Action of adrenal and gonadal steroid hormones on kainic acid-evoked seizures in a rat model of epileptogenesis

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ABSTRACT
Epilepsy is one of most reported neurological disorders after migraine, stroke and Alzheimer’s disease. Empiric clinical data reveal that seizures and epilepsy more likely affect men than women. The aim of present study was to investigate the effect of steroid adrenal and gonadal hormones on the intensity, dynamics and latency of kainic acid-evoked seizures and lethality in a rat model of epileptogenesis. After surgical adrenalectomy/gonadectomy, male rats were at random assorted in groups and treated from postoperative day 1 to day 5 with corticosterone (30 mg/kg), estradiol (0.03 mg/kg), progesterone (75 mg/kg), dihydroprogesterone (75 mg/kg) and dihydrotestosterone (0.75 mg/kg). Spontaneous recurrent seizures generated by kainic acid were assessed. The treatment with corticosterone eliminated the aggravation of kainic acid-evoked seizures produced by adrenalectomy/gonadectomy. The application of corticosterone decreased the seizure intensity by 31% and prevented seizure-associated animal death. The effect of estradiol treatment was quite opposite. Estradiol treatment exacerbated the somatic and behavioural aspects of kainic acid-evoked epilepsy-like syndrome. The hormone increased the intensity of kainic acid-evoked seizures by 31%, decreased the latency of clonic weak seizures by 49% and enhanced the associated lethality by 133%. The treatment with progesterone or dihydroprogesterone produced minor alterations in intensity and latency of kainic acid-evoked seizures in the operated male rats. The application of dihydrotestosterone significantly aggravated the kainic acid-evoked seizures. In summary, hormonal unbalance could play an important role for seizure susceptibility in epileptogenesis. Corticosterone has better anti-seizure activity than progesterone and testosterone has significant pro-convulsive activity.

Introduction
Epilepsy is one of most reported neurological disorders after migraine, stroke and Alzheimer’s disease [1], affecting over 65 million patients worldwide with some 150 thousand new cases diagnosed annually [2]. Epilepsy is a chronic devastating disease diagnosed by occurrence of unpredictable (two or more) and recurrent (separated by at least 24 h) seizures unprovoked by any immediate identified cause [3]. The generalized or partial tonic/clonic seizures are main pathognomonic features of most forms of epilepsy. The therapy is symptomatic and neither effective mechanism-based cures, nor safe prophylaxis are available thus far.

Empiric clinical data reveal that seizures and epilepsy more likely affect men than women and incidents of epilepsy are less in women [4] regardless of the fact that catamenial epilepsy is exclusive for females [5]. Several experimental findings show that picrotoxin or bicuculline evoke generalized seizures more easy in male than in female rats [1,5]. However, experiments with mice reported quite opposite findings [6]. Further data are obviously needed to reach definitive conclusions about the possible gender predisposition to generalized seizures [5]. On the contrary, it is general knowledge that sex hormones play significant roles in the pathogenesis of epileptic seizures. Steroid hormones can easily cross the blood–brain barrier and accumulate in structures responsible for initiation of seizures such as amygdala, hippocampus, area postrema as well as in structures involved in the control or termination of seizures such as substantia nigra, striatum and superior colliculus.

Estrogens are known to exacerbate seizures in women with epilepsy and proconvulsant and epileptogenic effects have been reported for estradiol [7]. Experimental
data have shown that estradiol has diverse effects varying from significant proconvulsant effect, no effect to mild anti-seizure effect depending on the use of physiological or supra-physiological doses, age, species, therapy, etc. [5]. The action of estradiol in epileptogenesis comprises non-genomic and genomic effects. The non-genomic proconvulsant effects are mediated by allosteric stimulation of N-methyl-D-aspartate NMDA receptors with increase in membrane excitability and augmentation of Ca\(^{2+}\) channel conductance [8]. Swift onset and short duration are principle characteristics of these effects. On the contrary, other experimental data have shown that single doses might occasionally evoke anti-seizure effects due to increased neuropeptide Y (NPY) release and enhanced expression of glutamic acid decarboxylase [9]. Despite the reported diversity of estradiol action on epileptogenesis, it is a well-known fact that estradiol pretreatment can alleviate seizure severity and decrease mortality in the episodes of status epilepticus [10]. The genomic epileptogenic effects involve up/down regulation of gene expression of nuclear estradiol receptors. In contrast to the former principle, characteristics of these effects are delayed onset and long duration [11]. Data have been reported that estradiol supply protects the hippocampal neurons against seizure-induced damage in female rats, but augments the hippocampal damage in male rats [5].

Progesterone is known from experimental models [12] or clinical studies [13] as an endogenous anticonvulsant hormone. The effects are fast, non-genomic and robust. The anti-seizure action is indirect following progesterone conversion to the active metabolite allopregnanolone, which acts as an allosteric agonist of \(\gamma\)-aminobutyric acid GABA\(_A\) receptors. The molecular mechanism of progesterone action is still incompletely revealed and might potentially include activation of progesterone receptors, increased synthesis of neurosteroids, modulation of oxidative cascades and decrease of prostaglandin synthesis by inhibition of Phospholipase A\(_2\) (PLA\(_2\)) [3,12]. Progesterone has been shown to support the normal neuronal development and protect against neuronal damage in traumatic brain injuries [14]. The opposite finding for positive correlation of serum progesterone levels and seizure severity has also been reported [15]. The ubiquity of progesterone neuroprotection remains fairly unproven. On the other hand, tolerance and enhanced neuronal excitability evoked by long-term progesterone treatment have been fairly proven [5].

Testosterone has been demonstrated to have protective action against seizures at lower doses, but to be able to enhance seizure activities at higher doses due to its conversion to estrogens [3]. Results from animal models have shown that temporal lobe seizures induced by pilocarpine or kainic acid are more frequent and severe in male than in female rats most likely due to \(\alpha\)-aromatization of testosterone to estradiol [16]. Endogenous steroid hormones are synthesized in gonads and adrenal glands can cross the blood–brain barrier due to higher lipid solubility and act as neuroactive steroids.

Few clinical studies report that corticosteroids known for their anti-inflammatory and immune-suppressing action suppressed the epileptogenesis of West syndrome, Landau–Kleffner syndrome, electrical status epilepticus during sleep, Lennox–Gastaut syndrome and epilepsy with myoclonic-astatic seizures [17]. The mechanism of action is associated with increase of dendritic sprouting and myelination, regulation of the action and metabolism of different neurotransmitters, and changes in membrane permeability and signal transduction. In addition, adrenocorticotropic hormone (ACTH) decreases the neuronal excitability via multiple mechanisms [18]. Clinical data showed that the treatment of 44 patients with epileptic syndromes of continuous spike-waves during slow-wave sleep with decreasing doses of hydrocortisone for 21 months had rather beneficial effects. The seizures were suppressed in 77.2% of patients with electroencephalogram normalized in 45.4% in the course of the first three months of the treatment [19]. The authors suggested that corticosteroids offered better efficacy and longer lasting anticonvulsant action than conventional anti-epileptic drugs. Similar data have been reported elsewhere as well [20,21]. The authors [20,21] concluded that steroid treatment of epilepsy remains yet undefined and needs further studies. Acknowledging this line of evidence, the aim of the present study was to investigate the effect of steroid adrenal and gonadal hormones on the intensity, dynamics and latency of kainic acid-evoked seizure and lethality in a rat model of epileptogenesis.

**Subjects and methods**

**Animals**

Male rats (Wistar, 8–10 weeks of age (WoA), 180–210 g body mass were housed (3/cage) at 12 h light/dark cycle (light off at 8:00 pm) in a laboratory environment (18–20 °C) with free access to food and drinking water until 24 h before surgery. After surgery, the rats were kept for 3 days in single cages and were then grouped in larger cages (4/cage) with unrestricted access to chalk food and drinking water. After surgery, the rats were given lightly salted (4.5 g/L) drinking water.

**Surgery**

All surgical manipulations were performed on rats maintained in deep anaesthesia with ketamine (90 mg/kg, i.p.) and thiopental (24 mg/kg, i.p.). The adrenal glands
were removed via dorsal musculature followed by bilateral in block removal of vas deferens, epididymis and testis via scrotum. The organs remained intact in sham operated rats. All animals received once gentamicin (10 mg/mg, i.m.) and topocine (in-wound spray).

**Hormonal treatment**

The steroid hormones were dissolved in sterile olive oil and injected subcutaneously through 5 consecutive days at 8:30–9:30 pm. The hormonal doses were applied in a final volume of 0.1 mL/100 g. Rats were at random assorted (9/group) in 7 groups (G) and treated from post-operative day 1 to day 5 as follows:

- (G1) sham operated received olive oil;
- (G2) operated received olive oil;
- (G3) operated received corticosterone at a dose of 30 mg/kg;
- (G4) operated received estradiol at a dose of 0.03 mg/kg;
- (G5) operated received progesterone at a dose of 75 mg/kg;
- (G6) operated received dihydroprogesterone at a dose of 75 mg/kg;
- (G7) operated received dihydrotestosterone at a dose of 0.75 mg/kg.

The hormones used were corticosterone, 5α-dihydroprogesterone, 5α-dihydrotestosterone, 17β-estradiol and progesterone. All substances were from Sigma.

**Kainic acid-evoked seizures**

It is widely accepted that (a) kainic acid, (b) pentylenetetrazole, (c) pilocarpine, (d) fluoroethyl and (e) electro-shock are mostly used as epileptogenic challenges in experimental models of seizure in rats. In preliminary experiments kainic acid-evoked seizures proved a reliable model of epilepsy-like syndrome in rats. The proconvulsive challenge of 24 mg/kg kainic acid was applied subcutaneously as bolus in 0.2 mL buffer (pH 7.4) on day 5 of the hormonal treatment. The behavioural reactions were witnessed for three hours by two observers who were unaware of the previous hormonal treatment. Seizure intensity was evaluated by a 6-point scale (Table 1) and seizure latencies as well as death latency were measured by a stopwatch.

**Ethics approval**

Rats were purchased from a licensed supplier and were kept in accordance with the Guides for Care and Use of Laboratory Animals of the Bulgarian Ministry of Agriculture and Food. After completion of experiments, the rats were exterminated by excess CO₂ gas. The experimental protocol and surgical manipulations employed in the present study were approved by the Committee of Ethics at the Medical University of Sofia.

**Data analysis**

The values are expressed as means with standard error of the means (±SEM). Statistical analysis was performed by two-way analysis of variance (ANOVA) followed by a Bonferroni multiple comparison test. The differences were considered significant at \( P \leq 0.05 \).

**Results and discussion**

Data of seizure and death latencies, severity and lethality are summarized in Tables 2 and 3. The results showed that removal of suprarenal glands and testes (G2) decreased the latency of clonic weak and tonic submaximal seizures by 53% and 35%, respectively, compared to sham operated rats (G1). The intensity of kainic acid-evoked seizures increased by 50% and the lethality rate tripled in non-treated operated rats (G2) compared to sham operated rats (G1). The treatment with corticosterone (G3) eliminated the aggravation of kainic acid-evoked seizures produced by adrenalectomy/gonadectomy (G2). The application of corticosterone decreased the seizure intensity by 31% and prevented seizure-associated animal death. The hormone counteracted kainic acid-triggered epileptogenesis and suppressed the appearance of submaximal and maximal tonic seizures.

The effect of estradiol treatment was quite opposite. The obtained data showed that estradiol treatment exacerbated the somatic and behavioural aspects of kainic acid-evoked epilepsy-like syndrome (G4). The hormone increased the intensity of kainic acid-evoked seizures by 31%, decreased the latency of clonic weak seizures by 49% and enhanced the associated lethality by 133%. The treatment with progesterone (G5) or dihydroprogesterone

**Table 1. Point scale for evaluation of seizure intensity in kainic acid-evoked experimental epileptic syndrome in rats.**

| Motor reaction                                                                 | Points |
|--------------------------------------------------------------------------------|--------|
| Locomotor excitation                                                           | 1      |
| Myoclonus of facial muscles, and/or convolution of head muscles, and/or contraction of neck or forelimb muscles | 2      |
| Contraction of masticatory muscles and/or ‘wet dog’ shake, and/or clonus     | 2.5    |
| Weak generalized clonic seizure                                               | 3      |
| Strong generalized clonic seizure with rearing, screaming and falling on case floor | 4      |
| Submaximal tonic seizure (tonic flexion of forelimbs)                         | 5      |
| Maximal tonic seizure (tonic flexion of forelimbs and tonic extension of hind limbs) | 6      |

The treatment with progesterone (G5) or dihydroprogesterone...
removal of the adrenal/testis axis was extremal stress that drastically aggravated the kainic acid-evoked convulsive syndrome. Previous studies have also shown that castration of male rats augmented the susceptibility to specific seizures [22]. The deterioration of convulsive syndromes triggered by steroid hormone deficiency was effectively reversed by corticosterone administration. In the same line of evidence, several clinical studies have shown that long-term treatment with corticosteroids significantly mitigated the convulsive syndrome in epileptic patients [17,20,21,23]. The present research did not reveal the precise mechanism of the anti-epileptic effects of corticosterone. It is plausible to suggest that central and peripheral anti-stress effects contribute to its anti-seizure action. However, multiple diverse effects may hamper the use of corticosteroids in the routine anti-epileptic therapy.

The role of estrogens and gestagens on epileptogenesis in male rats has been reported in few studies. The present data showed that treatment with estradiol intensified the convulsive syndrome triggered by kainic acid challenge in the adrenalectomized and castrated male rats. Similar proconvulsant action of estradiol in castrated female rats has been reported elsewhere [24]. It is well known that estradiol could stimulate glutamate release and inhibit GABA synthesis, which is most likely the principal mechanism of its pro-convulsive action. The results from the present study revealed a trend towards a decrease of the latency of tonic maximal seizures in progesterone treated rats and the latency of tonic weak seizures in dihydroprogesterone treated rats. Insignificant alterations of seizure intensity and a borderline increase in lethality rate were found in both groups. Experimental and clinical studies have demonstrated that progesterone produces powerful anti-convulsive effects in females [3,12,13]. It has also been found in vivo experiments that progesterone and 5α-

### Table 2. Effect of adrenal and gonadal sex steroid hormones on seizure latency and time of animal death in kainic acid-evoked experimental epileptic syndrome in rats.

| Hormonal treatment | First | Clonic weak | Tonic submaximal | Tonic maximal | Death end |
|--------------------|-------|-------------|------------------|---------------|-----------|
| G1. Sham not treated | 31.2 ± 3.9 | 130.2 ± 11.5a | 95.0b | – | 97.0b |
| (n = 9) | (n = 9) | (n = 5) | (n = 1) | (n = 0) | (n = 1) |
| G2. Operated not treated | 34.0 ± 3.3 | 61.2 ± 5.1 | 62.5 ± 18.5 | 60.0 | 74.0 ± 3.5 |
| (n = 8) | (n = 6) | (n = 2) | (n = 1) | (n = 3) |
| G3. Corticosterone | 30.5 ± 1.8 | 63.2 ± 2.6 | – | – | – |
| (n = 8) | (n = 6) | (n = 0) | (n = 0) | (n = 0) |
| G4. 17β-Estradiol | 29.0 ± 4.3c | 31.4 ± 4.5a | 48.4 ± 8.6 | 82.3 ± 37.9 | 87.3 ± 24.1 |
| (n = 8) | (n = 6) | (n = 7) | (n = 3) | (n = 7) |
| G5. Progesterone | 44.2 ± 10.8 | 66.2 ± 14.2 | 80.0 ± 28.2 | – | 87.0 ± 32.5 |
| (n = 9) | (n = 9) | (n = 4) | (n = 0) | (n = 4) |
| G6. 5α-Dihydroprogesterone | 35.2 ± 5.2 | 44.2 ± 5.2c | 69.1 ± 14.1 | 92.0c | 86.5 ± 13.0 |
| (n = 9) | (n = 9) | (n = 6) | (n = 1) | (n = 4) |
| G7. 5α-Dihydrotestosterone | 19.0 ± 2.2b | 33.6 ± 5.3b | 35.0 ± 4.8b | – | 56.5 ± 13.1b |
| (n = 9) | (n = 9) | (n = 4) | (n = 0) | (n = 4) |

Note: n, number of rats.

*P ≤ 0.001, †P ≤ 0.01, ‡P ≤ 0.05 vs. operated non-treated rats.

### Table 3. Effect of adrenal and gonadal sex steroid hormones on seizure intensity and lethality in kainic acid-evoked experimental epileptic syndrome in rats.

| Hormonal treatment | Intensity (points) | Lethality (%) |
|--------------------|--------------------|---------------|
| G1. Sham not treated | 2.66 ± 0.31b | 11.1a |
| (n = 9) | (n = 1) |
| G2. Operated not treated | 4.0 ± 0.42 | 37.5 |
| (n = 8) | (n = 3) |
| G3. Corticosterone | 2.75 ± 0.09a | – |
| (n = 8) | (n = 0) |
| G4. 17β-Estradiol | 5.25 ± 0.25b | 87.5a |
| (n = 8) | (n = 7) |
| G5. Progesterone | 3.71 ± 0.43 | 44.4c |
| (n = 9) | (n = 4) |
| G6. 5α-Dihydroprogesterone | 4.55 ± 0.33 | 44.4c |
| (n = 9) | (n = 4) |
| G7. 5α-Dihydrotestosterone | 4.11 ± 0.3 | 44.4c |
| (n = 9) | (n = 4) |

Note: n, number of rats.

*P ≤ 0.001, †P ≤ 0.01, ‡P ≤ 0.05 vs. operated non-treated rats.
dihydroprogesterone suppress amygdala-kindled seizures in female rats [25]. It is widely known that progesterone action is mediated via specific receptors. The finding that progesterone is less effective in the suppression of seizures in male than in female rats might be correlated with much sparser cerebral expression of progesterone receptor in males. The present data showed that treatment with dihydrotestosterone exacerbated the convulsive syndrome and moderately enhanced kainic acid-associated lethality. Experimental and clinical studies indicate that testosterone could increase the seizure activity after cleavage to estrogens by the enzyme 5α-reductase [26]. The pro-convulsive effect of dihydrotestosterone reported in the present study could apparently be mediated by the same mechanism.

Conclusions
The results from this study showed that corticosterone has better anti-seizure activity than progesterone and that testosterone has significant pro-convulsive activity in male rats. This should be considered carefully by subjects receiving anabolic steroids. It could be hypothesized that hormonal imbalance could play an important role for seizure susceptibility in epileptogenesis.

Disclosure statement
The authors declare no conflicts of interest.

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