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Steroids and ion channels in evolution: From bacteria to synapses and mind

Evolutionary role of steroid regulation of $GABA_A$ receptors

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Review

Abstract. Ion channels are vital components of plasma membranes. This article presents an evolutionary view of the biochemical mechanism of controlling activity of ion channels by rigid lipids, such as steroids or biophysically similar molecules, which were instrumental in formation and control of ion channels in cell membranes at the very origin of life. Such regulatory mechanisms exist in all cellular forms of life from ancient bacteria to humans and participate in a diversity of biological functions, from the most basic, such as maintenance of cell shape, homeostasis, feeding, cell fusion, and reproduction to the most intricate, such as the mind. Learning about the regulation of membrane ion channels by steroids and like molecules is important for understanding the evolution of life and various aspects of cell and organism physiology, for unraveling the mysteries of mind, and for practical purposes such as developing new pharmacotherapies.

Key words: steroids, neurosteroids, ion channels, $GABA_A$ receptors, regulation, evolution, bacteria, reproduction, brain, mind

INTRODUCTION

In past two decades endogenous steroids have been shown to be potent bimodal controllers of ionotropic receptors (ligand-gated ion channels) in the central nervous system (CNS). Originally this type of regulation was documented for the $GABA_A$ receptors ($GABA_AR$), subsequently also for glycine, NMDA, nACh and $5HT_3$ receptors, and it was shown to participate in numerous CNS functions. Because ligand-gated ion channels (LGICs) are evolutionarily very ancient and they participate in many vital cellular activities in unicellular and multicellular organisms, I was curious to explore the steroid interaction with such channels in evolutionary perspective and reveal here its origin at the very beginning of life on Earth.

ION CHANNELS

Separation of the intracellular molecular content from extracellular milieu is an essential attribute of all living cells, whether they exist as unicellular organisms or as tissue components. The intracellular-extracellular electrochemical gradient of ions is a driving force for numerous cellular activities. The ion gradient is generated by their active transport by specialized pumps located in plasma membranes, which utilize cellular energy, and is sustained by the lipid bilayers largely impermeable to inorganic ions. Ions can diffuse into or out of cells down their gradient *via* ion- and charge-selective protein channels embedded in cell membranes. The transmembrane flux of inorganic ions (primarily Na^+, K^+, Ca^{++}, and Cl^-) regulates many aspects of cellular physiology, including excitability, metabolism, osmolarity, volume, shape, movement, fusion, and other functions.

The ion channels of prokaryotic and eukaryotic cells share many common features. They are ion-selective homo- or oligomeric protein structures, containing several integral transmembrane domains, which form water-filled ionic pores. The hydrophobic surfaces of the pore interact with lipids of the membrane bilayer, whereas the charged hydrophilic pore lumen serves as an ion selector and gating mechanism. Lumen's charge of these channels, created by their lining with charged residues of amino acids, facilitates the selective attraction of different ions and their rapid transport (Cascio 2004, Taly et al. 2005).

The ion channels set in plasma membranes can be divided into the categories of voltage-dependent, mechano/pressure- or thermo-sensitive, and LGICs. The latter group, which in multicellular metazoans plays a critical role in inter-cellular communication, constitutes a family of cationic and anionic channels (Ortells and Lunt 1995, Tasneem et al. 2005). In mature mammalian nervous system they encompass fast acting ionotropic receptor-channel complexes, such as $GABA_A$, $GABA_C$ and glycine receptors that conduct Cl^-, and nACh, 5HT3, glutamate (NMDA, AMPA, kainite) and purinergic P2X receptors, which conduct cations (Na^+, K^+, and Ca^{++}).

The LGICs found in contemporary eukaryotic cells manifest a high degree of structural homology (Langosch et al. 1988). They are oligomers, which can be divided into three superfamilies based on their subunit quaternary structure. In mammalian cells they can be pentamers ($GABA_A$, glycine, nACh, and $5HT_3$ receptors), tetramers (glutamate receptors), or trimers (P2X receptors) composed of homologous or heterologous subunits. Each of these subunits spans the plasma membrane several times (two times in the trimeric family, three times in tetrameric, or four times in pentameric family of receptors). In the best characterized pentameric LGICs superfamily, the membrane-spanning domains (M1–M4) form the channel, where the M2 domain mainly contributes to lining of the pores. The N and C termini of each subunit are extracellular, and the intracellular loop usually exists between M3 and M4 domains. Characteristic for this family of channels is conserved 15 aminoacid-spaced disulfide loop in their extracellular ligand-binding domain (Cascio 2004, Schofield et al. 2003).

The ligand (neurotransmitter, hormone, drug) binding to the extracellular receptor domain alters the conformation of the receptor/channel complex from closed to open (Karlin and Akabas 1995, O'Shea and Harrison 2000, Taly et al. 2005, Wagner and Czajkowski 2001). It is believed to cause lateral twisting of the M2 domain, which exposes different residues to the pore lumen and enlarges its size (Keramidas et al. 2000, Lynch et al. 1997, Unwin 1995). The principal ion-gating mechanism is thought to be located in the narrowest region of the pore, close to its cytoplasmic end (Keramidas et al. 2000, Wilson and Karlin 1998, Xu and Akabas 1996), whereas a cloud of charges located at the channel's mouth serves as the ion selector (Imoto et al. 1988, Keramidas et al. 2000). The high degree of structural and functional similarity between different LGICs is evidenced by the fact that replacement of just

a few aminoacid residues in critical regions of the pore results in conversion of their ion selectivity from cations to anions and *vice versa* (Galzi et al. 1992, Gunthorpe and Lummis 2001, Keramidas et al. 2000).

Molecular evolutionary analysis of LGICs based on DNA and aminoacid data revealed their heritage from a common ancestor, which existed at least 2.5 billion years ago (Ortells and Lunt 1995), before origin of eukaryotes (Hedges et al. 2001). It is hypothesized that ancient LGICs receptors existed on cell membranes of early living organisms and served nutrient seeking and osmotic regulation functions (Ortells and Lunt 1995). With growth of organismal complexity, these LGICs have changed their function to serve predominantly intercellular communication.

In prokaryotes the lipid bilayer serves not only as a physical barrier separating intracellular and extracellular milieus, but also as a mechanism controlling functions of membrane proteins, which protects cell's osmotic homeostasis and permit movement. Cell membranes of prokaryotes are equipped in a diversity of cationic and anionic channels, which function as osmoregulators/volume protectors and mechanosensors (Koprowski and Kubalski 2001, Martinac 2001). The gating mechanism, which detects the deformation of lipid bilayer, seems to be evolutionarily the oldest mode of operating ion channels, as mechano-sensitive channels are present already in bacteria Archea (Martinac 2001), which originated close to the dawn of life on Earth about 4 billion years ago (Hedges et al. 2001). Some bacteria possess in their membrane also LIGCs similar to those found in metazoan cells, which serve chemotactic functions (Tasneem et al. 2005).

EVOLUTION OF STEROLS

The lipid bilayer of mammalian plasma membranes is a complex structure, containing more than 2000 lipid molecular species (Barenholz 2002). Sterols are vital elements of these membranes. They dynamically modulate physicochemical properties of membranes, restrict the permeability of water and solutes, control cellular volume and govern functions of membrane embedded proteins. Deficit of sterols is pathogenic and in extreme cases – lethal to eukaryotes. The critical significance of sterols in these cells is best illustrated by the fact that in mammals nearly a 100 proteins support the metabolism and transport of cholesterol. Membranes of prokaryotes do not contain classical sterols, but they are furnished with a variety of compounds, which play analogous roles. Haines (2001) proposed that the principal role of rigid lipid molecules, like sterols, in biological membranes is to reduce proton and sodium leaks through lipid bilayers and in this way to conserve biological energy.

Sterol-like molecules emerged early in evolution. Archaebacteria do not contain such molecules in their simple cellular membranes, but have a class of diphytanyl lipids, which inhibits proton leakage. Eubacteria may contain sterol-like molecules, such as non-cyclical squalene, or cyclical hopanoids and tetrahymanol, synthesized anaerobically from squalene, as well as lipids with branched-chain fatty acids (Barenholz 2002), which effectively inhibit water and proton/sodium leaks across membranes. In membranes of eukaryotes this function is served by various sterols – by isoprenes in organellar membranes (dolichol in peroxisomes and lysosomes, ubiquinone in mitochondria), and by plastoquinone in chloroplasts (Haines 2001). Membranes of yeasts contain ergosterols and plants have a diversity of phytosterols.

Sensu stricto sterols appeared in evolution together with eukaryotic cells circa 2.5 billion years ago, contemporarily with rise of atmospheric oxygen levels, required for sterol synthesis (Hedges et al. 2001). Oxygenation or hydroxylation of steroid molecules at various positions resulted in the formation of a diversity of compounds, endowed with a remarkable variety of cellular functions – from regulators of membrane properties to genome controllers, growth and reproductive controllers, signal messengers, stress adaptors, and sculptors of evolution. In animals, sterols play roles as cell membrane stabilizers and regulators, hormones, parahormones, autohormones, agents of intercellular communication, transducers of intracellular signals, and metabolic and reproductive regulators.

STEROLS INSTRUMENTAL IN THE FORMATION OF ION CHANNELS

Amphiphilic sterol molecules partition easily to the phospholipid bilayer and orient perpendicularly to its plane, with the rigid nucleus of steroid rings positioned in the region of the hydrocarbon core, and the hydrophilic groups – usually hydroxyl or sulfate – oriented toward phospholipid head groups at the membrane surface (Barenholz 2002, de Kruijff 1990, Golden et al. 1998). Such orientation obstructs the

intrinsic movement of fatty acids chains and allows steroid molecules to interact with both the aqueous milieu of cell surface and the hydrophobic membrane interior. Presence of cholesterol in the membrane increases separation of phospholipid head groups and thus increases water's freedom of movement on the membrane surface (Huang 1976). The amphiphilic nature of the sterol molecules also makes them ideal partners of membrane proteins.

de Kruijff and subsequently other investigators noticed that cholesterol is a target of polyene antibiotics (filipin, amphothericin B) and bacterial toxins, and that interaction of these molecules with sterols results in the formation of aqueous pores in the lipid bilayer (rev. de Kruijff 1990, Dufourc and Smith 1985). He proposed a model of ion channel creation, in which molecules of an antibiotic or a peptide of bacterial toxin partition to membrane, so that their hydrophobic sites interact with the hydrophobic core of sterols, which orients them perpendicularly to the membrane plane.

A hydrophilic pore can be cooperatively shaped by several molecules of toxins or antibiotics interacting with sterols in the lipid bilayer (de Kruijff 1990, Dufourc and Smith 1985). In his model of channel formation de Kruijff points to the functional stereoselectivity of sterol's 3-OH group. 3β-OH groups (as in native cholesterol) favor the formation of pores, because they can form stable hydrogen bonds with carbonyl oxygen of phospholipids, proteins, or antibiotic molecules. In contrast, 3α-OH may not form such bonds, because sterol isomers with such a group have a tilted, energetically unfavorable orientation in the membrane, and interact differently with phospholipids and proteins (Huang 1976). It is likely, that interactions of different sterols with peptides/proteins were critical in the evolution, stabilization, and regulation of ion channels in cell membranes. The above model may explain some aspects of the stereoselective regulation of these channels by different steroids.

STEROLS AS 'DOORMEN' OF ION CHANNELS

The body of research accumulated over the past 20 years documented that certain endogenous steroids are allosteric modulators of LGICs. This phenomenon was first observed for Cl^- channels associated with $GABA_A$ receptors by two independent approaches. I found it in biochemical experiments, while studying the influence of cholesterol and endogenous steroid hormones on $GABA_AR$ function (Majewska et al. 1985), whereas Harrison with colleagues observed it while examining the electrophysiological effects of steroidal anesthetics on the $GABA_AR$ (Harrison and Simmonds 1984). Subsequently, while collaborating we made further discoveries (Harrison et al. 1987, Majewska et al. 1986). This work was discussed in several reviews (Majewska 1987, Majewska 1992, 1999). Soon it became evident that other LGICs are also subjects of steroid control, which was not surprising, considering their structural homology.

Our research established that different endogenous steroids modulate the function of the $GABA_AR$ associated Cl^- ionophore in the opposite directions. At physiological, nanomolar, concentrations the steroids with a reduced A ring at 5α or 5β position, hydroxyl at 3α position and electronegative atom (usually oxygen) at C-17 or C-20, such as 3α,5α-tetrahydroprogesterone (THP; allopregnanolone; 3α-OH-DHP), 3α,5α-tetrahydrodeoxycorticosterone (THDOC), or androsterone (5α-androstan-3α-ol-17-one), promote channel opening and increase Cl^- flux (Harrison et al. 1997, Majewska 1992, Majewska et al. 1986). In contrast, the steroids which have sulfate at 3β position and D5 configuration, such as pregnenolone sulfate (PS) and dehydroepiandrosterone sulfate (DHEAS), at physiological, low micromolar, concentrations act primarily as allosteric antagonists of the $GABA_AR$-associated Cl^- channel and facilitate its closing (Demirgoren et al. 1991, Majewska 1992, 1995, Majewska and Schwartz 1987, Majewska et al. 1988, 1990a,b). Figure 1 illustrates mechanistic model of THP-mediated potentiation of Cl^- transport *via* $GABA_AR$ and PS-induced closing of Cl^- channels linked to this receptor.

In vertebrates steroid modulators of the $GABA_AR$ and of other LGICs are synthesized in steroidogenic glands, such as adrenals, gonads and placenta, but also in the liver, skin and in the CNS (Corpechot et al. 1981, 1983). The steroids, which originate in the brain and(or) are neuroactive, have been named neurosteroids.

While the steroid regulation of LGICs has been proven beyond any doubt and pharmacodynamics and structure-function relationships of this regulation have been established in biochemical, electrophysiological and behavioral experiments, the specific domains of steroid action at these channels are still a subject of

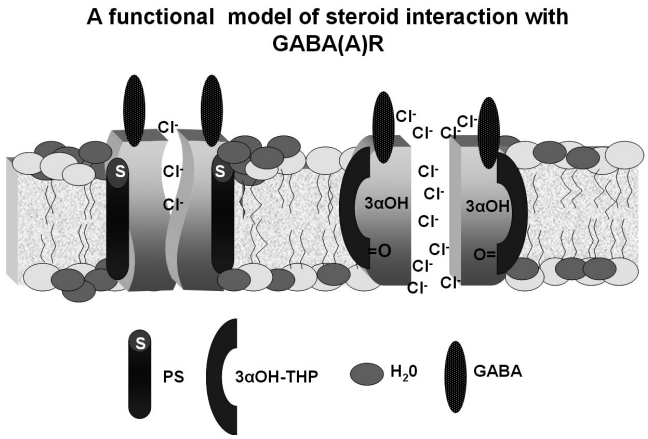

Fig. 1. The mechanistic model of interaction of the inhibitory neurosteroid, 3α-OH-THP, and the stimulatory steroid, PS, with the $GABA_AR$ (gray; shown in open and closed conformation) and the phospholipid bilayer. 3α-OH-THP works as allosteric receptor agonist – it changes conformation of the receptor complex in favor of widely open channel, allowing more intense Cl^- influx into the neuron (or other cell) than permitted by action of GABA alone. The functional groups of this steroid may disturb hydrogen bonding between the receptor subunits and phospholipids, promoting open conformation of the Cl^- channel. The excitatory neurosteroid PS interacts with the $GABA_AR$ complex as allosteric antagonist to close its channel. The 3β-sulfate group of this steroid is shown to interact with the external surface of the membrane to increase water trapping. This swelling of membrane surface may promote slow conformational change of the receptor complex, which facilitates channel closing.

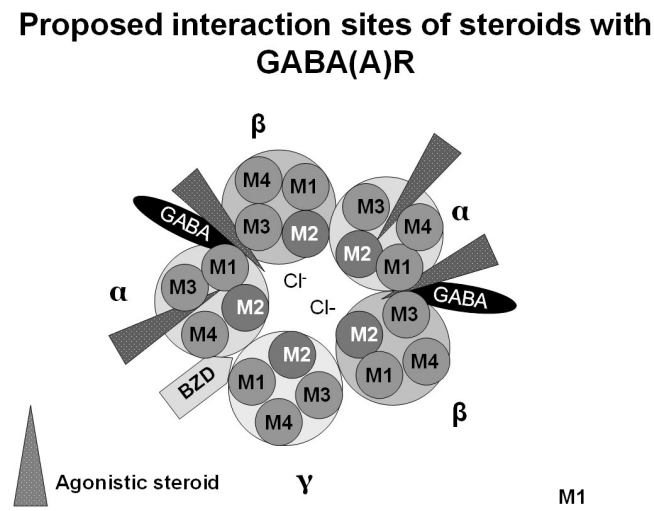

Fig. 2. The schematic representation of interaction sites of the inhibitory neurosteroids (3α-THP, 3α-THDOC) with the $GABA_AR$ complex according to Hosie and others (2006). The planar view of the $GABA_AR$ shows five receptor subunits: two α, two β, and one γ. Within each subunit are shown four transmembrane domains M1, M2, M3 and M4, where M2 domain is facing ion-conducting areas of the Cl^- lumen. BDZ determines a benzodiazepine. In this model four steroid molecules (shown as triangles) interact with one receptor pentamer. One steroid molecule binds within the hydrophobic cavity of α subunit to cause potentiation of GABA effect, and another steroid molecule interacts at the interface between α and β subunits to cause direct ion channel activation. Typically each receptor complex contains 2α and 2β subunits, hence maximally 4 inhibitory neurosteroid molecules may interact with one receptor to cause the potentiation of GABA effect and direct receptor activation.

investigations and discussions. The main difficulty with identification of these sites ensues from the amphiphilic nature of steroids, capable of interacting both with the lipid bilayer and protein receptors. Our early biochemical studies indicated existence of distinct binding sites for different steroids at the $GABA_AR$-Cl-ionophore complex (Demirgoren et al. 1991, Majewska 1992, Majewska et al. 1990a,b). The conclusions of others were similar (Covey et al. 2001, Park-Chung et al. 1999). Based on these observations I proposed that the steroids interact at the interface of proteins and lipids and this interaction promotes the conformational change of the receptor, facilitating channel's opening or closing (Majewska 1992).

Originally we identified two water-soluble steroid sulfates, DHEAS and PS, which at physiological (micromolar) concentrations act as allosteric antagonists of the $GABA_AR$-ionophore. Of these two, the larger molecule of PS seems to be more deeply rooted in the lipid bilayer, as its binding sites were insensitive to thermal and proteolytic denaturing. In contrast the binding sites for DHEAS were obliterated by such treatment (Demirgoren et al. 1991, Majewska 1992, 1999), which suggested theirs location closer to membrane surface. Such a conclusion was confirmed by the observations of others (Morissette et al. 1999).

The location of binding site for GABA and its agonists at the $GABA_AR$ has been characterized with help of aminoacid substitution studies (Amin and Weiss 1993, O'Shea and Harrison 2000, Boileau et al. 2002). It is thought to be situated in the pocket between α and β subunits of the receptor complex. Ligand binding to this site has been proposed to cause conformational change and opening of the Cl^- channel.

Studies attempted to explain in a similar fashion the locus and the mechanism of allosteric modulation of the $GABA_AR$ by the steroids, but this problem has been tantalizing. Chang and coauthors (2003) proposed that the anesthetic steroid bind at the hydrophobic pockets of the M1 transmembrane domains of the β subunits. However, in recently published study, Hosie and colleagues (2006) identified two types of discrete binding sites for agonistic steroids in the transmembrane area of the $GABA_AR$, which are required for high efficacy receptor activation. The site positioned in the hydrophobic cavity of α subunit (involving Gln241 and Asn407) mediates GABA-potentiating effect of allosteric steroid agonists such as THP and THDOC, whereas the site located in the pocket at interface between α and β subunits (involving αThr 236 and βTyr 284) mediates direct activation effect of the Cl^- channels (Hossie et al. 2006). The hypothetical interaction sites of inhibitory steroids with the $GABA_AR$ are illustrated on Fig. 2.

Likewise, it was not easy to identify loci and exact mechanism of action of allosteric steroid antagonists at the $GABA_AR$. The functional antagonism of this receptor by PS and DHEAS does not seem to be due to blockage of the Cl^- channel in its open state, but rather from conformational change of the receptor that facilitates channel's closing (Akk et al. 2001, Covey et al. 2001, Majewska et al. 1990b, Spivak 1994). Electrophysiological experiments conducted with recombinant $GABA_AR$ with mutated aminoacid residues (V256S) in the channel lining suggested that a residues near intracellular end of the channel of the α1 subunit are critical for the channel closing by PS (Akk et al. 2001). Because this region seems to function as a channel gating mechanism, the authors speculated that it participates in conformational receptor alteration induced by PS.

One universally observed feature of the channel blockade by PS and DHEAS is its temperature dependency and relatively slow action kinetics (Akk, et al. 2001, Demirgoren al. 1991, Majewska and Schwartz 1987, Park-Chung et al. 1999). Such characteristics suggest involvement of the membrane compartment or the site of the receptor that is deep-rooted in the membrane. Infrared spectroscopy investigation of the influence of steroids on biophysical properties of artificial phospholipid membranes or natural membranes from rat brains revealed a difference in the pattern of interaction between DHEA and DHEAS (Dicko et al. 1998, Morissette et al. 1999). At quasi-physiological temperatures DHEA behaved like cholesterol and it increased the order of fatty acid chains in membrane bilayer, whereas more hydrophilic DHEAS, which binds closer to the membrane surface, decreased the order (Dicko et al. 1998, Morissette et al. 1999). A possible explanation for this phenomenon may derive from the study of Faure and others (1996), who examined with biophysical methods the effects of cholesterol and its sulfate on phospholipid membranes. They found that the presence of sulfate at the 3β position in cholesterol molecule increased water trapping at the surface of membrane in the region of phospholipid head groups (Faure et al. 1996). Theoretically, such swelling at the membrane surface may contribute to the slow conformational modification of the receptor-ionophore, resulting in channel closing. It is conceivable therefore that DHEAS and PS, bound at the interface of the receptor protein and phospholipids, may be responsible for such effects. The ring of negative charges of steroid sulfate groups located near external mouth of the $GABA_AR$-associated channels may additionally act to reduce Cl^- attraction to the positively charged channel interior.

Some studies suggest that membrane phospholipids may participate also in the channel-opening effect of steroid agonists of the $GABA_AR$. The functional 3α-OH group of these steroids, which is necessary for their allosteric GABA-agonistic properties (Harrison et al. 1987, Majewska et al. 1986), disturbs the hydrogen bonding between sterols and phospholipids or proteins in the membrane (Huang 1976). Such an affect may facilitate conformational alteration of the channel promoting its opening. Quaternary structure of the proteins implanted in lipid bilayer is shaped by biophysical properties of the membrane. Because sterols interact with both proteins and lipids, they have the capacity to alter membrane order and hydration, and in this way to change conformation of membrane proteins. Collectively data suggest that steroids govern activity of LGICs through dual domains – the receptor proteins and their lipid milieu.

STEROID REGULATION OF ION CHANNELS IN EVOLUTION

Eukaryotic cells are assumed to have emerged in evolution through symbiogenesis by syntrophic union between several prokaryotes, such as anaerobic

archaebacteria and eubacteria before the biogenic rise of atmospheric oxygen on Earth (Lopez-Garcia and Moreira 1999, Margulis et al. 2000). While different hypotheses stress distinct nature of the primary eukaryotic organizing principle, most point to cell fusion as its vital element. Chimeras of loosely associated prokaryotes probably existed as intermediate cellular formations, which eventually fused forming a composite nucleus and organelles, typical for eukaryotes. In the model of Margulis, the earliest protoeukaryotes originated from the union of archaebacteria forming the "cell body" and several spirochete-like eubacteria forming protokinetosomes, which equipped the symbionts with motility (Margulis et al. 2000).

Cellular fusion thus lays at the basis of the development of complex forms of life. Biophysical experiments show that efficient fusion of phospholipid vesicles in aqueous environment requires presence of ion channels (Cohen et al. 1989, Woodbury and Hall 1988). Translocation of ions trough those channels generates the hyperosmotic environment inside the vesicles and their subsequent swelling, which promotes fusion with the membrane of another vesicle. Since sterol and sterol-like molecules control the activity of ion channels in cell membranes, they likely have been involved in the bacterial fusion at the dawn of eukaryotes' origin. Perhaps it is not incidental that classical sterols are present only in eukaryotes, but not in prokaryotes, and that they appeared in evolution concurrently with rise of atmospheric oxygen, which coincide with emergence of mitochondria from bacterial endosymbionts (Haines 2001, Hedges et al. 2001, Lopez-Garcia and Moreira 1999). The oxidized sterols synthesized in the eubacterial symbiont, which became the mitochondria, may have efficiently controlled ion channels in membranes of archaebacterial hosts, thus facilitating the cellular fusion and genesis of eukaryotes and complex forms of life.

It is interesting to trace the regulation of LGICs and other membrane channels by steroids in evolution. Oliver and coauthors (1991) examined the sensitivity of different animal species to the anesthetic effects of THP, which potentiates $GABA_AR$ function in mammals, and he concluded that it appeared late in evolution in the phylum Chordata, but was absent in evolutionarily more ancient animals. However, other studies documented control of the $GABA_AR$ and other LGICs by steroids also in invertebrates. In fact, this biological mechanism seems to be primeval. It also exists in mitochondria from different organisms, where sterols are essential for proper folding of porin polypeptides to create functional channels (Popp et al. 1995). The hydroxyl groups of sterols, which form hydrogen bonds with porin aminoacids, stabilize their tertiary and quaternary structures. Protozoa, such as Ciliates and other singe-celled eukaryotes (e.g. Paramecium), are auxotrophic for phytosterols, which support growth, movement and thermal sensoring in these organisms *via* membrane mediated phenomena (Hennessey et al. 1983, Whitaker and Nelson 1988). Membranes of the primitive metazoan *Hydra vulgaris* possess the $GABA_AR$, which control feeding. The function of these receptors is potentiated by the steroids THP and THDOC, which prolong the duration of the mouth opening and enhance feeding in this animal (Concas et al. 1998).

Many species of nematodes, which are plant and animal parasites, are incapable of *de novo* sterol synthesis, but have nutritional requirements for these compounds and are capable of their metabolism to different hormones (Chitwood 1999). The function of sterols in nematodes is poorly understood, but their cellular membranes possess ionotropic $GABA_A$ and nACh receptors, which participate in their movement and feeding, and are sensitive to sterols (Bamber et al. 2003). In a mollusk, the land snail *Cepaea nemoralis*, THP acting *via* $GABA_AR$ exerts antinociceptive effect similar to that in mammals (Kavaliers et al. 2000). Also the function of $GABA_AR$ from *Drosophila melanogaster* was potentiated by anesthetic neurosteroids (Hosie and Sattelle 1996). These examples provide evidence that regulation of membrane ion channels by steroids is a primordial phenomenon, which governs many different cellular and organismal functions.

STEROIDS AND ION CHANNELS IN FERTILIZATION AND REPRODUCTION

Fertilization, i.e. union of two gametes, in some sense recapitulates cellular fusion, which took place early in evolution to create complex forms of life. Steroid-ion channel duets are prominently involved in the genesis of diploid cells from haploid gametes. GABA and its receptors are present in abundance in female reproductive tracts of mammals. They are found in the ovaries, fallopian tubes and the uterus. GABA

changes the polarity and membrane conductance of unfertilized human oocytes (but not in fertilized ones), indicating that it directly participates in fertilization (Dolci et al. 1985). THP, whose concentration increases along with progesterone during the ovulation, may support this process by potentiating $GABA_AR$ function.

GABA and $GABA_AR$ are also present in various tissues of the male reproductive tract (Napoleone et al. 1990). Mammalian spermatozoa possess $GABA_AR$ and the GABA transport system. GABA increases sperm motility and initiates its capacitation – an essential step of fertilization (Burrello et al. 2004, Hu et al. 2002, Kuroda et al. 1999, Shi et al. 1997). In the fallopian tubes, GABA, whose gradient increases toward the ampula (Erdo 1989), acting *via* the $GABA_AR$ induces hyperactivated state of the sperm (Calogero et al. 1996). After traversing the cumulus oophorus, the spermatozoon reacts with the egg envelope, zona pellucida, inducing acrosome reaction in the sperm (Roldan et al. 1994, Vacquier 1998), which is coactivated by progesterone and its metabolites present in large quantities in cumulus oophorus (Baldi et al. 2002, Calogero et al. 2000, Roldan et al. 1994). This process involves the activation of $GABA_AR$-linked Cl^- channels on the sperm, leading to Cl^- efflux, which triggers an influx of Na^+ and Ca^{++}, and subsequent activation of phospholipases and other metabolic events leading to exocytosis and transfer of sperm genetic material to oocyte (Baldi et al. 2002, Vacquier 1998). Participation of the $GABA_AR$ regulated by steroids in the oocyte fertilization and initiation of new life shows how fundamental is this biological phenomenon.

We have documented that steroid regulation of the $GABA_AR$ also plays a critical role in pregnancy and birth. The reduced metabolite of progesterone, THP, by enhancing the activity of uterine $GABA_AR$, inhibits uterine contractions, whereas the GABA antagonistic steroids, such as PS, activate contractions (Majewska and Vaupel 1991). Because during pregnancy the plasma levels of progesterone and its metabolites are very high, these hormones play a vital quieting role on uterine motility. In contrast, during parturition relatively lowered plasma or tissue levels of the inhibitory steroids, combined with increased secretion of stimulatory ones, such as DHEAS and PS, promote uterine contractions and delivery (Doganay et al. 2004, Hill et al. 2002, Majewska and Vaupel 1991).

GABA, STEROIDS, AND FEEDING BEHAVIORS

In many animal species GABA system participates in feeding behaviors and GABA generally increases food consumption. The GABA agonistic steroids acting *via* $GABA_AR$ enhance feeding in a primitive organism such as *Hydra vulgaris* (Concas et al. 1998) and in mammals *via* the hypothalamic neurons (Chen et al. 1996, Reddy and Kulkarni 1998). In contrasts, GABA antagonists, including excitatory neurosteroids, generally inhibit feeding in mammals (Chen et al. 1996, Reddy and Kulkarni 1998). However, in the worm *Caenorhabdis elegans*, GABA agonists reduce food intake (Avery and Horvitz 1990), which may be due to the fact that GABA neurotransmission is excitatory in these animals (Reiner and Thomas 1995).

The mechanism of feeding control by steroids acting *via* $GABA_AR$ may come prominently into play during physiological states, which require alteration of food ingestion. For example, during pregnancy prolonged exposure to GABA-agonistic metabolites of progesterone may be responsible for increased appetite and eating, necessary for fetal growth. Plasma levels of various neuroactive steroid hormones also dramatically change during stress, which distinctly influences appetite in different individuals. Because normal individuals markedly vary in their expression of steroid synthesizing enzymes, it is not difficult to imagine, that the persons, whose adrenals secrete more GABA-agonistic steroids, may manifest increased appetite, whereas those, producing more neuroexcitatory steroids, may have suppressed appetite during stress.

NEUROSTEROIDS AND $GABA_A$ RECEPTOR CHANNELS IN THE CNS

Direct interaction of steroids with the $GABA_AR$-chloride channel in neurons has been studied most extensively. Because GABA is a ubiquitous neurotransmitter in the vertebrate CNS and the $GABA_AR$ are present on most neurons in the brain, regulation of these receptors by steroids is of paramount neurophysiological importance for all vertebrate species. Since our discovery of the interaction of steroids with the $GABA_AR$ and later with other LGICs by other investigators, a great body of evidence has been accumulated, documenting its physiological and pathological significance. These issues were discussed in many review

articles (Majewska 1987, 1992, 1999, 2002, Rupprecht et al. 1996, Schumacher et al. 2003), hence I will only briefly outline its significance here.

Research generally supported my early theoretical predictions regarding biological implications of the mechanism of rapid regulation of neurotransmission by steroids interacting with membrane ionotropic receptors. Synthesis of GABA-agonistic (inhibitory) steroids, such as THP and THDOC, has been shown to be markedly increased in women during the luteal phase of the menstrual cycle and pregnancy, in both sexes during stress and in some other physiological states, when they exert anxiolytic and hypnotic effects (Barbaccia et al. 2001, Gilbert Evans et al. 2005, Paoletti et al 2006, Parizek et al. 2005). These steroids also possess neuroprotective and anticonvulsive properties (Frye 1995, Landgren et al. 1987). Lowered levels of progesterone and its inhibitory metabolites or their sharp decline before and during menstruation may be contributing to catamenial seizures in women, whereas pharmacotherapy with such steroids might be beneficial in treating these forms of epilepsy (Backstrom et al. 2003, Nohria and Giller 2007, Reddy 2004, Rosciszewska et al. 1986). GABA-agonistic steroids also may be efficacious in treating intractable infantile spasms and other forms of epilepsy (Kerrigan et al. 2000).

Inhibitory steroids may participate in the regulation of sleep cycles (Lancel et al. 1997, Soderpalm et al. 2004) and influence mood. They may potentiate or alleviate depression, and their withdrawal before menstruation or post partum may induce anxiety and other mood disturbances in some women (Andreen et al. 2006, Rasmusson et al. 2006). Inhibitory steroids may be responsible for relaxation, sedation and increased appetite during pregnancy, and may have proamnesic effect in some stress situations (Johansson et al. 2002). Interactions of steroids with the $GABA_AR$ may also come into play in the regulation of peripheral systems, particularly the reproductive system. For example, during pregnancy THP acting *via* $GABA_AR$ quiets uterine activity (Majewska and Vaupel 1991) and may produce antinociceptive effects (Winfree et al. 1992).

Inhibitory metabolites of progesterone are neuroprotective during brain trauma or hypoxia (Djebaili et al. 2005, Rhodes et al. 2004) and regulate neuronal survival (Leskiewicz et al. 2006). It is interesting to note that the synthesis of these steroids in the brain and the spinal cord rapidly increases after ischemia or trauma, indicating that the CNS is equipped with its own neuroprotective mechanisms, which utilize steroids (di Michele et al. 2000, Meffre et al. 2007, Schumacher et al. 2000). Such mechanisms may also be responsible for the protection of fetuses and newborns from hypoxic insults (Nguyen et al. 2003). The neuroprotective effects of progesterone and its inhibitory metabolites in animals and humans (Wright et al. 2007) resemble those of general anesthetics (Majewska et al. 1978), which have been used with some success for many years to reduce brain damage after trauma (Hoffman WE et al. 1998, Koerner and Brambrink 2006). Such steroid effects may be due to synergistic GABA-agonistic and NMDA-antagonistic actions of inhibitory neurosteroids, resulting in decreased neuronal metabolism and activity.

In contrast, the neuroexcitatory steroids, PS and DHEAS, exert analeptic and invigorating effects during daily cycles and stress, and may have antidepressant actions in some situations (reviewed in Majewska 2002). Due to their neurostimulant actions as $GABA_AR$ antagonists and NMDAR agonists, and interactions with the sigma receptors, these steroids seem to improve learning and memory in experimental animals (Farr et al. 2004, Flood et al. 1995, Maurice et al. 2001, Sliwinski et al. 2004). However, prospective clinical studies did not show consistent effects of DHEA improving cognition in a healthy aging population (Grimley-Evans et al. 2006). As expected, excessive concentrations of the excitatory steroid DHEAS in plasma were shown to exert an anxiogenic and proconvulsant influence (Jacobs et al. 1999, Steffensen 1995). DHEA also appears to have a therapeutic utility promoting recovery from traumatic brain injury (Fiore et al. 2004, Hoffman SW et al. 2003, Lur et al. 2006, Malik et al. 2003), due to a complex mechanism of action, which combine mild neurostimulant, trophic and antiapoptotic effects (Suzuki et al. 2004, Zhang et al. 2002).

DHEA is of particular interest for neurophysiology and neuropsychiatry, because it is promoted and used by millions of people around the world as antiaging panacea without a solid proof of its therapeutic efficacy. DHEA is often called a mother hormone, because in addition to its own physiological and neuronal activity, it serves as a precursor of other steroid hormones, including estrogens and androgens. Several studies indicated that higher levels of endogenous DHEA and DHEAS in blood correlate with physical

Table I

Cellular and organismal functions, in which interactions between steroids and LGICs may come into play
Evolution
• Endosymbiosis, Eukaryote origin • Cell fusion • Creation of multicellular organisms
General Functions
• Intercellular communication • Integration of tissue and organ functions • Feeding • Mineral and energy balance • Maintenance of cell volume and integrity • Movement • Homeostasis • Stress survival • Physiological cycles (diurnal, monthly, seasonal)
Reproduction and development
• Gamete movement • Gamete fusion • Development • Reproductive cycles • Adaptation of maternal organism to pregnancy
Brain functions
• Cell integrity • Neurotransmission and neuromodulation • Neuroplasticity • Neurodevelopment • Cognition • Emotions • Sleep/diurnal cycles • Pain • Behavior • Neuroprotection and neuroregeneration

and psychological resiliency. In this regard we have shown that cocaine addicts, who had higher concentrations of endogenous DHEAS in blood during therapy for drug dependence, were more likely to succeed in maintaining prolonged abstinence after the therapy than those, who had lower levels of this hormone (Wilkins et al. 2005). However, treatment of cocaine addicts with high pharmacological doses of DHEA was not therapeutic and even resulted in increased cocaine use (Shoptaw et al. 2004). These apparently discordant results suggest the existence of a complex relationship between the levels of different neuroactive steroids in the blood (and presumably in the CSF) and intricate mental phenomena such as drug dependence, craving and use.

Wealth of accumulated scientific and medical data shows that alterations of the biosynthesis and release of diverse and metabolically linked neuroactive steroids as hormones, parahormones or autohormones during development, aging and in different physiological states serves to shape brain architecture and tune brain functions and the mind to those of the whole body in order to harmonize an organism's complex responses to environmental or physiopathological challenges.

CONCLUSIONS

Modulation of ion channels in plasma membranes by endogenous and exogenous steroids or steroid-like molecules is a biological mechanism, which seems to have emerged at the dawn of life. It is implicated in many basic cell functions, which guarantee maintenance of the cell's shape, volume, homeostasis and survival. This mechanism is involved in feeding, waste removal, cell fusion and division, reproduction, movement, defense, cellular communication and other functions in both simple monocellular and complex organisms. This primeval mechanism, which supports an amazing variety of organismal functions from the very basic to the most intricate, such as the mind, represents a true evolutionary wonder and is intimately interwoven in the very definition of life. Table I shows examples of cellular and organismal activities in which steroid-$GABA_AR$ interactions may come into play.

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