides Y and other metabolic peptides in the brain (Attele et al., 2007). Valproic acid (VPA) and carbamazepine are known to cause obesity which is associated with increase in serum leptin level in epileptic women (Aksoy et al., 2011). VPA treatment has been shown to increase blood leptin and insulin levels, whereas TPM was observed to cause reverse effects (Leskiewicz et al., 2008). In a recent report, administration of valproate was shown to reduce FSH and LH in males and decreased estradiol release and enhanced testosterone level in female epilepsy patients (Leskiewicz et al., 2008). Decreased serum free estradiol concentration in carbamazepine treatment may alter the feedback regulation of pituitary secretion in patients with menstrual disorders (Verrotti et al., 2011).

Recent studies reported that TPM also possesses antidiabetic and neuroprotective activity (Gensel et al., 2012; Shafik, 2012). Conventional AEDs like VPA, carbamazepine, benzodiazepines and phenobarbital are well known to enhance inhibitory events through GABAergic system, whereas novel AEDs acting selectively through GABAergic inhibition are TPM, tiagabine, gabapentin and vigabatrin. The proposed anticonvulsant effects of TPM include inhibition of neuronal voltage-dependent sodium channels and L-type calcium-channel activation, enhancement of GABA-A receptor transmission and reduction of glutamatergic AMPA and kainate receptor neurotransmission (Shank et al., 2000). Gabapentin is derived by addition of cyclohexyl group to the carbon backbone of GABA and was originally designed as a GABA-mimetic compound that could readily cross the blood-brain barrier (Sills, 2006). In humans, gabapentin causes a dose-dependent increase in the brain concentration of GABA that correlates with improved seizure control, as evident by functional magnetic resonance imaging studies (Petroff et al., 1996).

Previously our lab has reported that GnRH neurosecretory system undergoes structural and functional remodelling during different phases of estrous cycle (Kaur et al., 2002; Parkash and Kaur, 2005). Moreover, GnRH secretion is regulated through the activation of specific glia-to-glia and glia-to-neuron signalling pathways (Ojeda et al., 2000). Recent reports from our lab reported that PSA-NCAM, a glycoprotein which greatly reduces cell adhesion is a key candidate to intervene in dynamic remodelling of GnRH neurons and glial cells in ME region (Parkash and Kaur, 2007; Kumar et al., 2011).

The present study was aimed to explore whether long term use of second generation AEDs such as TPM and gabapentin in cycling female rats causes adverse effects on their reproductive function by disrupting estrous cyclicity as well as through their central action on GnRH pulse generator and on hormonal profiles such as estrogen and LH, and ovarian function. Keeping in view the inhibitory role of GABA as a neurochemical regulator of GnRH neurons and the GABAergic mechanism of action of TPM and gabapentin, the effects of these novel AEDs were studied on the expression of GAD, the GABA synthesizing enzyme and GABA level in ME region of hypothalamus to understand the underlying molecular mechanism(s) of neuroendocrine disturbances associated with the use of these drugs. Further, the expression of molecular markers of GnRH-glial plasticity such as PSA-NCAM and GFAP was studied in the ME region to ascertain the basis of structural remodelling of GnRH neurons in TPM and gabapentin treated young adult female rats. The experimental models were normal cycling young adult female rats in the age group of 3–4 months and the study was aimed to elucidate the adverse effects of these novel AEDs independent of the underlying disease phenotype.

2. Experimental procedures

2.1. Experimental animals

Wistar strain young adult virgin cycling female albino rats in the age group of 3–4 months and weighing 150–200 g were used for these experiments. Animal care and procedures were followed in accordance with the guidelines of Animal Ethical Committee, Guru Nanak Dev University, Amritsar. The estrous cycle was monitored by daily inspection of vaginal cytology and the animals were orally treated with AEDs, TPM (100 mg/kg) and gabapentin (60 mg/kg) at 10.00 h every day for 2 months by using a gavage needle. The rats of age matched control group were treated with 0.9 N saline.

2.2. Ovarian histology

After 2 months of TPM and gabapentin treatment, drug treated animals (n=5 each) alongwith control female rats (n=5) in proestrous phase were sacrificed via anesthetic overdose with sodium pentobarbital (100 mg/kg). All the animals were weighed before sacrificing and ovaries were removed thereafter. Ovaries were weighed and mid-ovarian sections were cut for studying follicular morphology by Eosin and Haematoxylin staining.