NEUROSTEROIDS, A NEW ANTIEPILEPTIC THERAPY?

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Abstract

Neurosteroids represent a class of hormones demonstrated to be synthesized in brain and having specific cerebral functions. There are three major categories of neurosteroids depending on molecule structure: pregnane, androstane and sulfatate derivatives. The first two are inhibitors and the last one is excitatory. The inhibitor ones are acting on specific GABA (gamma-amino butyric) receptors, different from classical antiepileptic drugs as benzodiazepine and barbiturates.

Epilepsy is a chronic disease where the level of excitation is above the one of inhibition, and this is why the neurons "fires" and the patients have seizures with different clinical signs depending on epileptogenic focus.

There is a bipolar relation between epilepsy and neurosteroids, because different types of seizures could influence the level of those and also the neurosteroids could have an antiepileptic role.

The article focuses on the possible function of neurosteoids in epilepsy, describing a possible new antiepileptic formula.

Rezumat

Neurosteroizii reprezintă o clasă de hormoni sintetizați la nivelul sistemului nervos central și având funcții cerebrale specifice. Există trei mari categorii de neurosteroizi, în funcție de structura moleculei: derivați de pregnan, androstan și derivații sulfatați. Primele două categorii sunt neurosteroizi inhibitori, ultimii fiind excitatori. Cei inhibitori acționează pe receptori GABA (acidul gama-amino butiric) specifici, alții decât receptorii medicamentelor antiepileptice clasice (benzodiazepine, barbiturice).

Epilepsia este o boală cronică în care nivelul de excitație centrală este superior celui inhibitor, și acesta este motivul pentru care pacienții au convulsii cu diferite semne clinice, în funcție de focalizare epileptogene.

Există o relație bipolară între epilepsie și neurosteroizi, deoarece diferitele tipuri de crize ar putea influența nivelul neurosteroizilor pe de o parte, iar pe de altă parte, aceştia ar putea avea un rol antiepileptic.

Articolul descrie posibilele implicații biochimice ale neurosteroizilor în epilepsie, precum și potențialul lor farmacologic antiepileptic.

***Keywords**: neurosteroids, GABA receptors, allopregnenolone, galaxolone, epilepsy*

Introduction

Neurosteroid is a term described for the first time by Etienne Baulieu in 1990; they are organic soluble molecules with a structure formed with 17 carbons similar to steroids. They are called "neuro" because they are synthesized in brain, in neocortex but also in white matter [1].

There are three major categories of neurosteroids: pregnane (alopregnanolone, alotetrahidrodeoxi-corticosterone), androstane (androstanediol, etiocolanone) and sulfatate (pregnenolone, dehidroepi-androsterone) derivatives. Pregnane and androstane are acting on the inhibitory GABA-A (gamma-amino butyric) receptors. They are acting on phasic synaptic receptors, with a rapid action and short duration, but also on tonic extrasynaptic receptor, which has slow activity, but long duration. These actions could be summarized as short acting synaptic, but with long duration inhibitory extrasynaptic effect with possible role on anxiety, stress, depression, and not least, epilepsy [2,6,19].

The sulfatate compounds are acting on NMDA (N-methyl D-aspartate) receptors on depolarization, therefore they are excitators (with possible implication in epileptic seizure, cognition, Alzheimer disease) [3].

These compounds are synthesized from progesterone, deoxycorticosterone and testosterone in principal from adrenal glands, but the enzyme responsible for the first step of metabolisation, alfa-reductase exists in brain, respectively in neocortex, white matter, hippocampus, so it does exist the possibility to synthesize the first metabolites directly in *cerebrum*. This is also demonstrated by the fact of presence of cerebral allopregnenolone in the cases with adrenalectomies, so without any possibility of making it peripherally, so it has to be a way of cerebral local metabolites [4].

On other hand, other metabolites of steroids, like allopregnenolone, tetrahidroxideoxicorticosternone, androstanediol pass the blood-brain barrier, so they could reach the brain after being synthesized in periphery [4, 5].

In the *cerebrum* exists a balance between excitation (principally glutamates) and inhibition (principally GABA), but when excitation exceeds the level of inhibition, the cortical network "fires" without any control and becomes clinically active as epileptic seizure.

Epilepsy is a chronic disease with repetitive seizures due to different causes: either structural or functional (genetic or metabolic diseases). Epileptogenesis represents the latency from the end of spontaneous seizure to the period of clinically manifested epilepsy.

Depending on etiology, the seizures are classified in focal, which means the onset on seizures is very limited to some cerebral areas, from where could migrate to different ones or even to the whole brain, or generalized with onset of seizures simultaneously on the both cerebral hemispheres.

Treatment of epilepsy consists in: specific conditions of life, more than 20 antiepileptic drugs or other alternative therapies as surgery, ketogenic diet or vagal nerve stimulation. Despite the evolution in this domain and so many drugs, they are all acting on almost the same *locus*, the same synaptic cerebral receptors and there are still approximately about 20% of seizures still resistant to treatment, so it's a need for an alternative therapy on different mechanisms.

How epilepsy could influence or be influenced by the level the neurosteroids?

It has been demonstrated that neurosteroids, molecules which act and maybe even synthesized in brain have a dual relation with epilepsy.

On one hand, in general, all seizures can modify the level of neurosteroids related to stress or inflammation. In response to stress or during the ovarian cycle there are fluctuations in neurosteroids synthesis which determine an increase in seizure threshold. Increased neurosteroid synthesis delays the appearance of recurrent spontaneous seizures in an animal model of temporal lobe epilepsy [7]. Also, after stress there is an increase level of cortisol, which has different effect depending on duration or repetition of stress. Only one episode decreases the epileptogenic level, but after repetitive stress there is an opposite effect, with a decrease of the frequency of seizures [8]. Also, ACTH (adrenocorticotropic hormone) is used as antiepileptic drug on particular epilepsy, being demonstrated having action on different GABA receptors [9].

Neuroinflamation is detected both in the latent period and also in the chronic phase of epilepsy. After brain injury, there is a significant increase in the level of allopregnanolone in tissues, which act against epileptogenesis in the latent period and also in the chronic period [7].

For focal seizures, in rodent models of temporal lobe epilepsy, glia derived neurosteroids have proved to exert antiepileptogenic actions. This is particularly relevant to date; there are no pharmacological agents capable of stopping epileptogenesis. In fact, endogenous neurosteroids and their synthetic analogs are modulators of neuronal excitability influencing the neurotransmission, depending on their class (sulfated or nonsulfated). The sulfated as it was mentioned, primarily enhance the GABAergic inhibitory

tone. Still, GABA-ergic mechanisms have specific contribution to epileptiform synchronization to different epileptic syndromes, so the previously mentioned capacity can yield opposite effects [7].

It has been demonstrated that in rats, allopregnenolone could provoke epileptiform discharges on electroencephalogram as 3 Hz generalized spike-wave complexes typical to absences in humans, so there is a connection between generalized seizures and neurosteroids (Figure 1) [10].

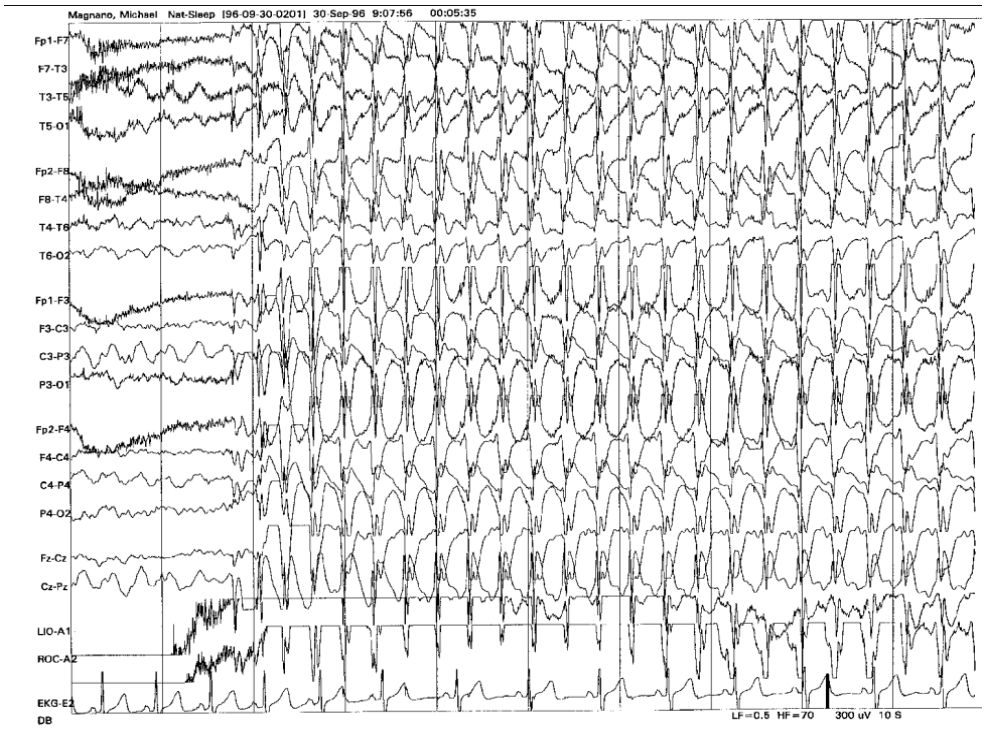

Figure 1

Epileptiform discharges as 3 Hz spike-wave complexes typical to absences on a human electroencephalogram

In the treatment of benzodiazepine resistant *status epilepticus*, intravenous allopregnanolone may stop seizures and in particular, protects against seizure induced neural injury. In non-seizure related brain injury models, neuroactive steroids confer a protection not done by benzodiazepines [11].

Catamenial epilepsy is a multifaced neuroendocrine condition. In the most common form, the perimenstrual catamenial epilepsy, there is cyclical occurrence of seizure exacerbations near the time of menstruation.

In the catamenial epilepsy model, the anticonvulsivant drugs, including benzodiazepines and valproate have a reduction in their potency against seizures, and currently there are no specific approved treatments to prevent seizure exacerbation. This could be explained by fluctuation of plasmatic level of antiepileptic drugs, fluid and electrolyte balance or hormone levels. At the time of menstruation there is a fall in progesterone and consequently a withdrawal of the progesterone – derived GABA-A receptor which modulates allopregnanolone but with increased receptor expressivity of this specific receptor in the first 24 hours, a different pathway from other antiepileptic drugs. After 72 hours of menstruation, there is a decrease of expressivity of these receptors with effect of lower level of inhibition. This type of fluctuation excitation *versus* inhibition makes this period specific maybe to a different therapy [12].

There are some model researches which prove that a high level of estrogen is proconvulsivant similar to finasteride (inhibitor of synthesis of pregnane neurosteroids). Progesterone may be effective, but is poorly absorbed orally and has a short half – life, its activity seems to be dependent on its conversion to allopregnanolone (thus dependent on individual variation on metabolism), not to mention the undesired hormonal side effects [13] (Table I – after Herzog, 2009).

Tabel I

Effect of different types of progesterone on catamenial epilepsy [13]

	medroxy-progesterone	**progesterone suppositories**	**progesterone lozenges**	**progesterone lozenges**
Regimen	5 mg to 10 mg once daily *days 15 to 28 of cycle*	100 mg to 200 mg three times/day *days 15 to 28 of cycle*	100 mg to 200 mg three times/day *days 15 to 28 of cycle*	100 mg to 200 mg three times/day *days 15 to 28 of cycle*
Assessment	at 3 months	at 3 months	at 3 months	at 3 years
Subjects (no.)	24	8	25	15 of original 25
Number improved	10 (42%)	6 (75%)	18 (72%)	15 (100%/60% overall)
Seizure frequency	10%	68%	−54% partial seizures −58% secondary generalized	−62% partial seizures −74% secondary generalized

Is any synthetic neurosteroid effective?

All the studies mentioned proved that neurosteroids, and also progesterone has positive effect on epilepsy. But because of the systemic side effects, it becomes necessary to create synthetic analogs, like ganaxolone (available as oral formulations).

Ganaxolone is a beta-methylated synthetic analog of allopregnanolone (Figure 2). Ganaxolone and allopregnanolone act as allosteric modulators of the GABA-A receptor complex augmenting chlorine conductance. They work at sites distinct from the benzodiazepine and barbiturate biding site, more on extrasynaptic than synaptic.

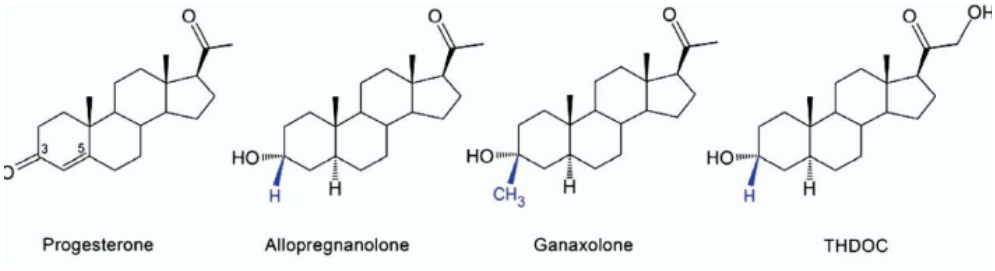

Figure 2

Chemical structure of progesterone, allopregnanolone, ganaxolone and THDOC (tetrahydrodeoxycorticosterone)

The molecule has been synthesized in 1997 and has been used since 2004 in different clinical studies. It is a water-insoluble molecule commercialized as tablets or syrup, without any of hormonal classical effect [14].

The animal studies showed a positive effect on seizures induced by drugs or electrical shocks, catamenial epilepsy and also on electroencephalographic epileptiform discharges [16, 17].

In rat models of catamenial epilepsy, during menstruation, there is an abrupt withdrawal of neurosteroids related to the level of progesterone. The enhanced potency of neurosteroids may be due to a relative neurosteroid withdrawal in the expression of neurosteroid-sensitive delta-subunit-containing perisynaptic or extrasynaptic GABA-A receptors. Positive allosteric modulatory neurosteroids and synthetic analogs such as ganaxolone may be administered to prevent catamenial seizure exacerbations, in what it is called “neurosteroid replacement therapy”. In rats there is also a phase similar to menstruation, which means lower

progesterone. It was observed that when ganaxolone was administered, the percentage of seizures was lower comparing to the ones treated with diazepam [18].

Considering all the positive effects as antiepileptic on animals studies, it was raised the idea of using this molecule also as antiepileptic in humans, an antiepileptic drug with different mechanism of action from the classical one.

The first clinical study was performed in 2000, on 52 patients with resistant focal epilepsy, surgery candidates. They were treated only with ganaxolone or placebo for up to 8 days, without any other antiepileptic drugs and it has been proven a lower frequency in the treated group, summarizing that the molecule has also anticonvulsant effect in humans [19].

The first clinical study in children was done also in 2000 for infantile spasms in a multicentric, open-label, add-on trial in 20 patients with ages between 7 months to 7 years. This type of seizures is generalized one, very sensitive to ACTH. It was an add-one study, the children being treated also with other antiepileptic drugs and it was add ganaxolone for 12 weeks. The drug was well tolerated, the most common side effect being somnolence. Clinically, it lowered the frequency of seizures: 30% of patients had a decreased frequency of seizures with more than 50%, 30% of patients developed 25-50% fewer seizures, and 30% patients had only a reduction with 25% of the number of seizures [20].

From 2000 to 2014 there were performed more than 119 studies in humans and animals, with positive effect, but not a complete control of seizures.

In Romania, in the department of pediatric neurology it was used ganaxolone for the treatment of infantile spasms in 3 children for 2 months, but the drug had the same effect as published in previous international studies, fewer seizures, but still persistent.

Conclusions

Neurosteroids are active molecules which are synthesized both in brain and in periphery. These molecules have implications in epilepsy, being related to some specific GABA receptors which are not active for the classic antiepileptic drugs. There is a direct relation between neurosteroids and seizures; convulsions can lower the level of neurosteroids and increase excitation. There has been proved that progesterone, and its synthetic analog, ganaxolone had antiepileptic activity. More studies with more patients are needed to be performed with ganaxolone as antiepileptic on

specific types of seizures and with specific antiepileptic drugs combinations. It will also be interesting to study the effect of ganaxolone on epileptogenesis, prophylaxis of epilepsy, a field not covered in the present by the common drugs.

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