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CELLULLAR AND MOLECULAR MECHANISMS OF NEUROSTEROIDS IN DIFFERENT PARTS OF CENTRAL NERVOUS SYSTEM OF RATS (Part 1)

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Neuroactive steroids are a class of endogenous steroids which are synthesized in nervous tissue or\and able to modulate the brain functional activity. The first part of the review is dedicated to less investigated genomic and non-genomic mechanisms of action of pregnenolone, pergnenolone sulfate as well as their synthesized analogs and highlights the peculiarities of their production in different regions of rat central nervous system in ontogenical aspect. Some effects of pregnenolone and pregnenolone sulfate modulation of NMDA, GABA, kainate and AMPA receptors, the inhibition of potential-dependent Ca2+ channels both in vivo and in vitro are presented in the review. We've also made an attempt to systematize basic effects of neurosteroids in dependency with the region of central nervous system.

Keywords: neurosteroids, neurotransmitters, regions of central nervous system, ontogenesis, pregnenolone, pergnenolone sulfate, PREG, PREGS, KK-169, NMDA-receptor, GABAA-receptor, AMPA-receptor.

Abbreviations: AMPA – -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GABAA – γ-aminobutyric acid's receptor; mEPSCs – miniature excitatory postsynaptic currents; NMDA – N-methyl-D-aspartat; PREG, PREGS – pregnenolone, pregnenolone sulfate; OATP – organic anion transporting polypeptides; TRP-channel - Transient receptor potential channels; CREB - cAMP response element-binding protein.

1. Introduction. Currently, the main neurosteroids are pregnane steroids, which include dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS), pregnenolone (PREG) and pregnenolone sulfate (PREGS). Messenger RNAs, protein components of neurosteroids have been found in neurons of such brain regions as the cerebral cortex, hippocampus, thalamus, amygdala, hypothalamus and nuclei in spinal cord. Detected neurosteroids' concentrations in nervous tissue were several times the size of their concentrations in blood serum, which did not change after adrenal and orchiectomy. Neurosteroids modulate the activity of many neurotransmitters and nuclear receptors without being specific or obligate ligands, that is why their regulatory functions are optional and may be suppressed by other endogenous mediators, many of the targets for the action of neurosteroids have not yet been identified [1].

2. Pregnenolone and pregnenolone sulfate. Pregenolone's (PREG) synthesis from cholesterol takes place in a mitochondrion with an involvement of enzyme named cytochrome P450cc (cholesterol side-chain cleavage enzyme), after that the conversion of PREG to other neuroactive metabolites follows [5]. Noteworthy that limiting stage of steroidogenesis is cholesterol transportation through the mitochondrial membrane, not the P450scc activity. Mechanisms of this process still remain unclear, but it's widely known that at least two proteins - StAR и TsPO take part in the cholesterol's transportation. [8]. Sulfotransferase converts PREG into PREGS, which is one of the most important neurosteroids synthesized in CNS. Consequently, the presence of the PREGS in rats' brain (male rats and mice 2 months old) were confirmed by the tandem of liquid chromatography and mass spectrometry(LC -MS\MS), concentrations in spinal fluid, blood serum, hippocampus, cerebral cortex were 3, 1, 20 и 17 ng\g respectively [1]. The presence of the PREGS in the human was confirmed as well [4].

It should be notified that the PREGS, which effects neurons in the CNS, doesn't always have neuronal origin. PREGS may also be transported to CNS through the BBB by OATP - organic anion transporting polypeptides expressed in the vascular plexus cells from peripheral tissue. Other representatives of the same transporter proteins family OSTα and OSTβ are more specific to PREGS and for this reason contribute to PREGS elimination from neurons in the mice's brain [7].

3. Major molecular targets. In spite of the huge amount of information about PREG's metabolites' molecular mechanisms of action, we lack data about PREG itself. It was identified in the experiment with antagonists of sigma-1 and sigma-2 receptors named DuP734 and Lu28179 respectively that PREG suppresses NMDA-mediated dopamine release in the extrapyramidal system [Table 1] by the interaction with those receptors, because the antagonists' usage led to the total abolishment of this effect. Intracellular cascade, which supports the sigma-receptors' signal transduction, consists of proteinkinase Cβ, which was established by another experiment with the LY379196 enzyme inhibitor. It also resulted in suppressing PREG's effect on dopamine's release [3]

PREGS interacts with different receptors of multiply ligand-activated ion channels both presynaptic and postsynaptic [4]. PREGS is an excitatory neurosteroide and for this reason it is a negative modulator for $GABA_A$, kainate and AMPA

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receptors and a positive one for NMDA receptors, although its binding site differs from the glycine's [3]; also PREGS is an agonist for transient receptor potential channels (TRP-channels) [9] and may provoke a dopamine release in rat's black substance both *in vivo* and *ex-vivo* in nanomolar and picomolar concentrations.

Cellular penetration of Ca2 + mediated by NMDA receptors in the cell increases neuron's activation and its "synaptic power". This process is called long-term potentiation, it's basic for neurons' training, shor-term and spatial memory formation [8]. Also NMDA-receptors' activation leads to the increase in an intracellular cAMP's level, which consistently increases both a concentration and activity of the transcription factor, which binds CRE genome fragments (CREB) and regulates transcription of various neuropeptids, and for this reason also regulates synaptic plasticity. This is the excellent example of genomic PREGS' activity [6]. Studies, focused on the effects of pregnenolone sulfate and its synthesized analogs (KK-169, KK-181) on NMDA-receptors, have serious clinical relevance, because many processes, which are basic for neurons' training in a physiological state, may be impaired in patients with schizophrenia or autism, therefore PREGS and its synthesized analogs may be estimated as a probable cure for this diseases. Application points, molecular mechanisms of PREGS interaction with NMDA receptors have to be defined accurately to use those substances for treatment purposes. It is widely known that sulfated molecules are more hydrophilic that non-sulfated ones, for this reason it's understandable, why PREGS and the other sulfated neurosteroids are more likely to interact with intracellular receptors, not with extracellular receptors. Nevertheless, it was stated that PREGS' synthesized analog called KK-169, which has almost identical effects on NMDA, AMPA receptors and synaptic transmission, may be stored intracellularly despite of its hydrophility. Such things as the presence of intracellular targets for PREGS and KK-169, the influence of their intracellular storage on neuron's functions and its structure, possibility of their usage in clinical practice are still under research. The main restricting factor for their introduction into therapeutical schemes is their negative inhibitory side effect on $GABA_A$ receptors. Furthermore, some of KK-169' features, which may be used in a description of PREGS' mechanisms of interaction with NMDA-receptors, were described. KK-169 changes glutamate's interaction with NMDA-receptor in such a way as the receptor's reaction on glutamate's presence is maximum possible and leads to more continuous and effective neuron membrane's depolarization with the minimum glutamate's quantity possible in a synaptic gap, which prevents receptors from early desensitization. Desensitization takes place only when non-physiological high concentrations of both KK-169 and glutamate are in synaptic gap in the same moment. It doesn't occur if the glutamate's concentration is high and KK-169 is absent from a synaptic gap, which underlines the neurosteroids' regulatory effect on a synaptic functioning. Therefore, the more NMDA-receptors are in a synaptic gap, the less is the PREGS' effect, because in a physiological state this neurosteroid increases the effectiveness of NMDA receptor's antagonist [3]. Interestingly that KK-169 also causes an outstanding increase in the amplitude and decay rate of an action potential in the autapse-synapse. KK-169 decreases time of the interaction between NMDA-receptor and its antagonist called memantine in a presence of N-methyl-D-aspartate, but this effect may be rather ambiguous as it can be explained by both increased ability of ion channels' opening, mediated by the interaction between NMDA-receptors with its antagonists in a KK-169's presence, and increased number of NMDA-receptors in a KK-169's presence [2].

It was showed that PREGS may change the functioning ability of recombinant NMDA receptors in geterological cells, formed by a combination of the NR1 subunit with one of NR2 subunits (NR2A, NR2B, NR2C or NR2D). PREGS modulates ion currents, induced by NMDA, glutamate and glycine through the NR1/NR2A and NR1/NR2B receptors, and inhibits a receptor's activity, if they belong to NR1\NR2C and NR1\NR2D subclasses. Effects of PREGS may change over time and differ from each other in CNS regions or on stages of ontogenesis, because NR2 subunits' expression has its topological features and changes during the ontogenesis as well [2]. Other pregnenone neurosteroids also modulates the NMDA receptor's activity

PREGS may mediate its influence on the NMDA receptor by preventing the an agonist's detachment or effecting kinetics of deactivation or macroscopic NMDA receptor's desensitization [Table 2], which were demonstrated on the transfected HEK-293 culture, NR1a/NR2A и NR1b/NR2B subclasses [3,8]. It is widely known that NMDA receptor's activity is influenced by various endogenous molecules, such as zinc, polyamines and protons. Zinc and polyamines modulates receptor's activity through the increase and decrease of tonic proton inhibition, on the contrary, PREGS' influence does not connected with proton sensor. The region of receptor's subunit NR2B, named as steroid modulatory domain SMD1 and formed by J/K loops in the S2 zone of glutamate recognition site and forth transmembrane region, may mediate both steroid and proton regulatory mechanisms, and in this way it contributes to forming of hydrophobic recess for the interaction with PREGS[9].

PREGS excitory effects on NMDA receptors are rather clear nowadays, but data, which implies on a bidirectional PREGS' action in a presence of high extracellular calcium concentrations (more then 0,5 mmol\l), still exists. PREGS boosted all NMDA-receptor-dependent peak and constant ion currents in perforated whole-cell modification of a patch clamp technique without Ca^{2+}, whereas in a presence of extracellular Ca2+ in concentration of 0,5mmol\l and higher PREGS inhibited ion currents. The same research [10] stated that bidirectional PREGS' effects on constant ion currents are conditioned by an influence on the average time of ion channel opening, which is a constant unit for each receptor type. Data was obtained by the usage of cell-attached modification of patch clamp technique, which allows to analyze an ion current in a single NMDA-receptor-de-

Table 1

List of pregnenolone's (PREG) main effects in various regions of the central nervous system (without developmental characteristics)

Location	Basic effect	Required conditions	Receptors	Meaning
Striatum	NMDA-stimulated Dopamine Release (-)***	-	Sigma-1 (+)* Sigma-2 (+)	Influence on the processes of neuronal and behavioral plasticity

Table 2

List of pregnenolone sulfate (PREGS) main effects in various parts of the central nervous system (without developmental characteristics)

Location	Basic effect	Required conditions	Receptors	Meaning
Hippocampus	Spontaneous release of glutamate (o** \ +)	Culture of hippocampal neurons in newborn rat pups. Sections of the hippocampus of 3-4 day old rat pups	Sigma-1 (+)	Regulation of synaptogenesis, formation of synaptic neural networks
	Stimulated release of glutamate (+)	Mature hippocampal neurons	Sigma-1 (+)	Improvement of learning and memorization processes due to paired relief (PPF) in mature hippocampal neurons
	Spontaneous release of γ-aminobutyric acid (-)	Mature hippocampal neurons	Sigma-1 (+)	Modulation of the excitability basal level, enhancement of long-term potentiation, improvement of memory and learning
	Release of acetylcholine (+)	Intraventricular injection, infusion into the medial septal nucleus, bodies of acetylcholinergic neurons		Improvement of memorization processes
	Spontaneous release of norepinephrine (o) NMDA-stimulated norepinephrine release (-)	Hippocampal slices, synaptosomes	Sigma-1 (+)	Influence on the processes of memorization and learning, participation in the pathogenesis of epilepsy
Prefrontal cortex	Spontaneous release of glutamate (+)	Concentration ~20 μM	Sigma-1(+), α1-adrenoreceptors (+), σ1-receptor (+)	Improvement of synaptic transmission, cognitive functions
	Dopamine-stimulated Glutamate Release (-) 5-HT-stimulated Glutamate Release (-) Spontaneous release of glutamate (o)	Concentation ~1 μM	Activation of the Gi-mediated signaling cascade	Suppression of stimulated release of glutamate may play an important role in the pathogenesis of neuropsychiatric diseases
Фронтальная кора	Release of acetylcholine (+)	-	-	Improvement of cognitive processes
Стриатум	Spontaneous release of glutamate (o)	-	-	-
Гипоталамус	Dopamine release (o)	-	-	-
Nucleus accumbans	Dopamine release (+)	Intracerebro-ventricular injection	-	Mediation of behavioral responses (motivation, encouragement)

Note. *(+) stimulating effect; ** (-) inhibitory effect; ***(o) no effect.

pendent ion channel. Ca^{2+} influence on PREGS' effects may vary because of the NMA-receptor structure. If DRPEER and exon-5 motives are present in the GluN1 subunit of the receptor, either Ca2+ binding occurs (as DRPEER is a calcium-binding motive), or changes in the receptor's conformation takes place, which leads to inhibition of ion influence on the receptor (H+, Zn2+, Ca2+). In other words, the presence of any of these motives leads to the potential effect during the interaction between PREGS and NMDA-receptor, even if a Ca^{2+} concentration is high. These results were obtained in the experiment with GluN1-GluN2A modifications of NMDA-receptor [6,10].

Showed that PREGS causes long-term postsynaptic potentiation mediated by AMPA receptors [2]. Patch clamp technique in the culture of CA1 pyramidal neurons was used to find out that short-term 5-minutes PREGS exposition induces long-term potentiation in the hippocampal neurons culture, which was selected from 3 to 5 days old rat pups, but not 6 days old ones. There is information about a transient increase in a glutamate release from presynaptic terminal under the PREGS' influence, which may be the trigger for long-term potentiation of postsynaptic AMPA-receptors. These postsynaptic effects are mediated by NMDA receptors with NR2B in its structure. The mechanism of presynaptic PREGS' activity embraces an intracellular Ca^{2+} increase through the NMDA-receptors with NR2D subunits, the regulation of its expression may be also conducted postnatally. Same effects may be mediated by PREG and PREGS synthetized in hippocampal neurons. Depolarization of hippocampal slices leads to enduring increase in the frequency of minor excitory postsynaptic ion currents, interestingly that these effect occurs only in neurons, selected from 3-4 days old rat pups and was absent in hippocamps of 6019 days old animals [1, 2]. Moreover, preincubation of cultivated slices with antibodies to PREGS led to eradication of the long-

term potentiation effect, which allows to conclude that local production of PREGS after the depolarization trully effects the synaptogenesis process [2].

P450scc is an enzyme, which converts cholesterole into pregnenolone, may be identified in rat's brain in early ontogenesis' stages, whereas maximum NMDA receptors expression is detected much later, therefore, endogenous synthesis of PREG and PREGS is likely to influence a functioning activity of NMDA, AMPA, kainite and $GABA_A$ receptors on the last stages of intranatal development.

Besides of the glutamatergic transmissions, PREGS' influence on both spontaneous and potential-dependent release of GABA [Table 2], main inhibitory mediator of the CNS was identified in hippocampus by decreasing of spontaneous inhibitory postsynaptic ion channels' frequency in PREGS' presence [10]. This effect is mediated by the same mechanisms as spontaneous glutamate's releasing, such as sigma-1 receptor activation and $G_{i/o}$ signaling cascade. PREGS also speeds up receptor's desensitization to glutamate by contributing to disulfide bonding between M2-M3 loops in receptor's subunits (M2 domain covers the ion channel, M3 domain is large intracellular domain) and extracellular cysteine loops 7 and 2. Thus effect is absent if Val256Ser mutation is present in α-subunits of the receptor [10]. Glutamatergic transmission's suppression leads to strengthenig of long-term potentiation in CA1 hippocampal neurons, which may have positive effect on learning processes and neuronal plasticity [3].

4. Functions of PREG and PREGS *in vivo.* PREGS increases convulsive potency of NMDA receptors, extends mice's long-term memory, rat's reproducing memory if PREGS is injected directly into the gigantocellular nucleus. PREGS also prevents lack of NMDA effects [Table 2], induced by its antagonists in the passive avoidance test, and antagonistic dizocilpine-induced rat's amnesia. The necessity of the sulfation is approved in chemical sulfation inhibitory tests. It was found that chronical inhibition of the neurosteroids' sulfatase activity by estrone sulfamate improves memory in the passive avoidance test. Experiments, mentioned above, allow to suggest that animal's behavior correlates with the positive modulation of NMDA receptors.

PREG may have functions, which are not connected with GABA, NMDA or other neurotransmitters' receptors. The pregnenolone-linking protein with high affinity and low capacity was identified in the rodent's brain (both in fetus and a sexually mature individuals) [5], it was calledMAP2. The interaction between pregnenolone and MAP2 is intense and becomes even more intense in an association with tubulin.

Conclusion. Pregnenolone and pregnenolone sulfate are major components of the normal rat's neurosteroid status. Nowadays, such questions as a PREG and PREGS' interaction with NMDA-receptors, both excitory and bidirectional, their influence on releasing of basic neurotransmitters in the CNS (glutamate and GABA), on the CNS' ontogenesis process. obtained results may be successfully used in the description of the normal and pathophysiological human neurosteriod profile, because neurosteroid profiles of rats and human being are alike. Nonetheless, many molecular mechanisms of PREG and PREGS' action as well as their side effects are still not clear, that is why their clinical application is rather controversial.

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