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Neuro(active)steroids actions at the neuromodulatory sigma$_1$ (σ_1) receptor: Biochemical and physiological evidences, consequences in neuroprotection

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Abstract

Steroids from peripheral sources or synthesized in the brain, i.e. neurosteroids, exert rapid modulations of neurotransmitter responses through specific interactions with membrane receptors, mainly the γ-aminobutyric acid type A ($GABA_A$) receptor and *N*-methyl-D-aspartate (NMDA) type of glutamate receptor. Progesterone and 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) act as inhibitory steroids while pregnenolone sulfate or dehydroepiandrosterone sulfate act as excitatory steroids. Some steroids also interact with an atypical protein, the sigma$_1$ (σ_1) receptor. This receptor has been cloned in several species and is centrally expressed in neurons and oligodendrocytes. Activation of the σ_1 receptor modulates cellular Ca^{2+} mobilization, particularly from endoplasmic reticulum pools, and contributes to the formation of lipid droplets, translocating towards the plasma membrane and contributing to the recomposition of lipid microdomains. The present review details the evidences showing that the σ_1 receptor is a target for neurosteroids in physiological conditions. Analysis of the σ_1 protein sequence confirmed homologies with the ERG2/emopamil binding protein family but also with the steroidogenic enzymes isopentenyl diphosphate isomerase and 17β-estradiol dehydrogenase. Biochemical and physiological arguments for an interaction of neuro(active)steroids with the σ_1 receptor are analyzed and the impact on physiopathological outcomes in neuroprotection is illustrated.

Keywords: Neurosteroids; Sigma$_1$ (σ_1) receptor; Sequence homologies; Non-genomic effects; Neuroprotection

1. Introduction

The sigma$_1$ (σ_1) receptor has now a long history, since its initial denomination proposed in the pioneering work of Martin et al. (1976) using the morphine-dependent chronic spinal dog. This thirty years long history could be a posteriori divided into three periods. During the initial ten years long, very confusing period, the protein was first identified as an opiate receptor, then closely related to the phencyclidine (PCP) binding site associated with the *N*-methyl-D-aspartate (NMDA) type of glutamate receptor. Finally, the σ site was considered as clearly distinct from any other receptor (Quirion et al., 1987), but putatively of similar nature as membrane-bound receptors. It remained clearly of enigmatic nature. During the second ten years long period, a limited number of research teams kept their interest on σ sites, but major breakthroughs were obtained. Pharmacological studies revealed the existence of at least two subtypes of σ sites, named σ_1 and σ_2 (Quirion et al., 1992). Endogenous peptides, like neuropeptide Y and calcitonin gene-related peptides, and neuroactive steroids, including progesterone (PROG), pregnenolone (PREG) and dehydroepiandrosterone (DHEA), were found to interact with the σ_1 site, at least in in vivo physiological tests (Su et al., 1988; Bouchard et al., 1993, 1997). Numerous high affinity and often very selective compounds were described, for each type of σ site. The neuromodulatory effect of σ_1 ligands, on NMDA responses particularly, was shown and extensively analyzed (Monnet et al., 1990, 1992). Finally, behavioral effects of σ_1 ligands, in learning and memory, depression, anxiety, stress, addiction or psychoses were described (for reviews, see Walker et al., 1990; Su, 1991; Debonnel and de Montigny, 1996; Maurice and Lockhart, 1997; Maurice et al., 1999, 2001, 2002; Skuza, 2003; Maurice, 2004; Maurice and Romieu, 2004; Bermack and Debonnel, 2005). The last ten years long period

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started with the publication of the σ_1 protein cloning in guinea-pig and human (Hanner et al., 1996; Kekuda et al., 1996) and, then, in rat and mouse (Seth et al., 1997, 1998). These recent years were mainly characterized by very precise studies of the cellular role of the σ_1 receptor in neuron-like cells (for reviews, see Hayashi and Su, 2004a,b; Takebayashi et al., 2004; Su and Hayashi, 2003), and of its putative role in tumorigenesis (Aydar et al., 2004), generating a renewal of interest for the field.

The possibilities that neuro(active)steroids could be endogenous activator/inactivator of the σ_1 receptor, if not "the" endogenous ligands (not yet identified at present), and, conversely, that the σ_1 receptor could be a target for neuro(active)steroids has generated a major interest. Biochemical, physiological and behavioral studies examined, first, the involvement of the σ_1 receptor as a target for neuro(active)steroids and, second, how the steroidal tonus affects the pharmacological activities of σ_1 receptor ligands. The field has been covered by several exhaustive review articles (Maurice et al., 1999, 2001, 2002; Frye, 2001; Vallee et al., 2001; Schumacher et al., 2003; Barbaccia, 2004; Maurice, 2004; Maurice and Romieu, 2004; Dubrovsky, 2005; Monnet and Maurice, 2006). In the present article, after a brief overview concerning neuro(active)steroids and the σ_1 receptor, we would like to detail the arguments identifying the σ_1 receptor as a molecular target for neuro(active)steroids actions, at the structural, biochemical and physiological levels. We will, in particular, re-examine the structural similarities between the σ_1 receptor sequence and steroidogenic enzymes. The behavioral consequences in learning and memory, stress, depression, and addiction, will not be presented, but the importance of the σ_1 receptor in the neuroprotective effects of neuro(active)steroids will illustrate the putative importance of the question.

2. Neuromodulatory effects of neurosteroids

2.1. Biosyntheses of neurosteroids

The brain is considered to be a target site of steroids. In contrast to this classical concept, new findings over the past decade established that the brain also synthesizes steroids de novo from cholesterol or from steroid precursors through mechanisms partly independent of peripheral steroidogenic endocrine glands, gonads and adrenal glands. Such steroids synthesized in the brain or in other areas of the nervous system, are designed as "neurosteroids" (Baulieu, 1981, 1998; Majewska, 1992). DHEA, androstenedione, PREG, their sulfate esters (DHEAS, PREGS; note that DHEA/S and PREG/S will refer to both the free and sulfated forms) and lipoid conjugated forms, as well as reduced metabolites of PROG and deoxycorticosterone were found at high levels in the brain long after gonadectomy and adrenalectomy (Corpechot et al., 1981; Cheney et al., 1995). Two requirements are necessary to qualify as a neurosteroid: (i) persistence of the steroid in the nervous system in the absence of the steroidogenic endocrine glands; and (ii) expression and activity of the enzymes involved in its synthesis. The presence of the steroidogenic enzymes, including cytochrome P450 side-chain cleavage (P450 scc), aromatase, 5α-reductase, 3α-hydroxysteroid dehydrogenase (3α-HSD), and 17β-hydroxysteroid dehydrogenase (17β-HSD) in the human brain has now been firmly established by molecular and morphological studies. Their presence in the cerebral cortex and in the subcortical white matter indicates that various cell types including oligodendrocytes, Schwann cells, type I astrocytes, and neurons are involved in the biosynthesis of neurosteroids in the brain (Schumacher et al., 1999; Zwain and Yen, 1999). The syntheses of steroids, adrenal and gonadal, as well as neurosteroids, depend upon tissue-specific and cell-specific array of steroidogenic enzymes. Steroidogenic enzymes can be classified as cytochrome P450 and non-P450 enzymes. The presence of these enzymes in the nervous system has been documented by mRNA and protein analysis in a variety of species (Compagnone and Mellon, 2000; Mellon and Griffin, 2002). The steroidogenic P450 enzymes, such as the cytochrome P450 scc, are found as single genes in multiple species but can mediate multiple enzymatic steps (Mellon and Griffin, 2002; Stoffel-Wagner, 2003). Moreover, the non-P450 enzymes, such as 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-HSD and 3α-HSD, are found as multiple genes encoding different proteins. Each of these proteins mediates specific reactions. PREG biosynthesis, for instance, is regulated via a glial mitochondrial benzodiazepine receptor (MBR), expressed at high levels in steroid synthesizing tissues. MBR binds cholesterol with high affinity and facilitates the intra-mitochondrial transfer of cholesterol to the P450 scc enzyme located in the inner mitochondrial membrane of glial cells, thereby stimulating PREG formation (Papadopoulos et al., 1992; Romeo et al., 1994). MBR is a key element of the cholesterol mitochondrial import machinery responsible for supplying the substrate cholesterol to the first steroidogenic enzyme, thus initiating and maintaining neurosteroid biosynthesis. In the brain, PREG can be converted by 3β-HSD into PROG, which is a neuroactive steroid because in nanomolar concentrations it can bind to intracellular, nuclear, PROG receptors and control the transcription of specific genes in neurons or glial cells (Koenig et al., 1995; Rupprecht and Holsboer, 1999).

Neurosteroids synthesized in the central and peripheral nervous system, particularly in myelinating glial cells, astrocytes and several neuronal types, act directly in the nervous system. Their syntheses may start from cholesterol or from steroidal precursors imported from a peripheral source. It is probable that several cell types participate in the synthesis of a given neurosteroid by sequential chemical modifications. However, study of the biosynthetic pathways remains to be completed. Until now, only DHEA/S, PREG/S, PROG and several PROG metabolites have been considered bona fide as neurosteroids. They are still present in the absence of steroidogenic gland secretion. Selective changes in the concentrations of neurosteroids in certain parts of the brain have been found in different behavioral or environmental situations, such as sexual behavior (Baulieu, 1981, 1987), developmental, memory enhancing effects (Flood et al., 1995; Vallee et al., 1997), pregnancy (Herbison, 2001; Stoffel-Wagner, 2001) and stress (Urani et al., 2001; Higashi et al., 2005).

2.2. Genomic and non-genomic actions of neurosteroids

Neurosteroids exert several biological actions in the brain during embryogenesis as well as in adults. These have been

reported in several species, including human. Measured concentrations of neurosteroids are consistent with the affinities of receptor systems with which they interact in the nervous system. Both intracellular and membrane receptors responding to neurosteroids can be distinguished. Some receptors are identical or similar to steroid receptors found in peripheral target organs, such as the PROG receptor, which is likely to be involved in auto/paracrine PROG action.

Mechanisms by which neurosteroids affect neuronal and brain functions include both genomic actions mediated by nuclear steroid receptors and nongenomic actions, mediated by neurotransmitter receptors (Majewska et al., 1986). Steroid action involves binding of the steroids to their respective intracellular receptors, which, in turn, change their conformation, by dissociation from the heat shock proteins, translocate to the nucleus and bind to the respective response elements located in the regulatory regions of target promoters (Truss and Beato, 1993; Rupprecht and Holsboer, 1999). Steroid receptors act as transcription factors in the regulation of gene expression (Evans, 1988).

In the last decade, considerable evidence has emerged that certain steroids may alter neuronal excitability through a nongenomic mechanism, by interacting at the cell surface with some neurotransmitter receptors (Majewska et al., 1986; Paul and Purdy, 1992; Rupprecht and Holsboer, 1999). The term "neuroactive" steroid therefore refers to steroids that, independently of their origin, are capable of modifying neural activities. Although the action of steroids at the genome requires a time period from minutes to hours limited by the rate of protein biosynthesis (McEwen, 1991), the modulatory effects of neuroactive steroids are fast occurring events within milliseconds to seconds (McEwen, 1991). Thus, the genomic and nongenomic effects of steroids within the central nervous system provide the molecular basis for a broad spectrum of steroid action on neuronal function and plasticity.

Receptors for circulating neuroactive steroids have been described, such as estrogen receptors, responding to both circulating estrogens and those synthesized in the hypothalamus. PROG receptors have been described, which are inducible by estradiol in hypothalamic neurons but not in the cortex (Rainbow et al., 1982). PROG receptors can be activated by phosphorylation in the absence of a ligand (Power et al., 1992). Experiments on mixed glial cell cultures from rat brain indicated the estrogen inducibility of the PROG receptor in oligodendrocytes, and inhibition by PROG of estrogen-directed cell growth and morphological differentiation of both oligodendrocytes and astrocytes (Jung-Testas et al., 1991, 1992). However, it is difficult to determine whether nuclear receptors found in the nervous system are or not identical to those of the peripheral target tissues, since they have not yet been cloned: neuroisoform (s) would be of great pharmacological interest. In contrast to the circulating steroids, which act at a distance from their gland of origin, on brain and neurons at relatively low concentrations (endocrine effect), neurosteroids act in the nervous system in an auto/paracrine configuration. For example the sulfate esters of DHEA or PREG, and the reduced metabolites 3α,5α-tetrahydro-PROG (allopregnanolone) or its 5β isomer have their own distinct activities. These steroids act as potent modulators of the $GABA_A$ receptor, NMDA receptor and σ_1 receptor activities (Monnet et al., 1995; Baulieu et al., 1999; Gibbs et al., 1999; Majewska, 1999) and their levels are compatible with a physiological neuromodulatory role for these interactions.

3. The σ_1 receptor, molecular structure and homologies with steroidogenic enzymes

3.1. Structure of the σ_1 protein

The σ site has been defined 30 years ago by Martin et al. (1976) as a subtype of opiate receptor. Although it was rapidly evident that σ sites were unrelated to classical opiate receptors (for a recent historical review, see Leonard, 2004), the observation that numerous, chemically unrelated compounds bind with moderate-to-high affinities to σ sites brought confusion on its exact pharmacological nature. However, early structure/activity relationship studies allowed the distinction of two classes of σ sites, designated σ_1 and σ_2 (Quirion et al., 1992). The two sites were distinguished based on their different drug selectivity patterns and molecular weights. In fact, it is now accepted that only the σ_1 receptor represents an identified protein, that was recently extensively characterized after its cloning and a series of cellular biology studies (Su and Hayashi, 2003). The σ_2 sites have been defined by exclusion, and may likely represent several pharmacological entities those remain to be defined.

The σ_1 receptor is a single polypeptide with a low molecular weight protein of 29 kDa. It has now been purified and cloned with high homology and identity from several species (guinea pig liver, human placental cell line and brain, from mouse kidney and brain and from rat brain) and completely sequenced (Hanner et al., 1996; Kekuda et al., 1996). The gene, located on chromosome 9 in human and 2 in rodents, is 7 kpb-long and contains 4 exons and 3 introns (Prasad et al., 1998). Exon 2 codes for the single transmembrane domain, identified at present, but two other hydrophobic regions exist and one of them may putatively constitute a second transmembrane domain (Yamamoto et al., 1999). The receptor is a unique protein composed of 223 amino acids highly preserved, with 87–92% identity and 90–93% homology among tissues and animal species. The protein sequence does not show homology with any classical neurotransmitter or neuropeptide receptor sequences and limited homology with only a few number of protein present in mammalian brain (see following paragraph), outlining the unicity of the σ_1 receptor as compared with any other known protein (Moebius et al., 1993, Seth et al., 1998).

3.2. Sequence homologies between the cloned σ_1 receptor and steroidogenic enzymes

3.2.1. ERG2 and σ_1 receptor like protein/EBP family

Molecular comparisons of the cloned σ_1 receptor sequences using protein data banks revealed that very few protein families able to exhibit significant similarity or identity with the σ_1 receptor. In their initial report describing the σ_1 receptor cloning in guinea-pig, Hanner et al. (1996) noticed a significant a similarity, 33% identity and 66% homology, with a sterol

Table 1
Summary of the structure–activity comparative analysis of σ_1 receptor and steroidogenic enzymes

Protein	σ_1 receptor	C8 sterol isomerase Δ8–Δ7 sterol isomerase	Emopamil binding protein 3β-hydroxysteroid-Δ8, Δ7-isomerase	Emopamil binding protein-like	Isopentenyl diphosphate isomerase isopentenyl pyrophosphate isomerase	Estradiol 17β-dehydrogenase 1
Identity		45%	35%	35%	35%	45%
Function	Neuromodulatory protein	Ergosterol biosynthesis	Cholesterol biosynthesis	No activity	Isoprenoid biosynthesis	Estrogens biosynthesis
Activity	Regulates Ca^{2+} mobilization and modifies lipid microdomains composition. No enzymatic activity.	Catalyzes the reaction, which results in unsaturation at C-7 in the β ring of sterols.	Catalyzes the conversion of Δ8-sterols to their Δ7-isomers.	Lacks of sterol Δ8–Δ7 isomerase activity.	Catalyzes the 1, 3-allylic rearrangement of the homoallylic substrate isopentenyl (IPP) to its highly electrophilic allylic isomer.	Favors the reduction of estrogens and androgens
Family	ERG family?	ERG family	EBP family	EBP family	IPP isomerase type 1 family	Short-chain dehydrogenases/reductases (SDR) family
Ligand	*Steroid*: DHEA(S), Preg(S) PROG, dihydrotestosterone *Synthetic*: (+)SKF-10,047, haloperidol, pentazocine, ditolylguanidin, …	*Steroid*: DHEA PROG and testosterone *Other*: haloperidol, pentazocine, and ditolylguanidine	*Steroid*: PROG and testosterone *Other*: haloperidol, pentazocine, and ditolylguanidine	Does not bind σ_1 receptor ligands.	*Steroid:* bromohydrine Nipp Eipp	*Steroid:* DHEA

Adapted from Hanner et al., 1996; Moebius et al., 1996, 1998, 1999, 2003; Adimoolam et al., 1998; Grebenok et al., 1998; Kass and Sampson, 1998; Peelman et al., 1998; Han et al., 2000; Bonanno et al., 2001; Shi and Lin, 2004; Laggner et al., 2005; Wouters et al., 2005.

Δ8–Δ7 isomerase with the yeast ERG2p and with emopamil binding protein (EBP). ERG2 and σ_1 receptor like protein family consists of the mammalian σ_1 receptor and the fungal C8 sterol isomerase, i.e. Δ8–Δ7 sterol isomerase, involved in ergosterol biosynthesis. In parallel, EBP is a family of protein that displays Δ8–Δ7 sterol isomerase activity. This family plays also a role in cholesterol biosynthesis and several mutations of EBP are known to cause the genetic disorder of X-linked dominant Conradi–Hunermann–Happle syndrome (Braverman et al., 1999; Derry et al., 1999). Several experiments demonstrate the common pharmacological relationship between the σ_1 receptor, yeast ERG2p and EBP (Hanner et al., 1996; Moebius et al., 1996, 1998; Grebenok et al., 1998). σ_1 Receptor ligands, from synthetic or steroidal origins, DHEA, PROG or testosterone (see Section 5), bound ERG2 and EBP (Laggner et al., 2005), suggesting homology in their binding site. Interestingly, the EBP-like protein lacks sterol Δ8–Δ7 isomerase activity and does not bind any σ_1 receptor ligands (Moebius et al., 2003). Overall structures of these proteins and the steroid binding site are completely unrelated and it is unclear how steroid bind the σ_1 receptor. To answer this question, we examined the sequence homology between the ERG2 and σ_1 receptor like protein family with proteins known to interact with steroid.

3.2.2. Sequence homology with enzyme of steroid biosynthesis: isopentenyl diphosphate isomerase and 17β-estradiol dehydrogenase

HMM-HMM® and Basic Local Alignment Search Tool® (BLAST®) research (Altschul et al., 1997; Söding et al., 2005) confirmed sequences homologies between the σ_1 receptor protein, ERG2 and EBP (Table 1). Research in the Protein Data Bank® (PDB; Bernstein et al., 1977) led us to identify sequence homology that reach 45% of identity, revealing two enzymes known to play a role in steroid biosynthesis (Han et al., 2000; Bonanno et al., 2001; Shi and Lin, 2004): isopentenyl diphosphate isomerase (PDB code: 1I9A) and 17β-estradiol dehydrogenase (PDB code: 3DHE). Isopentenyl diphosphate isomerase is an α/β metalloenzyme involved in the first steps of sterol/isoprenoid biosynthesis and 17β-hydroxysteroid dehydrogenase is responsible for the last step in the formation of androgens and estrogens by the reduction of the 17-ketone group to 17-hydroxyl. Interestingly, the 17β-hydroxysteroid dehydrogenase converts PREG into 17-hydroxy-PREG, precursor of DHEA (Compagnone and Mellon, 2000). Isopentenyl diphosphate isomerase and the human estrogenic 17β-hydroxysteroid dehydrogenase have been co-crystallized with their ligands present in the steroid binding site, respectively bromohydrine, a steroid precursor (Wouters et al., 2005), and DHEA (Han et al., 2000).

3.2.3. Evolutionary conservation of the steroid-binding site

By sequence comparison between the σ_1 receptor, 17β-estradiol dehydrogenase and isopentenyl diphosphate isomerase with the multi-align program, we predicted the localization of the steroid-binding site of ERG2/σ_1 receptor. Sequence homology was consistent with a conservation of the steroid-binding site, where some amino acids essential for binding are conserved. On the one hand, especially hydrophobic amino acids such as tryptophane, phenylalanine or leucine are necessary to form the hydrophobic cavity that binds the steroid. The interaction between the central hydrophobic core of the steroid with the cavity has been described to contribute to the thermodynamic force of the binding (Han et al., 2000). On the other hand, the specificity of the binding required hydrogen bonds carry out by amino acids such as histidine or glutamic

acid. Most of the homology is localized in four regions of the ERG2/σ_1 receptor corresponding to the steroid-binding site of 17β-estradiol dehydrogenase (Fig. 1). The first region contains a conserved histidine. Moebius et al. (1999) showed the role of histidine but also tryptophan, glutamic acid, threonine and asparagine for the sterol Δ8–Δ7 isomerization of the human EBP. Histidines are involved in the specificity of the interaction with steroids and their orientation, and play a role in the catalytic function of the enzyme such as isomerization (Adimoolam et al., 1998; Kass and Sampson, 1998; Han et al., 2000). Catalytic center required the triad Ser/Asp–Glu/His (Peelman et al., 1998). The second region contains the conserved Tyr-155 required for the interaction between the O-17 atom of the phenolic D-ring of DHEA and the 17β-estradiol dehydrogenase (Han et al., 2000). The third region contains a conserved glutamic acid. Glu-282 of the 17β-estradiol dehydrogenase forms a possible hydrogen bond with the O-3 atom of the A-ring of DHEA. The last region is more specific of the 17β-estradiol dehydrogenase and contains the His-221 not conserved in ERG2/σ_1 receptor. This histidine has been described to contribute to the steroid orientation in the pocket and form a specific hydrogen bond between the 17β-estradiol dehydrogenase and the O-3 atom of the A-ring of DHEA. By sequences comparison with isopentenyl diphosphate isomerase, an additional region that contains the conserved motif His–Ala–Phe–Ser could be predicted to be required in the binding of steroid to the σ_1 receptor. Therefore, specific amino acids required for the binding of steroids have been conserved between the EBP/σ_1 receptor family and steroidogenic enzymes. Homologies over the entire sequence are observed between EBP and σ_1 receptor.

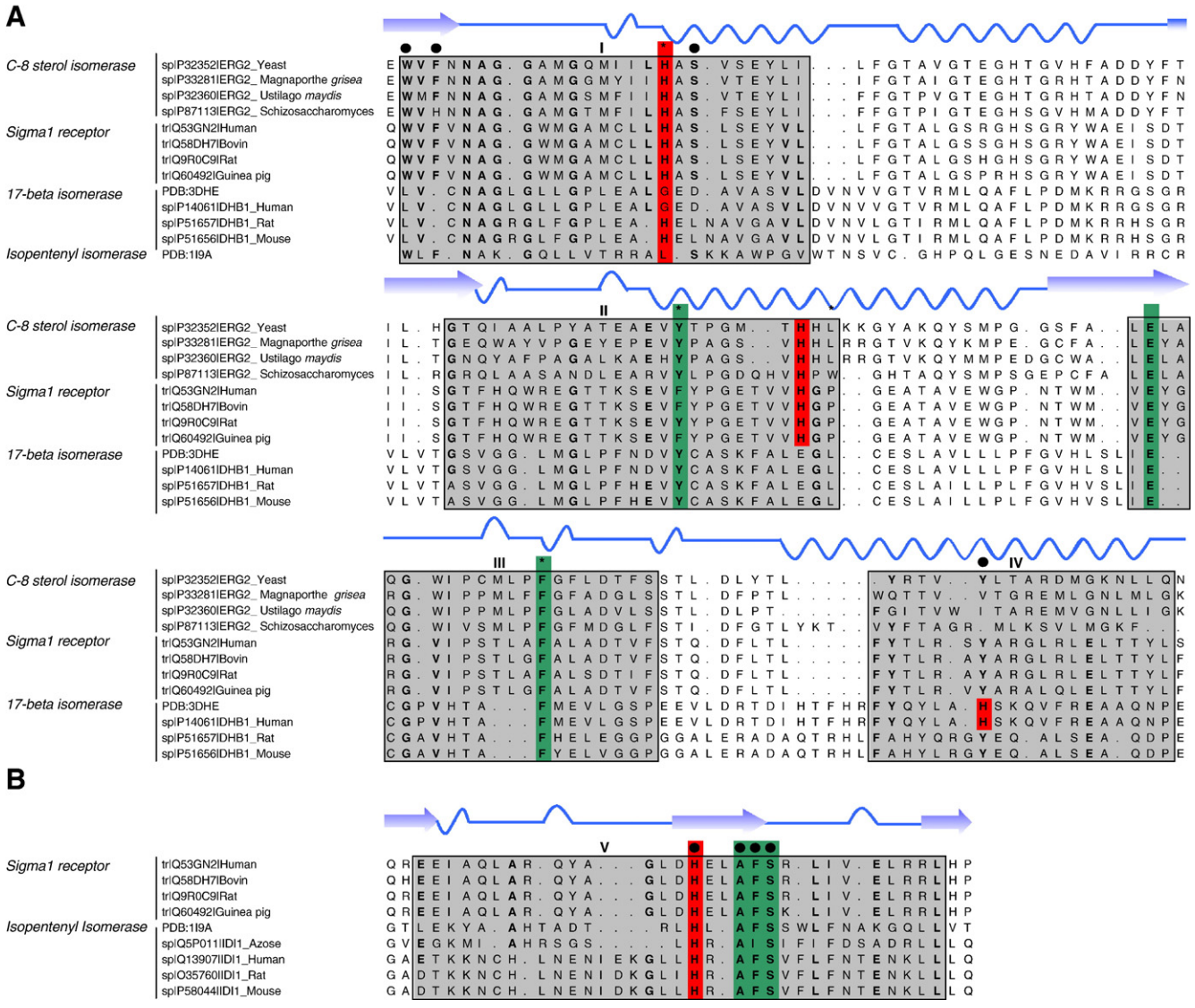

Fig. 1. Predictive steroid binding site of ERG2 and σ_1 receptor protein family. A multiple alignment of the steroid binding site was generated with Multi align® program and manually improved. The binding site of ERG2 and σ_1 receptor is predicted to be localized in four regions by homology with the 17β-estradiol dehydrogenase (A) and by one additional region by homology with the isopentenyl diphosphate isomerase (B). Conserved residues ≥ 50% are in bold. Conserved aromatic amino acids (W, Y, F, H) and (S, E), predicted for the binding, are represented by a black circle Y155 and H221 necessary for the binding of DHEA with specific orientation are shaded in green. Database accession numbers are given in right column and refer to the SwissProt/TrEMBL and to the PDB databases.

The organization of the binding pocket that could play a critical role in steroid discrimination showed specific homologies between isopentenyl diphosphate isomerase, 17β-estradiol dehydrogenase and the σ_1 receptor.

4. Pharmacological characteristics of the σ_1 receptor

4.1. Localization of the σ_1 receptor

The σ_1 sites are widely distributed both centrally and peripherally but particularly concentrated in the hippocampal formation and other limbic areas, in agreement with their suggested role in psychiatric disorders (Walker et al., 1990; Leonard and Nicholson, 1994; Maurice et al., 1994; Debonnel et al., 1996; Leonard, 1997). In peripheral organs, the σ_1 protein is expressed in the heart, lung, kidney, liver, intestines and sexual and immune glands. In the brain, it is expressed in neurons, ependymocytes, oligodendrocytes and Schwann cells (Alonso et al., 2000; Palacios et al., 2003, 2004; Hayashi and Su, 2004a,b). It is particularly concentrated in specific areas throughout limbic systems and brainstem motor structures. The highest levels of σ_1 immunostaining can be observed in the granular layer of the olfactory bulb, hypothalamic nuclei and pyramidal layers of the hippocampus (Alonso et al., 2000; Phan et al., 2003). At the subcellular level, the σ_1 receptor was found to be the mostly present within neuronal perikarya and dendrites, where it is associated with microsomal, plasmic, nuclear or endoplasmic reticulum (ER) membranes (Alonso et al., 2000; Phan et al., 2003). Indeed, the sequence of the receptor contains a 22 amino acid retention signal for the ER (Hanner et al., 1996). Cell biology and biochemical studies showed that the σ_1 receptor is an intracellular protein anchored on the ER and the translocation of σ_1 receptor from the cytoplasm to the membrane requires a Ca^{2+} efflux from the ER pools (Morin-Surun et al., 1999; Hayashi et al., 2000).

4.2. Pharmacology of the σ_1 receptor

The pharmacological identification of σ_1 sites was characterized by their ability to bind several chemically unrelated drugs with high affinity, including psychotomimetic benzomorphans, e.g. (+)SKF-10,047 or (+)pentazocine, the psychotomimetic drug phencyclidine, the psychostimulants cocaine, amphetamine and derivatives, certain neuroleptics, e.g. haloperidol, many new atypical antipsychotic agents, anticonvulsants, cytochrome P450 inhibitors, monoamine oxidase inhibitors, histaminergic receptor ligands, peptides from the neuropeptide Y (NPY) and calcitonin gene-related peptide (CGRP) families, substance P and several neuroactive steroids (Walker et al., 1990; Maurice et al., 1999, 2001). The σ_1/σ_2 subtype classification was initially mostly based on radioligand binding particularities: σ_1 sites exhibit a stereoselectivity for dextrorotatory isomers of benzomorphans, whereas the levorotatory isomers as well as haloperidol or 1,3-di-*o*-tolylguanidine (DTG) bind with high affinity also to the σ_2 sites (Hellewell et al., 1994; Quirion et al., 1992). DTG, (+)-3-PPP and haloperidol are non-discriminating ligands with high affinity on both subtypes. Several biochemical features were although proposed to be selectively observed with σ_1 receptors, which are still considered, such as an allosteric modulation by phenytoin (Musacchio et al., 1988) and sensitivity to pertussis toxin or G-proteins modulators (Itzhak, 1989; Itzhak and Stein, 1990). It also has been shown that several drugs, such as haloperidol, reduced haloperidol, α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine butanol (BMY-14,802), rimcazole, or *N*,*N*-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)ethylamine (NE-100) act as antagonists in several physiological and behavioral tests relevant to the σ_1 pharmacology (Taylor and Dekleva, 1987; Taylor et al., 1991; Okuyama et al., 1993; Klein et al., 1994). However, most of them are non-selective compounds, which also bind to other pharmacological targets. Only NE-100, *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino)ethylamine (BD1047) or *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*,*N*′,*N*′-trimethylethylenediamine (BD1063) appear as selective σ_1 receptor antagonists, although BD compounds also show some affinity for σ_2 sites (Okuyama et al., 1995; Matsumoto et al., 1995).

An in vivo electrophysiological model to study the pharmacological activity of selective σ receptor ligands has been proposed by Monnet and Debonnel (Monnet et al., 1990; Bergeron et al., 1993; Debonnel et al., 1996; Couture and Debonnel, 1998). Numerous results from this laboratory demonstrated that σ_1 ligands, applied by microiontophoresis or administered i.v. at low doses, potentiated the neuronal response to NMDA in the CA3 region of the rat dorsal hippocampus, but did not modify kainate—nor quisqualate—induced activations. The σ_1 antagonist, including NE-100 or haloperidol, could be discriminated since they did not modify NMDA-induced firing activity, but suppressed the potentiation of NMDA response induced by σ_1 receptor agonists. A majority of σ_1 receptor agonists tested thus far generated bell-shaped dose–response curves with respect to potentiation of NMDA response. It is worth emphasizing that this dose–response relationship seems characteristic of σ_1 receptor ligands, and was observed in some behavioral models as well (Maurice et al., 1994; Couture and Debonnel, 1998).

Many neuroleptics exhibit high affinity for σ_1 receptors. For several years, haloperidol remained the ligand showing the highest affinity and selectivity for σ receptors and was thus used in most of the radioligand binding studies. Furthermore, the selective loss of cerebral cortical σ binding sites in schizophrenic patients was reported (see Debonnel et al., 1996). Finally, the recent demonstration of an association between polymorphism in the σ_1 receptor gene and schizophrenia supported the idea that these receptors may play a role in pathogenesis of this disease (Ishiguro et al., 1998). For the last twenty years, a number of compounds acting as high affinity σ_1 receptor antagonists and selective towards other, including dopamine D_2, binding sites have been described, like rimcazole, NPC-16,377, SR-31742A, NE-100, BD1047, BD1063, E5842, MS-377...

4.3. Mechanisms of action of the σ_1 receptor

Activation of σ_1 receptors affect intracellular signaling through a mechanism involving translocation between different

cellular compartments, whereby they modulate inositol trisphosphates, protein kinases and Ca^{2+}(Hayashi et al., 2000; Hayashi and Su, 2001; Morin-Surun et al., 1999). Brent et al. (1997) demonstrated that protein phosphorylation, dependent on extracellular Ca^{2+}, may be one of the important mechanisms through which σ_1 receptor ligands produce their effects. Hayashi et al. (2000) showed that selective σ_1 agonists potentiated the bradykinin-induced increase in $[Ca^{2+}]_i$, thus mediated by activation of inositol-1,4,5 trisphosphate ($InsP_3$) receptors, in a bell-shaped manner. After depletion of intracellular Ca^{2+} from ER stores, the depolarization-induced increase in $[Ca^{2+}]_i$ in the cells could also be modulated by σ_1 agonists. Both effects were blocked by an antisense oligodeoxynucleotide targeting the σ_1 receptor (Hayashi et al., 2000). Therefore, activation of the σ_1 receptor resulted in a complex, bipolar modulation of calcium homeostasis. At the ER level, the σ_1 receptor activation facilitates the mobilization of $InsP_3$ receptor-gated intracellular calcium pools and at the plasma membrane level, the σ_1 receptor activation modulates extracellular calcium influx through voltage-dependent calcium channels. Co-immunoprecipitation studies further revealed that the σ_1 receptor could regulate the coupling of the $InsP_3$ receptor with the cytoskeleton via an ankyrin B anchor protein, a cytoskeletal protein originally attached to ER membranes (Hayashi and Su, 2001). Activation of the σ_1 receptor results in its dissociation with ankyrin B from $InsP_3$ receptor in NG-108 cells, and led to an increase of the ligand efficacy in potentiating the Ca^{2+} efflux induced by bradykinin. These results, coherent with the σ_1 receptor subcellular localization (Alonso et al., 2000), showed that the σ_1 receptor may act as a sensor/ modulator for the neuronal intracellular Ca^{2+} mobilizations and consecutively for extracellular Ca^{2+} influx.

Hayashi and Su (2003a,b, 2005) then used confocal fluorescence microscopy to examine the protein dynamics in NG108 cells and primary oligodendrocytes overexpressing tagged σ_1 receptors. They observed that endogenously expressed σ_1 receptors localize on the ER reticular network and nuclear envelope. They are seen particularly as highly clustered unique globular structures associated with the ER (Hayashi and Su, 2003a,b). These σ_1 receptor-enriched globules contain moderate amounts of free cholesterol and neutral lipids (Hayashi and Su, 2003a). Therefore, on the one hand, σ_1 receptors translocate from the ER lipid droplets to plasmalemma or nuclear membranes when stimulated by agonists. The translocation of σ_1 receptors, associated with the ankyrin B protein, consequently affects Ca^{2+} mobilization at the ER (Hayashi and Su, 2001). On the other hand, lipid droplets are formed by coalescence of neutral lipids within the ER membrane bilayer and may, when reaching a critical size, bud off to form cytosolic lipid droplets, serving as a new transport pathway of lipids between ER and Golgi apparatus or plasma membrane (Murphy and Vance, 1999; Ohashi et al., 2003; Hayashi and Su, 2005). Indeed, Hayashi and Su (2003a) observed that functionally dominant negative σ_1 receptors, which cannot target ER lipid droplets and cannot translocate, are transfected into NG108 cells, a large amount of neutral lipids and cholesterol is retained in the ER, causing the pathological aggregation of the ER and decreases of cholesterol in Golgi and plasma membrane. Therefore, σ_1 receptors on the ER may play a role in the compartmentalization of lipids into the ER lipid storage sites and in the export of lipids to peripheries of cells (Hayashi and Su, 2003a).

4.4. Behavioral effects of σ_1 receptor ligands

The behavioral role of σ_1 receptors in the central nervous system has been investigated extensively. The σ_1 receptors have been shown to play an important role in antidepressive effects since selective σ_1 receptor agonists, as well as typical antidepressants, reduced the immobility time in the forced swimming and tail suspension tests in rodents (Matsuno et al., 1996; Ukai et al., 1998; Urani et al., 2001). The reduction of immobility by σ_1 receptor agonists is antagonized by NE-100 or BD1047, σ_1 receptor antagonists or antisense oligodeoxynucleotide probes targeting the σ_1 receptor. In addition to the depressive animal model, phenytoin-sensitive σ_1-receptor agonists such as (+)-SKF-10,047 and dextromethorphan attenuate the conditioned fear stress (CFS) response, which is less influenced by typical anxiolytics and antidepressants, in rodents, the attenuating effects being mediated through phenytoin-sensitive σ_1 receptors, which are closely connected to the mesolimbic dopaminergic systems (Kamei et al., 1996). These findings suggest that σ receptors are involved in stress-induced pathophysiological changes such as depression and anxiety and that phenytoin-sensitive σ_1 receptor ligands are useful for the treatment of affective disorders, particularly those considered to be treatment-resistant.

Several σ_1 receptor agonists have been reported to possess anti-amnesic effects in rodents. In particular, the anti-amnesic effects induced by the novel σ_1 receptor agonists, such as (+)-pentazocine, SA4503 and PRE-084, were shown in β-amyloid peptide-induced, basal forebrain (BF)-lesioned and carbon monoxide (CO)-induced amnesia models and senescence-accelerated mouse (SAM) (for reviews, see Maurice et al., 1999, 2001). In addition, these σ_1 receptor agonists have good profiles for the central acetylcholine and dopamine systems. Moreover, they also have neuroprotective effects, which may lead to promising strategies for the treatment of dementia disorders such as Alzheimer's disease, senile dementia and vascular dementia (Maurice, 2002).

5. Biochemical and physiological evidences for a neurosteroid/σ_1 receptor interaction

5.1. Biochemical demonstration of the neurosteroid/σ_1 receptor interaction

The initial report describing a link between steroids and σ_1 receptors was provided by Su et al. (1988) who described in guinea pig brain and liver that PROG is a very active inhibitor of [^{3}H](+)SKF-10,047 binding to σ_1 receptors, with Ki values about 300 nM in membrane extracts. These Ki values are close to the physiological concentrations of the steroid during pregnancy or acute stress conditions. In addition, the variation of σ_1 binding site levels within the rodent brain, in the 100–600 fmol/mg of

tissue range, parallels that of PROG in the rodent brain (Walker et al., 1990; Monnet and Maurice, 2006). Scatchard analyses indicated that the steroid was acting in a competitive manner, thus suggesting that the steroid binding site on the σ_1 receptor was the synthetic ligand binding site. Testosterone, desoxycorticosterone and PREGS were almost equipotent, with Ki values within the micromolar range, while PREG, DHEA and estrogens (estriol, estrone and estradiol) appeared less efficient in these in vitro binding assays. The notion that the potent ligands for the PROG receptor, 11β-hydroxy-PROG, promegestone and RU-27987, failed to modify [^{3}H](+)SKF-10,047 binding to σ_1 sites underlined the specificity of the binding of PROG to the σ_1 binding site. Systemic administration of steroids dose-dependently inhibited the in vivo binding of [^{3}H](+)-SKF-10,047 to σ_1 sites (Maurice et al., 1996). PROG was also the most potent inhibitor, with a significant inhibition at the dose of 10 mg/kg. PREGS and DHEAS led to significant reduction of in vivo [^{3}H](+)-SKF-10,047 binding at 40 mg/kg. Interestingly, for these two steroids, the active doses in vivo appeared were clearly unrelated to their affinity, which questioned the relevance of the in vitro binding values. Binding levels of [^{3}H](+)-SKF-10,047 were significantly reduced in pregnant female mice as compared to non-pregnant ones or males (Maurice et al., 1996). Moreover, modulation of endogenous steroid levels affected in vivo [^{3}H](+)-SKF-10,047 binding parameters (Phan et al., 1999). Suppression of circulating steroids by adrenalectomy/castration (AdX/CX) enhanced [^{3}H](+)-SKF-10,047 binding. Finasteride, an inhibitor of the 5α-reductase enzyme involved in the conversion of PROG to 5α-pregnane-3,20-dione, was used to increase PROG levels (Jung-Testas et al., 1989a,b). Treatment of AdX/CX mice with finasteride led to a significant decrease of in vivo [^{3}H](+)-SKF-10,047 binding levels (Phan et al., 1999). Thus, neuroactive steroids directly interact with σ_1 sites, PROG acting as the most potent inhibitor. The concentration range might be expected to be reached in local physiological concentrations, suggesting a functional interaction with σ_1 receptors in the brain (Su et al., 1988).

At the same period, Wolfe et al. (1989) demonstrated a relationship in the immune and endocrine systems since high affinity binding sites for both σ receptor subtypes, i.e. labeled with [^{3}H](+)pentazocine for the σ_1 subtype or [^{3}H]DTG, [^{3}H]haloperidol for the σ_2 subtype, were found in lymphocytes and thymocytes (Wolfe et al., 1989; Carr et al., 1991). Hypophysectomy increased σ_1 binding in the adrenal gland and testis (Wolfe et al., 1989). In endocrine and immune systems, the rank order for steroid binding at the σ_1 receptor was PROG > 5α-dihydrotestosterone > testosterone > corticosterone > estradiol ≈ cholesterol. Interestingly, the hypothalamic–pituitary–adrenal (HPA) axis may possibly also be under the control of distinct σ receptor subtypes or distinct intracellular regulations triggered by the same σ receptor subtype. Indeed, several σ_1 receptor ligands dose-dependently and stereoselectively stimulated adrenocorticotropic hormone release in vivo (Iyengar et al., 1990, 1991). The σ_1 receptor-mediated modulation of the HPA axis has been demonstrated to be mimicked by steroids, since (+)SKF-10,047 and (+)pentazocine increased, as did PROG (after estrogen priming), testosterone and desoxycorticosterone, whereas (+)3-PPP and (−)butaclamol decreased prolactin release (Iyengar et al., 1991; Su, 1991). This was however not the case in vitro for corticotrophin releasing factor-evoked adrenocorticotropic hormone release from primary culture cells of the anterior pituitary (Iyengar et al., 1990, 1991).

Among peripheral tissues, liver presents the highest levels of σ binding sites, with a concentration of protein being appreciatively 120 times higher than that found in the brain (Samovilova et al., 1988; Hellewell and Bowen, 1990; McCann and Su, 1991; Yamada et al., 1994). The similar order of binding potency for σ ligands in inhibiting [^{3}H](+)SKF-10,047 binding suggests that central and hepatic σ sites might be identical (Walker et al., 1990; Ross, 1990; Klein et al., 1991). [^{3}H]PROG binds to solubilized σ receptor preparation from rat liver (McCann and Su, 1991). Moreover, Ross reported that the binding of [^{3}H]SKF-10,047, [^{3}H](+)3-PPP and [^{3}H]haloperidol was inhibited by PROG with apparent IC_{50} values in the 150–650 nM range in rat brain membranes.

Primary breast carcinomas have also been used to assess the putative inter-relationship between σ_1 receptor and human sterol isomerase. Simony-Lafontaine et al. (2000) have indeed found a close positive correlation between the σ_1 protein expression and the PROG receptor status but an inverse one between σ_1 receptor and human sterol isomerase in 95 patients using immunochemical analysis. Finally, Meyer et al. (1998) have described, in solubilized fractions of pig liver crude membrane preparations, that haloperidol, (+)3-PPP, DTG and rimcazole competed with PROG for [^{3}H]PROG binding with Ki values in the 20–500 nM range, while pentazocine, (+)SKF-10,047 and phenytoin exhibited low micromolar affinity. The most active steroids were PROG > corticosterone ≈ testosterone > cortisol >> 17β-estradiol. The only cytochrome P450 inhibitor active against the [^{3}H]PROG binding was SKF-525A with a Ki value of 140 nM, while methyrapone and cimetidine remained inactive. This unusual pharmacology profile did not fit with the typical order proposed for σ receptors, but rather corresponded to that described by Tsao and Su (1997) on a purified σ protein following chromatographic procedures using DAPE-containing column. The reported [^{3}H](+)SKF-10,047-sensitive σ protein had a molecular mass of 31 kDa, i.e. similar to the cloned σ_1 protein (Hanner et al., 1996), which was preferentially sensitive to dextrorotary benzomorphans and naloxone but not to DTG, (+)3-PPP and exhibited only high micromolar affinity for PROG. Therefore, in the liver and putatively other tissues, atypical σ proteins might exist with specific binding activity and specific physiological properties.

5.2. Physiological evidences for the neurosteroid/σ_1 receptor interaction

Selective σ_1 receptor ligands modulate NMDA receptor-mediated glutamate responses in the brain, as documented both in vitro on NMDA-induced [^{3}H]norepinephrine release from rat hippocampal slices, and in vivo from NMDA-induced neuronal firing of rat CA3 hippocampal neurons (Monnet et al., 1990, 1992; Debonnel, 1993). Although the mechanism of action remains to be fully understood, extensive pharmacological studies have

supported their close interrelationships. The selective σ ligands igmesine and DTG potentiated and inhibited, respectively, the NMDA response in a concentration-dependent manner, using the in vivo approach combining microiontophoresis and extracellular recordings of hippocampal pyramidal neurons (Monnet et al., 1992). Among butyrophenones showing close pharmacological profiles, haloperidol presents a high affinity for σ_1 sites, contrarily to spiperone. Only the first compound prevented the effects of igmesine and DTG. Among neurosteroids, DHEAS potentiated the [^{3}H]NE release induced by NMDA at nanomolar concentrations and in a concentration-dependent manner (Monnet et al., 1995). PREGS was also active, but inhibited the NMDA-induced release. Haloperidol and BD1063, but not spiperone, blocked both the potentiating effect of DHEAS and the inhibitory effect of PREGS (Monnet et al., 1995). The pre-treatment with pertussis toxin, injected in the dorsal hippocampus 3 to 11 days prior to sacrifice, totally abolished the effects of both DHEAS and PREGS, indicating that the neurosteroids action involved the σ_1 receptor subtype. Conversely to sulfated steroids, free DHEA or PREG, PROG and allopregnanolone did not affect NMDA-evoked [^{3}H]NE release in the 0.01–1 μM concentration range. However, PROG inhibited the potentiation of NMDA-evoked [^{3}H]NE release induced by DHEAS and the inhibition induced by PREGS or DTG. From this pioneer observation, PROG has been proposed to act as an endogenous σ_1 receptor antagonist (Monnet et al., 1995).

Bergeron et al. (1996) extended the initial observation of Monnet et al. (1995), using in vivo microiontophoretic application of NMDA and measures of the firing activity of hippocampal CA3 neurons. They showed that DHEA potentiated the NMDA-induced firing of pyramidal neurons in a PROG-, testosterone- and haloperidol-sensitive manner. PREG or PREGS were inactive in their experiments (Bergeron et al., 1996; Debonnel et al., 1996). They also investigated the electrophysiological responses of σ drugs in spayed, i.e. PROG-free, rats or during the menstrual cycle (Bergeron et al., 1996, 1999). Following ovariectomy, DTG potentiated the NMDA response in the hippocampal CA3 pyramidal layer more consistently. In pregnant rats or in animals treated during three weeks with PROG, the σ_1 agonists were significantly less effective on the NMDA response that in control animals. Furthermore, at day five postpartum the neuronal response to NMDA following σ_1 agonist administration was not only restored but also again enhanced (Bergeron et al., 1999), showing that σ_1 receptor was tonically inhibited by endogenous PROG (Bergeron et al., 1996, 1999). These in vivo data strengthened the initial statement that PROG was acting as a σ_1 antagonist.

A preliminary report indicated that σ_1 agonists, including DTG, (+)-pentazocine, and igmesine modulated LTP components, i.e. both the magnitude and slope of population spikes and field excitatory postsynaptic potentials, in the rat hippocampus (Monnet, 2002). The link with neurosteroids was reported very recently by Chen et al. (2006). They observed that chronically administered DHEAS significantly facilitated the induction of frequency-dependent LTP in rat hippocampal slices. Lower intensity tetanus was needed to induce LTP in DHEAS-treated animals. In contrast DHEA, had no facilitating effect on the induction of LTP. Chronically administered DHEAS did not alter the presynaptic glutamate release in response to both single pulse and tetanic stimulation, suggesting a postsynaptic mode of action. Co-administration of the σ_1 receptor antagonist haloperidol or NE100 with DHEAS completely inhibited the DHEAS-facilitated LTP. However, acute administration of the antagonists to the slices did not affect the induction of DHEAS-facilitated LTP, suggesting that the σ_1 receptor is a key target for the chronic actions of DHEAS but is not involved in the induction of DHEAS-facilitated LTP.

These data must be considered in line with other studies examining the electrophysiological effects of neurosteroids. On the one hand, Farb et al. (1992), using whole cell recordings from voltage-clamped spinal cord neurons, have observed no enhancing effect of DHEAS (at concentrations up to 10 μM) on the basal transmembrane potential, the spontaneous firing activity and no modulatory effect of DHEAS on the neuronal response to NMDA. On the other hand, Meyer et al. (1999) showed that DHEAS, in the 10 to 100 μM concentration range, weakly facilitated the activation of CA1 neurons in hippocampal slices after stimulation of the Schaffer collaterals. This enhancement was related however to a concomitant antagonistic activity of the neurosteroid on GABA-mediated inhibitory postsynaptic potentials as well as an augmentation of the glutamatergic excitatory postsynaptic potentials. Moreover, PREG and PREGS have no effect on spontaneous firing (Bergeron et al., 1996), but allosterically potentiated at micromolar concentrations NMDA-evoked currents in rat hippocampal neurons in culture (Wu et al., 1991; Farb et al., 1992; Irwin et al., 1992, 1994). More recently, Partridge and Valenzuela (2001) reported that PREGS, acting on both NMDA and AMPA ionotropic receptors, enhanced paired-pulse facilitation of EPSPs with an EC50 < 1 μM supporting the notion that the neurosteroid acts presynaptically to modulate neuronal excitability. Schiess and Partridge (2005) confirmed that PREGS acts through a Gi/o-coupled σ_1 receptor to enhance short-term presynaptic facilitation onto adult hippocampal CA1 neurons (Schiess and Partridge, 2005).

Therefore, DHEAS appears to potentiate NMDA responses, mainly through its agonist action at the σ_1 receptor, rather than through direct effects on NMDA receptors. PREGS exerts more complex effect on NMDA-induced neuronal activation: (i) a potent facilitation of NMDA-receptor activation, through a direct interaction on the NMDA receptor complex and (ii) an indirect σ_1 receptor-mediated modulation of the NMDA response, which in some experimental condition results in an inhibition of the NMDA response, PEGS acting as an inverse agonist. This last response may predominate under physiological conditions but, as argumented in Section 6.2, may be rapidly exceeded in pathological conditions.

Direct effects of σ_1 receptor agonists on GABAergic neurons have never been convincingly described. Neurosteroids interact directly with GABAergic receptors. Noteworthy, allopregnanolone is the most efficient positive modulator of $GABA_A$ receptors and devoid of affinity for σ_1 receptors. However, Mtchedlishvili and Kapur (2003) reported that low concentrations of PREGS inhibited presynaptic GABA release and the resulting miniature inhibitory presynaptic currents in hippocampal neurons cultures. This effect was abolished by the σ_1 receptor antagonist BD1063,

suggesting that the σ_1 receptor activation modulates GABA release and neurotransmission. This observation does not exclude that σ_1 receptor may also indirectly modulate $GABA_A$ receptors and deserves extensive studies using selective σ_1 ligands.

Finally, several studies demonstrated a σ_1 receptor-mediated neuromodulatory effect on potassium conductances. DTG, (+)3-PPP and haloperidol have been shown to facilitate hyperpolarisation with a reversal potential corresponding to that of K^+ or to block tonic outward K^+ currents in rat cortical synaptosomes, C6 glioma cells (Jeanjean et al., 1993) or NCB-20 cells (Morio et al., 1994). (+)pentazocine and (+)SKF-10,047 as well as DTG and haloperidol, but not DHEAS nor PROG, elicited a marked inhibition of K^+ currents in rat neurohypophysis (Wilke et al., 1999). This study suggested that σ_1 receptor ligands and neurosteroids may act at different molecular levels. Soriani et al. (1998, 1999) reported that σ_1 ligands affected K^+ conductance of IA, Ca^{2+}-activated K^+ currents and IM types in perforated patches of frog melanotropic cells. Using transfected *Xenopus* oocytes, Aydar et al. (2002) documented that σ_1 receptor, activated by (+)benzomorphans and inactivated by antisense probes, modulated voltage-gated K^+ channels (Kv1.4 and Kv1.5) depending on the presence or absence of ligands. They suggested that the σ_1 protein forms a stable complex with the K^+ channels serving as auxiliary subunits to voltage-gated K^+ channels with distinct functional interactions, depending on the presence or absence of ligand.

6. Does the neuroprotective activity of neurosteroids involves the σ_1 receptor?

Trophic and neuroprotective effects of neurosteroids have been described on the survival of brain and peripheral cells submitted in culture to toxic insults. The first model studied was the excitotoxicity induced by bath application of glutamate. However, recent studies also examined the neuroprotective ability of neurosteroids against oxidative stress and β-amyloid peptide toxicity, thus covering the toxic processes involved in many acute and chronic neurodegenerative pathologies (Choi et al., 1988; Meldrum and Garthwaite, 1990). In vivo studies have also been performed, extending the in vitro results to animal models of anoxia, ischemia or β-amyloid peptide central injection. The most promising candidates for a marked neuroprotective action are DHEAS and PROG, but some efficacy has also been observed with PREGS. Since numerous studies demonstrated robust neuroprotective properties for σ_1 receptor agonists in most of the above-mentioned toxicity models, the involvement of the σ_1 receptor in the neuroprotective abilities of steroids must be evoked.

6.1. Neuroprotection induced by DHEA/S

Mao and Barger (1998) reported that DHEAS, but not DHEA, protected hippocampal neurons against glutamate-induced toxicity. Interestingly, this protective action was correlated with the ability of DHEAS, but not DHEA, to increase the NFκB transcription factor expression, a mechanism previously shown to mediate neuroprotection (Mao and Barger, 1998). Kimonides et al. (1998) described that DHEA and, in a lesser extent, DHEAS protected hippocampal neuronal cultures from NMDA and glutamate toxicity. A protective effect of DHEAS was also reported on primary cultures of rat hippocampal neurons submitted to an oxidative stress induced by direct application of hydrogen peroxide or sodium nitroprusside (Bastianetto et al., 1999). Interestingly, the same authors reported that DHEA prevented the $H_2O_2/FeSO_4$-stimulated lipid peroxidation in human hippocampal slices from both control and Alzheimer's disease patient brains (Ramassamy et al., 1999). In a retinal pigment endothelial cell culture model, Bucolo et al. (2005) recently described that DHEAS and 17β-estradiol protected the cells against H_2O_2-induced apoptosis. These studies, demonstrating either a trophic activity of DHEAS by itself or a marked neuroprotective activity against in vitro toxicity, suggested that DHEAS levels in the brain have an important role on the viability of neurons in physiological or pathological conditions. In line with this observation, first, Brown et al. (2000) examined the ability of human glioma/glioblastoma cells to synthesize DHEA via an alternative pathway induced by treatment with Fe^{2+}. Oligodendrocytes and astrocytes make DHEA via this pathway, but neurons do not. In searching for a natural regulator of DHEA formation, they observed that treating oligodendrocytes with β-amyloid peptide increased DHEA formation in parallel with reactive oxygen species. These effects of the β-amyloid peptide were sensitive to vitamin E. Therefore, human brain makes steroids in a cell-specific manner and DHEA synthesis can be regulated by intracellular free radicals. The authors proposed that DHEA can potentially feedback onto the glial cells via an autocrine mechanism through glial neurotransmitter receptors or by effects on gene expression, and protect them against the toxic effects of β-amyloid peptide. Second, direct measurements of PREG/S, DHEA/S, PROG and allopregnanolone were performed in individual brain regions of Alzheimer's disease patients and aged non-demented controls, including the hippocampus, amygdala, frontal cortex, striatum, hypothalamus, and cerebellum (Weill-Engerer et al., 2002). A general trend towards decreased levels of all steroids was observed in brain regions of Alzheimer's disease patients compared to controls. PREGS levels were significantly lower in the striatum and cerebellum; DHEAS levels were significantly reduced in the hypothalamus, striatum and cerebellum; and PROG and allopregnanolone levels were markedly but non-significantly reduced in several brain structures, including the hypothalamus, striatum, frontal cortex, or amygdala. A significant negative correlation was found between the levels of cortical β-amyloid peptides and those of PREGS in the striatum and cerebellum and between the levels of phosphorylated tau proteins and DHEAS in the hypothalamus (Weill-Engerer et al., 2003). Since high levels of key proteins implicated in the formation of plaques and neurofibrillary tangles were correlated with decreased brain levels of PREGS and DHEAS, the authors confirmed the concept of a possible neuroprotective role of neurosteroids in Alzheimer's disease.

Several in vivo studies confirmed the potential neuroprotective activity of DHEA against hypoxic/ischemic insults. In

particular, we reported that a DHEA treatment protected mice from cell death in the hippocampal CA1 area and resulting learning and memory deficits induced by repetitive exposure to CO gas (Maurice et al., 2000).

The involvement of the σ_1 receptor in the neuroprotective effect of DHEA has been examined with contrasting results. The neuroprotective efficiency of σ_1 receptor agonists on in vitro or in vivo models of toxicity has been reviewed previously (Maurice and Lockhart, 1997; Maurice et al., 1999). Selective σ_1 receptor agonists present robust neuroprotective ability. In vitro, exposure of cultured rat brain neurons or retinal epithelial cells to selective σ receptor ligands protects cells against glutamate or NMDA exposure, hypoxic/hypoglycemic conditions, oxidative stress or β-amyloid peptide application (Long et al., 1992; Pauwels et al., 1992; De Loore et al., 1994; DeCoster et al., 1995; Klette et al., 1995; Lockhart et al., 1995; Nakazawa et al., 1998; Marrazzo et al., 2005; Bucolo et al., 2006). In vivo, σ_1 receptor agonists protected against ischemic damages in rodents (Lobner and Lipton, 1990; O'Neill et al., 1995, 1996). Different mechanisms could be evoked to describe the nature of the neuroprotective activity exerted by σ_1 receptor ligands, including inhibition of ischemia-induced glutamate release (Lobner and Lipton, 1990; Lockhart et al., 1995), attenuation of postsynaptic glutamate-evoked Ca^{2+} influx (DeCoster et al., 1995; Klette et al., 1995), depressed neuronal responsivity to NMDA receptor stimulation (Bhardwaj et al., 1998; Yamamoto et al., 1995), and reduced nitric oxide production (Bhardwaj et al., 1998; Goyagi et al., 2001). The effects of a pretreatment with a σ_1 receptor antagonist, such as NE-100 or BD1047, or a σ_1 receptor-directed antisense oligodeoxynucleotide before DHEA/S was performed in some studies to determine whether the neuroprotection induced by the steroid was mediated by the σ_1 receptor. Although some studies led to negative results, the DHEA-mediated neuroprotection against morphological and behavioral damages in CO-exposed mice appeared for instance insensitive to NE-100 (Maurice et al., 2000), other studies demonstrated a link between DHEA effects and σ_1 receptor activation. In particular, using the retinal epithelial cell culture model, Bucolo and Drago (2004) reported that the σ_1 receptor antagonist BD1047 blocked similarly the neuroprotective effect of PRE-084 and DHEAS, suggesting that most of the neuroprotective action of the steroid involved in this model its interaction with the σ_1 receptor. Interestingly, the neuroprotective effect of 17β-estradiol was also partly sensitive to BD1047, the drug blocking about 40% of the steroid's effect (Bucolo and Drago, 2004). Since 17β-estradiol has been shown to be devoid of affinity for the σ_1 receptor (Yamada et al., 1994), the steroid may either act as a precursor for a σ_1 receptor-active neuroprotective metabolites or induced its neuroprotective activity through a pathway involving σ_1 receptor activation and shared by σ_1 receptor agonists. This observation is of importance and suggests a particular importance for σ_1 receptor-mediated neuroprotection.

The σ_1 receptor-mediated neuroprotective action may involve a rather complex mechanism, due to the atypical mode of activation of the receptor. First, acute activation of the σ_1 receptor led to modulation of intracellular Ca^{2+} mobilization from the ER $InsP_3$ receptor-gated pools (Hayashi et al., 2000; Hayashi and Su, 2001). Imbalance of Ca^{2+} regulation is involved not only in the hyper-activation of glutamate receptor generating the excitotoxic cascade, but also in apoptosis when massive Ca^{2+} release from ER pools generates the production of cysteine proteases, critical mediators of programmed cell death, such as caspase-12 (Nakagawa et al., 2000). The most recent studies examining the σ_1 receptor activation demonstrated its efficacy in modulating the intensity of the ER pools Ca^{2+} mobilization. Whether the neuroprotective activity of σ_1 receptor ligands, and therefore DHEA/S, involves an effective regulation Ca^{2+} release through specific effects on the ER is a fascinating hypothesis that must examined in the future.

Second, chronic, or at least sustained, activation of the σ_1 receptor leads to its translocation, within lipid droplets, towards the plasma membranes where it modifies the composition of lipid rafts. These microdomains play a role in a variety of cellular functions including vesicle transport, receptor clustering and internalization, and coupling of receptor with proteins involved signal transduction (Simons and Ikonen, 1997). Since glycosylated moieties of gangliosides have been proposed to play a role in regulating, for instance, the localization of growth factor receptors in lipid rafts (Simons and Ikonen, 1997), chronic activation of σ_1 receptors may present substantial consequences in cell viability and differentiation, and therefore facilitate neuroprotection or neuronal recovery. Moreover, in the particular case of Alzheimer's disease, GM1 ganglioside-bound amyloid β protein (GM1/Aβ) has been suggested to be involved in the initiation of amyloid fibril formation in vivo by acting as a seed. Aβ recognizes a GM1 "cluster" in membranes, the formation of which is facilitated by cholesterol, and associated within lipid rafts on the plasma membrane (Kakio et al., 2001, 2002). Therefore, recomposition of lipid rafts after σ_1 receptor activation may be a mechanism by which selective ligands and DHEA/S induce their effective protection against Aβ toxicity. This hypothesis has also to be validated by extensive studies, but it may provide a new and original mechanism of action for neuroprotective drugs.

6.2. Neuroprotective/neurotoxic effects induced by PREG/S

Contrarily to DHEAS, and putatively due to its more direct interaction with the NMDA receptor, PREGS may rather facilitate the NMDA-induced toxicity. Several reports provided such evidence in rat hippocampal cultures after acute or chronic exposure (Weaver et al., 1998) or in the isolated retina (Guarneri et al., 1998a). NMDA receptor antagonists completely blocked of the PREGS-induced enhancement of NMDA receptor-mediated toxicity. The ability of PREGS to potentiate the NMDA-induced increase in intracellular Ca^{2+} concentration was clearly demonstrated on their model of cultured hippocampal neurons (Weaver et al., 1998) and, in the isolated retina model, the activation of NMDA receptors was shown to stimulate PREGS biosynthesis through a transneuronal mechanism implicating activation of the $GABA_A$ receptor (Guarneri et al., 1998b). On the contrary, blockade of PREGS synthesis by the $P450_{scc}$ inhibitor aminogluthetimine attenuated the acute retinal cell damage (Guarneri et al., 1998a). This early NMDA-

induced stimulation of the neurosteroid synthesis, together with its direct interaction with the NMDA receptor, may directly exacerbate the extent of acute excitotoxicity.

However, several reports showed some neuroprotective activity for PREG/S. First, controlled compressive injury to rat spinal cord was used to show some efficacy of a treatment combining indomethacin, an anti-inflammatory substance, bacterial lipopolysaccharide, a stimulator of cytokine secretion, and PREG (Guth et al., 1994). In this model, DHEAS alone or in combination and PREG alone were however ineffective. The anti-inflammatory effect of PREG, not attributable to the formation of corticosterone, was evoked to explain the steroid synergic action with indomethacin and lipopolysaccharide (Guth et al., 1994). Moreover, PREGS has been reported to attenuate the neurotoxicity in cortical slice cultures induced by bath application of α-amino-3-hydroxy-5-methyl-D-aspartate (AMPA) in a concentration dependent manner (Shirakawa et al., 2005). The effect was selective to the steroid and not shared by PREG or PREG hemisuccinate. Noteworthy, a similar effect was described for DHEA/S by Kimonides et al. (1998) in hippocampal cultures. Moreover, the neuroprotective effect of PREGS was not affected by cycloheximide, RU-486, picrotoxin or rimcazole, suggesting that it did not involve nuclear receptors, $GABA_A$ receptors or σ_1 receptor (Shirakawa et al., 2005). The authors suggested that the PREGS effect may result from a direct interaction with AMPA receptors, an hypothesis that remains to be examined.

In conclusion, PREG/S may not, on physiopathologically relevant excitotoxic models present a valuable neuroprotective activity, but rather acts as a worsening excitotoxic steroid. However that may be, the steroid neuroprotective/neurotoxic effect is unrelated to its σ_1 receptor agonist action.

6.3. Neuroprotection induced by PROG

PROG has also been reported to exert a sustained neuroprotective effect. Several toxicity models have been used. PROG was first shown to reduced kainic acid (KA)-induced seizure severity in rats and to reduce the susceptibility of hippocampal neurons to death from seizures (Hoffman et al., 2003). PROG was also tested in models of traumatic brain injury in rats and humans (Robertson et al., 2006). PROG reversed the early mitochondrial dysfunction observed in this model and preserved from hippocampal neuronal neurodegeneration in the CA1 area and in CA3 at the highest physiologic dose tested only. No effect was however observed against cortical tissue loss (Robertson et al., 2006).

The more extensive studies were conducted in a model of excitotoxic insult of the spinal cord. The steroid was, in particular, tested in the Wobbler mouse, a mutant presenting severe motoneuron degeneration and astrogliosis of the spinal cord (Gonzalez Deniselle et al., 2002). PROG pellet (20 mg) during 15 days produced substantial neuroprotection against the spinal cord motoneuron degeneration and astrogliosis. PROG also prevented the lesion-induced chromatolytic degeneration of rat spinal cord motoneurons as determined by Nissl staining (Gonzalez et al., 2004). In the normal, intact, spinal cord, PROG significantly increased brain-derived neurotrophic factor (BDNF) inmunoreactivity in ventral horn neurons, without changes in mRNA levels and the authors suggested that the PROG-induced enhancement of endogenous neuronal BDNF could provide a trophic environment within the lesioned spinal cord and might be part of the PROG activated pathways to provide neuroprotection. No link with its antagonist action at the σ_1 receptor has yet been evoked.

7. Conclusions

In the present article, we have reviewed the evidences demonstrating the role of σ_1 receptors as targets for the rapid non-genomic effects of several neuro(active)steroids. We detailed first the evidences showing that the σ_1 protein has a high level of structural homologies with the Δ7–Δ8 sterol isomerase, as initially proposed by Hanner et al. (1996) but provided a further analysis, suggesting that the σ_1 protein also presents homologies with the steroid binding site of two other steroidogenic enzymes, namely isopentenyl diphosphate isomerase and 17β-estradiol dehydrogenase. The biochemical and physiological arguments demonstrating that several steroids interact with the σ_1 receptor, shown using radioligand binding techniques, and act as σ_1 receptor ligands in physiological tests, either in vitro, using for instance the NMDA-induced [^{3}H]NE release from hippocampal slices or in vivo using the NMDA-evoked firing activity of rat hippocampal CA3 pyramidal neurons (Monnet et al., 1990, 1992). DHEA/S and, with a lower efficacy depending on the models, PREG/S act as σ_1 receptor agonists. The steroids may therefore directly modulate neuronal intracellular Ca^{2+} homeostasis and concur to the formation and recomposition of lipid microdomains through activation of the σ_1 receptor. These effects undoubtedly interfere, and putatively synergistically, with their direct effects on NMDA or $GABA_A$ receptors. PROG acts as an antagonist of the σ_1 receptor, and, therefore, the involvement of the σ_1 receptor in PROG effects may be sensible only when the physiopathological conditions result in over-expression or stimulation of the σ_1 receptor, such as in psychoses or drug addiction.

An important key issue in future studies will be to consider the σ_1 receptor involvement not only in the acute effects of steroids, but also in the long-term physiological effects of the steroids. Involvement of the σ_1 receptor in the cognitive effects of steroids, and particularly DHEA/S and PREG/S effects, has been reported and detailed in previous review articles (Maurice et al., 1999, 2001, 2002; Frye, 2001; Vallee et al., 2001; Schumacher et al., 2003; Barbaccia, 2004; Maurice, 2004; Monnet and Maurice, 2006). Conversely, the steroidal tonus has been shown to be a particularly important parameter determining the efficacy of pharmacological strategies involving selective σ_1 receptor ligands (Phan et al., 1999).

Analysis of the studies examining the neuroprotective potentials of steroids led to several interesting points. (i) The neuroprotective activity of DHEA/S seems to reliably involve its interaction with the σ_1 receptor, as observed in different toxicity models. Moreover, the recent examination of the retinal degeneration after ischemia in rats by Bucolo and Drago (2004) suggest that 17β-estradiol's effect also involves an activation of

the σ_1 receptor. (ii) PREG/S appears to exacerbate glutamate toxicity putatively through a direct facilitating effect on NMDA receptor, and to act as a neuroprotective steroid only selectively against AMPA toxicity, suggesting that the steroid effect on the σ_1 receptor is overtaken in pathological conditions. (iii) The neuroprotective effect of PROG has been shown to involved mainly regulation of the expression of trophic factors through classical stimulation of PROG receptors (Gonzalez et al., 2004).

Whether neuro(active)steroids constitute the endogenous ligands of the σ_1 receptor has still to be established unambiguously. However, increasing amount of studies demonstrated the importance of this atypical target in their non-genomic rapid effects. Among them, the two steroids most clearly interacting with the σ_1 receptor are DHEA/S, acting as an agonist and PROG, acting as an antagonist.

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