

Accepted Manuscript

Review

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PII: S0039-128X(16)30016-2

DOI: <http://dx.doi.org/10.1016/j.steroids.2016.04.005>

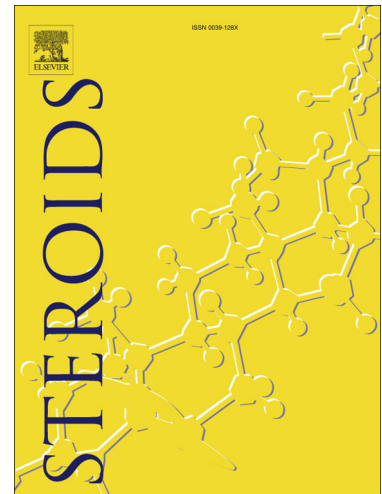
Reference: STE 7962

To appear in: *Steroids*

Received Date: 24 February 2016

Revised Date: 29 March 2016

Accepted Date: 11 April 2016



Please cite this article as: Coronel, M.F., Labombarda, F., González, S.L., Neuroactive steroids, nociception and neuropathic pain: a flashback to go forward, *Steroids* (2016), doi: <http://dx.doi.org/10.1016/j.steroids.2016.04.005>

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Neuroactive steroids, nociception and neuropathic pain: a flashback to go forward.

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Abstract

The present review discusses the potential role of neurosteroids / neuroactive steroids in the regulation of nociceptive and neuropathic pain, and recapitulates the current knowledge on the main mechanisms involved in the reduction of pain, especially those occurring at the dorsal horn of the spinal cord, a crucial site for nociceptive processing. We will make special focus on progesterone and its derivative allopregnanolone, which have been shown to exert remarkable actions in order to prevent or reverse the maladaptive changes and pain behaviors that arise after nervous system damage in various experimental neuropathic conditions.

Keywords

Neurosteroids; Progesterone; Allopregnanolone; Nociceptive Pain; Chronic Pain; Spinal Cord Dorsal Horn

Abbreviations

ALLO, Allopregnanolone; GABA_A, γ -aminobutyric acid type A; NMDA, N-methyl-D-aspartate; SP, substance P; CGPR, calcitonin gene-related peptide; KOR, kappa opioid receptor; DOR, delta opioid receptor; MOR, mu opioid receptor; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; DHT, 5 α -dihydrotestosterone; 3 α -diol, 5 α -androstane-3 α ,17 β -diol; THP, 3 α ,5 α -tetrahydroprogesterone; P0, myelin protein zero; PMP22, peripheral myelin protein 22; MAL, myelin and lymphocyte protein; PKC γ , gamma isoform of protein kinase C; iNOS, inducible isoform of nitric oxide synthase; COX-2, cyclooxygenase 2; IL, interleukin; TNF α , tumor necrosis factor α ; pNR1, phosphorylated form of NR1; PR, progesterone nuclear receptor; mPR, progesterone membrane receptor; PGRMC1, progesterone receptor-membrane component 1; PXR, pregnane xenobiotic receptor; StAR/STARD1, steroidogenic acute regulatory protein; TSPO, 18kDa translocator protein; PREG, pregnenolone; P450sc, P450 side-chain cleavage; 3 β -HSD, 3 β -hydroxysteroid dehydrogenase; 5 α -R, 5 α -reductase; 3 α -HSOR, 3 α -hydroxysteroid oxidoreductase; LXRs, nuclear liver X receptors.

1. Introduction

Almost 35 years have gone by since the pioneering works of Baulieu and colleagues showed that the rat nervous system is capable of synthesizing bioactive steroids [1,2]. This discovery gave birth to the term “neurosteroids”, e.g. steroids produced by the nervous system [3], while “neuroactive steroids” refers to steroids acting in the nervous system by modulating inhibitory or excitatory neurotransmitter receptors and neuronal excitability [4]. However, this notion has been extended to all steroids that directly act on neurons, either via membrane or nuclear signaling mechanisms [5], and includes those synthesized locally and in the peripheral glands (ovary, testis and adrenal glands) and also synthetic steroids [6].

Among these molecules, progesterone and its derivative allopregnanolone (ALLO) exert a wide range of fascinating actions in the central nervous system. In fact, an impressive number of pharmacological and behavioral studies have demonstrated that these neurosteroids /neuroactive steroids are involved in the control of several and diverse biological mechanisms such as cognition, stress, anxiety, depression, neuroprotection and myelination, which have been extensively reviewed elsewhere [6-12]. Furthermore, an important area of research has been devoted to explore the role of these steroids and related molecules in the modulation of both nociceptive [13-16] and neuropathic [17-30] pain, providing clear evidences towards the development of steroid-based therapies to counteract chronic pain conditions.

The present review discusses the potential role of neuroactive steroids in the regulation of nociceptive and neuropathic pain, and recapitulates the current knowledge on the main mechanisms involved, making reference mainly to those occurring at the spinal cord, a crucial site for nociceptive processing. We will make special focus on progesterone and ALLO, which have been shown to exert remarkable actions tending to prevent or reverse the maladaptive changes and pain behaviors that arise after nervous system damage in various experimental neuropathic conditions.

2. Steroids and nociceptive pain

Nociceptive pain represents a pivotal defensive mechanism intended to warn an individual of recent, ongoing or imminent damage to the body. In this context, the ability to detect noxious stimuli is essential to an organism’s survival and wellbeing [31,32]. Mechanical, thermal and chemical noxious stimuli are detected by a subpopulation of primary afferent neurons called nociceptors [31,33]. Nociceptors have cell bodies located in dorsal root ganglia, a peripheral axonal branch that innervates tissues and organs, and a central branch that synapse on second order neurons, and local interneurons, within the dorsal horn of the spinal cord [31,33] (Fig 1). Some second order neurons act as projection neurons and convey nociceptive information to the somatosensory cortex, via several brainstem and thalamic nuclei [31,34]. Supraspinal control of nociceptive signaling is relayed through the midbrain periaqueductal gray matter, the serotonergic raphe nucleus, the noradrenergic locus coeruleus and the rostral ventromedial medulla, that directly or indirectly project to the dorsal horn [35,36]

Therefore, nociception can be regulated at different levels of the nervous system through facilitating (pronociceptive) or inhibitory (antinociceptive) actions. Particularly, the dorsal horn of the spinal cord stands as a critical site for nociceptive modulation, receiving both information about painful stimuli from the periphery as well as descending feedback from supraspinal centers, which ultimately regulates (with inhibition or further facilitation) the output from the spinal cord [31].

Steroids play pivotal physiological roles in the nervous system, including the modulation of pain sensitivity [8,37,38]. Steroids are able to exert their effects by binding to intracellular/nuclear receptors thus influencing gene transcription and signaling pathways [39,40], or by rapidly modulating membrane excitability and synaptic transmission through their interaction with ionotropic neurotransmitter receptors, such as γ -aminobutyric acid type A (GABA_A) and N-methyl-D-aspartate (NMDA) receptors, or voltage-dependent Ca²⁺ or K⁺ channels [39-41]. Moreover, several steroids can also influence second-messenger pathways by directly interacting with specific membrane receptors [40,42].

Specifically, the spinal cord is not only a target for circulating steroids, but also an active steroid-producing site, that contains an array of regulatory proteins and enzymes involved in neurosteroid production and metabolism [37,38]. Accordingly, the impact of circulating steroids and neurosteroids on nociceptive processing at the spinal level has been largely studied, mainly in the context of stress response [9,43], pregnancy [44-46], estrous cycle [47,48] and sex-dimorphic responses to both noxious stimuli and opioid administration [49-51].

The impact of the stress response on nociception is multifaceted, and involves a large number of neurotransmitters and neuropeptides [52]. Acute stress is most commonly associated with the induction of analgesia, whereas chronic stress has been more frequently linked with the onset of hyperalgesia [52]. In this context, changes in circulating levels of glucocorticoids, occurring as part of the stress response, may impact on nociceptive processing at spinal level [43,53].

One recent study has shown that high levels of plasma corticosterone produce analgesia, by its conversion into its neuroactive 3 α ,5 α -reduced metabolite in the spinal cord, which enhances GABAergic spinal inhibitory transmission and decreases nociceptive inputs [43]. Further, chronic glucocorticoid administration can also modulate the spinal expression of neuropeptides i.e. substance P (SP) and calcitonin gene-related peptide (CGRP), involved in nociceptive transmission [54], confirming that these steroids regulate physiological pain processing at the dorsal horn level.

Less is known about the participation of aldosterone in nociceptive modulation. Various preclinical and clinical studies have documented the role of the renin–angiotensin–aldosterone system in pain processing [55] and the consequences of experimental activation of the mineralocorticoid receptor, which increases the severity of inflammation [56], a key component in pain generation. However, the participation of the mineralocorticoid receptor in nociception and pain conditions deserves further investigation.

Regarding the role of ovarian steroids, data arising from experimental models of pregnancy [44-46] found that, in late pregnancy, progesterone and estradiol recruit spinal anti-nociceptive systems involving dynorphin and both kappa and delta opioid receptors (KOR and DOR, respectively), a process associated with significant elevations in nociceptive response thresholds [57]. In particular, progesterone is crucial in mediating maternal antinociception and contributing to sex-based differences in pain sensation [49].

In fact, sexual dimorphism in nociception has been extensively documented in human and experimental animals [50,51,58]. Although several factors are involved in these complex differences, the fact that estrogens and progesterone have a multitude of effects on opioid systems [59-61], strongly point to these steroids as foremost determinants of sex-dependent nociception and sex-dimorphic opioid antinociception.

The spinal cord has been shown to be a central nervous system region in which the regulation of opioid antinociception displays a clear sexual dimorphism. In experimental conditions, the conformation of spinal mu- and kappa-opioid receptor (MOR/KOR) heterodimers, which results antinociceptive, is strikingly sexually dimorphic, being higher in spinal cord of females vs. males and also varying between stages of the estrus cycle, which suggests its regulation by estrogens and/or progesterone [60]. Further, endomorphin 2, the predominant endogenous MOR ligand in the spinal cord, not only mediates analgesia depending on the stage of the estrus cycle but also exhibits a sexual dimorphism in the mechanisms utilized to elicit antinociception [61-63]. Fluctuations in estrogen and progesterone in different phases of the menstrual cycle also affect pain perception [64] and the nociceptive thresholds differ across the estrous cycle of the rat as well [47,48,65]. However, inconsistent and paradoxical effects have been described, showing that these hormones generate both antinociceptive and pronociceptive actions [51,66]. Thus, the accurate role of these hormones and the underlying mechanisms mediating these events remain elusive and under permanent investigation [for a recent review see [67]].

In addition to these controversial results, the role of androgens in pain perception cannot be ignored [50,68]. In both sexes, androgens are primarily synthesized in the gonads but also by the reticular portion of the adrenal gland as dehydroepiandrosterone (DHEA). In addition, DHEA synthesis also occurs in neural networks of the spinal cord dorsal horn [37,69]. DHEA, its sulfate derivative (DHEAS) and testosterone, one of the major androgens deriving from DHEA, have been shown to impact spinal nociceptive transmission [69,70]. DHEA acute treatment induces a rapid pronociceptive and a delayed antinociceptive action in naive animals and also in pain conditions, whereas spinal intrathecal administration of testosterone induces a significant analgesic effect by increasing the nociceptive thresholds [69]. The biphasic effect observed after DHEA treatment suggests that DHEA itself is capable of decreasing both thermal and mechanical thresholds, while its androgenic metabolites may exert analgesic actions [69]. Recently, the individual differences in testosterone levels revealed that this steroid may also be related to antinociception and protective effects against noxious stimuli in healthy women [71].

In males, testosterone is also converted into its 5 α -reduced metabolites dihydrotestosterone (DHT) and 5 α -androstane-3 α ,17 β -diol (3 α -diol). These two testosterone metabolites have been reported to induce analgesia, in gonadectomized rats, when evaluated using the tail flick and the paw lick tests [72], as well as during different pain conditions [37,69]. Moreover, testosterone can be converted to estradiol by the enzyme aromatase, implying that the specific effects of testosterone are complex to be evaluated. Aromatization of testosterone into estrogen reduces the response to a noxious stimulus in the male quail [73,74] mediated at the spinal cord level by the interaction of estradiol with estrogen receptors and/or by fast nongenomic actions [74].

The use of positive allosteric modulators of inhibitory amino acid receptor channels, such as GABA_A receptors, appears to be particularly important in pain modulation. Specifically, GABA_A receptors located in spinal neurons represent an interesting target to limit pain expression. Endogenous progesterone-derived neurosteroids and synthetic analogs of 3 α ,5 α -reduced derivatives of progesterone, acting as potent allosteric modulators of GABA_A receptors and/or T-type calcium channels, are particularly efficient to elevate nociceptive thresholds in naive animals [13-15,75,76]. In addition, neurosteroids can also modulate GABA_A receptor subunit expression and reorganization [77].

Altogether, these findings point to the crucial actions of neurosteroids /neuroactive steroids on spinal nociceptive networks, and propose their use as agents able to modulate pain messages.

3. Neuropathic pain: definition, etiology and mechanisms

From an evolutionary point of view, nociceptive pain can be considered as a necessary evil since it provides a potent warning system that protects an individual from either actual or potential tissue damage [31]. However, not every type of pain is part of this adaptive response. Neuropathic pain, is a chronic pain that may arise after an injury or disease affecting the somatosensory system [78] and is considered a maladaptive response of the nervous system to damage [79,80].

Neuropathic pain classical etiologies include diabetic polyneuropathies, postherpetic neuralgia, trigeminal neuralgia, painful radiculopathies, central post-stroke pain and spinal cord injury pain, although traumatic, postsurgical and chemotherapy-associated neuropathies also represent common conditions [81,82]. Due to this diversity, neuropathic pain afflicts as much as 7-10% of the general population, worldwide. Despite the fact that diseases causing neuropathic pain vary substantially, patients present common clinical characteristics, with the presence of both negative and positive symptoms which respectively represent loss and gain of function of the somatosensory pathway. Negative phenomena arise as sensory loss, while positive phenomena include both ongoing (also called spontaneous) and evoked pain [81]. Two prominent neuropathic pain-associated symptoms are allodynia, pain induced by normally innocuous stimuli, and hyperalgesia, increased pain response elicited by noxious stimuli [81]. Pain with neuropathic characteristics is severe and constitutes a very important physical and psychological

burden. Furthermore, chronic pain generates an elevated and sustained economical cost for the individuals, their families and their communities [83]. In fact, pain represents the greatest economic burden of any pathological condition in the USA, with an estimated annual cost of \$600 billion. Unfortunately, the treatment of neuropathic pain is still unsatisfactory, with a substantial number of patients having decreased quality of life with little or no benefit from available treatments [84].

It is now clear that injury or disease of the somatosensory system results in maladaptive plasticity that profoundly alters signaling at different levels along the pain pathway, from the peripheral nerves to the cortex [32]. Maladaptive changes can affect either the peripheral or the central nervous system and lead to increased excitability of the neuronal pain processing circuitry, generating peripheral or central sensitization, which are symptomatically expressed as allodynia and hyperalgesia [31]. This plasticity involves multiple and diverse mechanisms such as: a) the ectopic activity of primary afferents, mainly related to altered trafficking of receptor ion channels; b) changes in the phenotype of sensory neurons, affecting different molecules and in particular neuropeptides such as SP and CGRP; c) the imbalance of excitatory and inhibitory neurotransmission at the dorsal horn level, mainly involving increased NMDA receptor-mediated currents and decreased GABA_A receptor activity; d) an exacerbated neuroimmune reaction, with glia activation and the production of pro-inflammatory mediators; and e) increased descending facilitation from the brainstem and supraspinal centers [32,79,80,85]. As a consequence of these different and often coexisting pathophysiological mechanisms, this type of pain commonly results refractory to conventional treatments [84].

4. Progesterone and allopregnanolone in neuropathic pain conditions

It has recently become clear that the treatment of neuropathic pain needs to move from merely suppressing symptoms to a disease-modifying strategy aimed at preventing the different events leading to maladaptive plasticity, described in the previous section. As extensively demonstrated, steroids control several aspects of the development, activity and plasticity of the nervous system [6,8,12] and thus appear as interesting candidates for these purposes.

Among steroidal compounds, synthetic glucocorticoids (i.e. dexamethasone, methylprednisolone) have been effectively used to reduce certain kinds of pain when given in large systemic doses, mainly through the inhibition of pro-inflammatory cytokines or the upregulation of lipocortin (annexin-1), an anti-inflammatory molecule that blocks the production of eicosanoids [86]. However, corticosteroids exert paradoxical effects during chronic pain [9,87] and may exacerbate neuropathic pain conditions [88-90].

Glucocorticoid receptors are up-regulated in the spinal dorsal horn during experimental neuropathic conditions and contribute to hyperalgesia [91]. Moreover, stress exacerbates neuropathic pain via glucocorticoid and NMDA receptor activation [90] and spinal glucocorticoid receptors have been shown to mediate morphine tolerance, a pharmacological phenomenon that hampers the clinical use of opioids [92]. These data

raise concern since glucocorticoid-mediated anti-inflammatory therapies, which are often used after nervous system injury, may produce maladaptive plasticity contributing to the onset and maintenance of neuropathic pain [88,89].

It is now a well-consolidated concept that a certain group of steroids, such as progesterone and its reduced derivatives, 5 α -dihydroprogesterone (DHP) and 3 α ,5 α -tetrahydroprogesterone (THP), also known as allopregnanolone (ALLO), are neuroprotective agents in the central and peripheral nervous system [6,8,12,93]. Progesterone is synthesized by ovaries and placenta in females, and by adrenal glands in both males and females. Progesterone is also locally synthesized by the nervous system which has the capacity to convert progesterone into its neuroactive metabolite ALLO as well. These molecules have been shown to exert beneficial and neuroprotective effects in experimental models of Alzheimer's disease, Parkinson's disease, multiple sclerosis and traumatic brain and spinal cord injury [6,94-97]. Recently, it has been found that these steroids also reduce pain-related behaviors in several neuropathic pain models (i.e. peripheral nerve injury, diabetes, spinal cord injury) [17-23,29,30] (Table 1).

In the peripheral nervous system, progesterone and its metabolites are able to restore biochemical, morphological and functional parameters after physical injury of peripheral nerves [98,99]. In animals subjected to a sciatic nerve crush, progesterone and/or DHP administration counteracts the injury-induced decrease in the expression of myelin proteins such as myelin protein zero (P0), peripheral myelin protein 22 (PMP22) and myelin and lymphocyte protein (MAL), as well as the injury-induced increase in the activity of Na⁺K⁺ ATPase pump [98]. In addition, the steroids are able to restore the impairments in nociceptive thresholds detected in vehicle treated injured animals.

Using an animal model of diabetic neuropathy, Leonelli and colleagues showed that progesterone, DHP and ALLO improve sciatic nerve conduction velocity, thermal nociceptive threshold, skin innervation density, P0 and PMP22 expression levels and Na⁺K⁺ ATPase activity [100]. Furthermore, Afrazi and colleagues recently reported that chronic treatment with ALLO prevents the diabetes-induced spinal down-regulation of γ 2 subunit of GABA_A receptor and counteracts thermal hyperalgesia, as well as motor impairment [29].

In addition, our laboratory has recently shown that progesterone treatment prevents mechanical allodynia and significantly reduces the number of painful responses to cold stimulation in male animals subjected to a sciatic nerve chronic constriction injury [18]. In correlation with the observed attenuation of pain behaviors, progesterone administration prevents the injury-induced increase in the expression of the NR1 subunit of NMDA receptor and the gamma isoform of the enzyme protein kinase C (PKC γ), both key players for chronic pain generation [18]. A report from Dableh and colleagues further supported progesterone antiallodynic effects after peripheral nerve injury, showing that the steroid prevents neuropathic pain-associated behaviors only if the treatment is started early and maintained for a critical period of time [20]. Interestingly, after nerve root ligation progesterone-dependent regulation of the growth factor neuregulin-1 in the spinal cord of

female rats was shown to mediate sex differences in the maintenance phase of central sensitization [101]. Therefore, additional research designed to elucidate the mechanisms driving sex differences after progesterone administration in pain conditions is needed.

ALLO has also been shown to reduce mechanical and thermal hyperalgesia after sciatic nerve ligation [21], probably through its blockage of neuronal low-voltage activated (T-type) Ca^{2+} channels and the potentiation of GABA_A ligand-gated channels.

Furthermore, progesterone and its derivatives DHP and ALLO suppress neuropathic symptoms evoked by antineoplastic drugs, such as vincristine [22] or oxaliplatin [23] in animal models of chemotherapy-induced painful neuropathy. The cited neurosteroids both prevent and revert mechanical allodynia and hyperalgesia, when they are administered either before or after the establishment of pain behaviors. Steroid treatment also counteracts vincristine-induced decrease in the number of intra-epidermal nerve fibers and nerve conduction velocity [22].

Early progesterone administration also prevents mechanical and thermal allodynia after spinal cord injury [17,19]. Notably, these anti-allodynic effects are maintained even after the treatment has stopped, supporting the relevance of targeting the key components of the central injury cascade occurring early after a spinal cord lesion [17]. In this regard, we have shown that progesterone administration is able to attenuate the injury-induced increase in the number of immunoreactive astrocytes and microglial cells and in the expression of pro-inflammatory mediators: enzymes, such as the inducible isoform of nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2), and cytokines, such as the interleukins (IL) IL-1 β , IL-6 and tumor necrosis factor α (TNF α), probably by decreasing the transactivation potential of the transcription factor NF κ B [17,30]. In addition, animals receiving progesterone show lower levels of expression of NR1, NR2A and NR2B subunits of the NMDA receptor, a key player in the process of central sensitization, when compared with vehicle-treated animals [19]. Progesterone administration also results in lower expression of PKC γ , increased KOR mRNA levels and reduced number of neuronal profiles exhibiting immunoreactivity for the phosphorylated (activated) form of NR1 (pNR1) in the chronic phase after injury [19]. NR1 phosphorylation, a post-translational modification that is essential to enhance NMDA receptor activity, results in increased neuronal responsiveness and pain [102]. Thus the steroid, by preventing the maladaptive changes in the expression and/or activity of several pain related molecules is able to prevent allodynia after spinal cord injury.

Overall, the molecular changes taking place after progesterone, DHP or ALLO administration to different animal models of neuropathic pain suggest that, by preventing maladaptive plasticity, these steroids could be favoring a molecular scenario that prevents the onset of neuropathic pain. As shown here, steroids stand as excellent candidates since they are able to block multiple cellular and molecular events leading to damage at the different control levels. Therefore, these steroids might provide a valuable tool for alleviating chronic pain in the clinical setting.

Progesterone in particular has several features that make it an attractive potential drug for the treatment of injuries or diseases of the nervous system leading to chronic pain. Due to the wide experience derived from its administration in hormone replacement therapy, its pharmacokinetic and pharmacodynamic properties are well known. In addition, progesterone can be administered systemically since it is able to cross the blood-brain/spinal barrier; it is widely available and has low cost and limited adverse effects. In fact, progesterone administration has already been shown to be safe and well tolerated in patients with traumatic brain injury [103,104]. In relation to ALLO, data showing safety and tolerability in patients is still needed. In fact, a phase II clinical trial designed to test safety and effectiveness of ALLO in patients with traumatic brain injury is now recruiting patients (<https://clinicaltrials.gov/ct2/show/NCT01673828%3Fterm=traumatic+brain+injury+and+all+opregnanolone&rank=1>).

In addition, the effects of a single, local administration of 17- α -hydroxyprogesterone caproate have been evaluated in patients with mild carpal tunnel syndrome, and compared with those observed after local treatment with corticosteroid [105]. Patients receiving the long-acting synthetic progesterone derivative showed a reduction in pain scores, similar to that observed after corticosteroid treatment, one month after injection, whereas only patients receiving 17- α -hydroxyprogesterone caproate still manifested symptoms of relief six months after treatment [105].

5. Potential mechanisms underlying progesterone and allopregnanolone actions towards pain control

As shown in the previous section, after a lesion or disease of the nervous system both progesterone and ALLO exert concerted beneficial influences on multiple processes, avoiding maladaptive changes and pain behaviors. Those pleiotropic effects are mediated via regulation of gene transcription, intracellular signaling pathways and neurotransmission [40].

In the spinal cord, an array of progesterone receptors are expressed in both neurons and glial cells [40,42]. These include: a) the classic nuclear receptor (PR) [106], that may be targeted to the nucleus and act as a ligand-activated transcription factor regulating the expression of target genes or may be delivered to the cell membrane where it interacts with Src/Ras/MAPK signaling pathway; b) progesterone membrane receptors (mPR) [107], which contain seven integral transmembrane domains and mediate signaling via an inhibitory G-protein coupled pathway and stimulate MAPK pathway; and c) the membrane-associated protein progesterone receptor-membrane component 1 (PGRMC1) [108], a progesterone binding site that has several potential signaling mechanisms, including Jak/STAT and Src pathways and the activation of protein kinase G (Fig 2). Thus, similarly to what has been observed in other nervous system structures, the spinal cord offers multiple sites for progesterone actions [8,40,42].

In addition, progesterone also inhibits the activity of the nicotinic acetylcholine receptor [109] and acts as an competitive antagonist of sigma-1 receptor binding site [110]. The activation of sigma-1 receptor, which is strongly expressed in the dorsal spinal cord [111]

and associated with central sensitization and pain [112], enhances NMDA receptor mediated currents via PKC-dependent phosphorylation of the NR1 subunit [113]. Therefore, progesterone, acting as competitive antagonist of sigma-1 [110,114], could be reducing pain behavior through this mechanism, among others.

As mentioned, gene expression regulation may be mediated through progesterone or DHP binding to the intracellular classical receptors PR-A and PR-B [40,42]. Thus, target genes exhibiting progesterone response elements in their promoters allow a direct control of their expression by PR. However, it still remains unclear whether these response elements are present in the promoters of the molecules previously mentioned to be modulated by progesterone in different animal models of pain. Nevertheless, since PR can cross-talk with members of the AP-1, NF κ B and Sp families of transcription factors or interact with the Src/Ras/MAPK and the cAMP signaling pathways [40], regulation of target genes could be achieved even in the absence of steroid-response elements. In fact, progesterone regulation of pro-inflammatory cytokines and enzymes could be mediated through PR modulation of NF κ B transactivation potential [17,115]. The steroid is a well-known regulator of NF κ B activity [116], through its ability to antagonize NF κ B by either a direct PR-mediated interaction with the transcription factor [117], or by the modulation of the expression and activity of NF κ B and its inhibitory protein I κ B α [118]. Since NF κ B regulates glia activation and the production of a wide array of pro-inflammatory mediators, these mechanisms could explain progesterone anti-inflammatory actions in different animal models of pain [17].

In contrast to progesterone and DHP, ALLO does not bind to PR and acts by modulating neurotransmitter receptors. In fact, at least part of progesterone's anti-allodynic and anxiolytic effects could be due to its rapid conversion to ALLO [119], metabolite that reinforces inhibitory neurotransmission by acting as a positive allosteric modulator of GABA_A receptor [120] and by enhancing specific GABA_A receptor subunits [121]. In fact, ALLO is one of the most potent endogenous positive allosteric modulators of GABA_A receptor function.

To further complicate this scenario, the pregnane xenobiotic receptor (PXR) has been shown to act not only as a novel target of ALLO in the brain, for functional, reproductively-relevant responses, but also in the biosynthesis of ALLO (i.e., acting upstream of TSPO, translocator protein involved in the first step of steroidogenesis) [122]. However, whether these mechanisms are involved in experimental neuropathic conditions remains to be explored.

Since progesterone and ALLO appear as novel and promising molecules in pain modulation, with several neuroprotective properties and exerting anti-allodynic and anti-hyperalgesic actions, further studies contributing to unravel their mechanisms of action in painful conditions should be carried out.

6. Spinal cord neurosteroidogenesis

It is now well-established that steroids can be synthesized both in the peripheral and the central nervous system either *de novo* from cholesterol or from circulating steroid hormones, that easily cross the blood-brain / spinal barrier and serve as precursors for neurosteroidogenic enzymes. Both neurons and glial cells (astrocytes, oligodendrocytes, Schwann cells and ganglia satellite cells) participate in neuroactive steroid biosynthesis and metabolism [12,37,123-126]. Neurosteroids include a broad group of molecules (androgens, estrogens, progestagens and their derivatives); in this section we will focus on the enzymatic pathways leading to the synthesis of progesterone and ALLO at the spinal cord level.

In the spinal cord, the presence and the biological activity of several key steroidogenic enzymes have been demonstrated [12,37,38,119,127-129]. As well as in other steroidogenic tissues, biosynthesis of steroid hormones in the spinal cord begins with the translocation of cholesterol from intracellular stores to the inner mitochondrial membrane. This transport, considered the rate limiting step for neurosteroidogenesis, is hormonally controlled and mediated by the steroidogenic acute regulatory protein (StAR/STARD1) [130-132] and the 18kDa translocator protein (TSPO), previously known as peripheral benzodiazepine receptor [133] (Fig 3). Cholesterol is then converted into pregnenolone (PREG), in a reaction catalyzed by the enzyme P450 side-chain cleavage (P450scc) located on the matrix side of the inner mitochondrial membrane. The enzyme 3 β -hydroxysteroid dehydrogenase (3 β -HSD) converts pregnenolone into progesterone, which is further reduced into 5 α -DHP by the action of 5 α -reductase (5 α -R). Finally, 3 α -hydroxysteroid oxidoreductase (3 α -HSOR) is responsible for the reduction of DHP into ALLO (3 α ,5 α -THP) [12,37,93].

Notably, several neurosteroids, in particular pregnenolone and progesterone are measurable in the rat spinal cord even in adrenalectomized and gonadectomized animals, further supporting their local biosynthesis [119].

7. Promoting neurosteroidogenesis as a therapeutic strategy

The local production of neurosteroids during several pain conditions has been extensively studied in the spinal cord [37,69,134,135], dorsal root ganglia [125,126,136] and peripheral nerves [98,100,137]. As previously mentioned, we will make special focus on the spinal cord dorsal horn, an active steroid-producing center that contains various key steroidogenic enzymes and where local steroid secretion is regulated in different chronic pain conditions [37,38].

In the spinal cord of animals subjected to a sciatic nerve ligature, there is a threefold increase in the mRNA levels of the enzyme P450scc [138], and this up-regulation correlates with a significant increase in the density of P450scc-positive fibers in the ipsilateral dorsal horn of neuropathic animals [127]. By using reversed-phase HPLC analysis coupled with flow scintillation detection to evaluate the conversion of

[³H]cholesterol into [³H]PREG by tissue homogenates from the lumbar dorsal horn, Patten-Mensah and colleagues showed that the newly synthesized [³H]PREG is 80% higher in the spinal cord of neuropathic animals [127,138]. In addition, there is an increase in the endogenous concentrations of PREG and ALLO in the dorsal horn of animals with neuropathic pain provoked by peripheral nerve injury, while their plasma levels do not change [138]. In correlation with these findings, the mRNA levels and biological activity of 3 α -HSOR, the enzyme involved in the biosynthesis of ALLO also increase in the spinal cord of animals subjected to a chronic constriction injury, and contribute to reduce thermal hyperalgesia and mechanical allodynia [27]. In the spinal cord of streptozotocin-induced diabetic rats the gene encoding for 3 β -HSD shows a ninefold increase and the amount of [³H]progesterone newly synthesized is 200% higher than in control animals [139].

Thus, in different painful states, there is an up-regulation of the biosynthetic pathways leading to progesterone and ALLO production, probably as an endogenous mechanism triggered to cope with the chronic pain condition [37,38]. In line with this concept, the local production of DHEA, a steroid with pro-nociceptive actions, is reduced in the spinal cord of neuropathic animals, due to the down-regulation of the enzyme involved in its biosynthesis [69]. Thus, during neuropathic pain conditions, neurosteroid biosynthesis appears to be selectively regulated in the spinal cord sensory networks, tending to increase the local production of antinociceptive steroids such as progesterone and ALLO, and to reduce the formation of pronociceptive neurosteroids such as DHEA.

Based on these findings, an attractive area of research has recently been dedicated to promote endogenous neurosteroidogenesis in the nervous system, as a potential therapeutic strategy with the intrinsic advantage of limiting the typical endocrine side effects of long-term systemic administered steroids [11,140]. In fact, the induction of neuroactive steroids synthesis has been recently evaluated both in the peripheral and central nervous systems [25,26,141-144].

The production of neurosteroids may be controlled by the endogenous activation of the translocator protein of 18 kDa (TSPO), localized predominantly in the outer mitochondrial membrane, and involved in the first step of neurosteroidogenesis by stimulating the translocation of cholesterol across the inner mitochondrial membrane [145]. With this perspective, the administration of TSPO ligands, such as Ro5-4864 or etifoxine, has been used as promising options to control pain conditions [26,143,144] (Table 2). In fact, the use of TSPO ligands increases the levels of endogenous PREG and progesterone in the sciatic nerve of diabetic animals, counteracting the impairments in thermal thresholds and nerve conduction velocity arising during diabetic neuropathy [142], reduces astrocyte activation and decreases established mechanical allodynia and thermal hyperalgesia after spinal nerve ligation [26] and exerts beneficial effects modulating vincristine-induced neuropathic pain [143].

Indeed, additional pharmacological strategies aimed to stimulate the local production of neurosteroids are now being developed [25,140,141]. The activation of the nuclear liver X receptors (LXRs) with the synthetic ligand GW3965, results in increased levels of the

neuroactive steroids PREG, progesterone, DHP and/or ALLO in the sciatic nerve, the spinal cord, cerebellum and cerebral cortex of diabetic rats, in association with neuroprotective effects on thermal nociceptive activity, nerve conduction velocity, Na,K-ATPase activity and the expression of myelin proteins both in peripheral nerves and the spinal cord [25,141].

LXRs are ligand activating transcription factors that belong to the nuclear receptor superfamily and serve as cholesterol sensors, preventing its excessive intracellular accumulation, by their ability to regulate the transcription of genes involved in cholesterol homeostasis. LXRs have been shown to regulate StAR expression in the adrenal glands, thus modulating the rate of steroidogenesis [146]. In agreement with those findings, GW3965 restores StAR mRNA levels in the sciatic nerve of diabetic animals, and increases the local levels of neuroactive steroids [25]. Interestingly, LXR activation does not affect plasma levels of the cited neurosteroids [25], thus avoiding possible systemic side effects which results advantageous in relation to TSPO ligands, that have been shown to increase both local and plasma neurosteroid levels in experimental diabetic neuropathy [142]. It is interesting to note that both pharmacological approaches are able to increase the local levels of neurosteroids, only in pathological situations when these levels are affected [141]. Therefore, the potential neuroprotective and pain-reducing activities of both TSPO and LXR ligands in other experimental models of neuropathic pain merit to be systematically explored.

Major future challenges for steroid-based therapy and/or steroidogenic-promoting strategies are related to the selection of the doses and treatment regimens that show the best benefit/risk ratio, in order to optimize favorable actions and reduce adverse side effects [12]. Sex-specific treatments and the type of injuries and/or the etiology of the diseases feasible of receiving steroid therapies should also be determined. In addition, different administration routes, feasible and practical in clinical settings, as well as physico-chemical properties for adequate formulation and delivery should also be evaluated [12].

8. Concluding remarks

-In spite of the increasing knowledge on pain mechanisms, the identification of appropriate targets for the prevention and /or treatment of neuropathic pain remains a major challenge.

-Exogenously administered progesterone and/or ALLO, or the stimulation of their local production in the nervous system may represent an attractive option to prevent or treat pain after peripheral or central nervous injuries.

-Major challenges for steroid-based therapy, including the evaluation of pharmacokinetics, bioavailability, safety and sex-differences in the levels or in the action of neuroactive steroids, should be faced for a successful translational approach in severe neuropathic pain conditions.

Acknowledgments

This work was supported by grants from Universidad de Buenos Aires (Grant No. 20020090200126), CONICET (PIP 201-101-00576) and Fundación René Barón.

ACCEPTED MANUSCRIPT

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Figure legends

Figure 1: Schematic representation of the pain pathway

Noxious stimuli are detected by nociceptors, a subpopulation of primary afferent neurons. They are located in dorsal root ganglia and have a peripheral axonal branch that innervates tissues and organs, and a central branch that synapse on second order neurons within the dorsal horn of the spinal cord, which convey nociceptive information to the brain.

Figure 2: Multiple receptors involved in progesterone and allopregnanolone actions

Progesterone pleiotropic effects are mediated via interaction with an array of progesterone receptors including the classic nuclear receptor (PR), progesterone membrane receptors (mPR) and the membrane-associated protein progesterone receptor-membrane component 1 (PGRMC1). In addition, progesterone acts as a competitive antagonist of sigma-1 receptor.

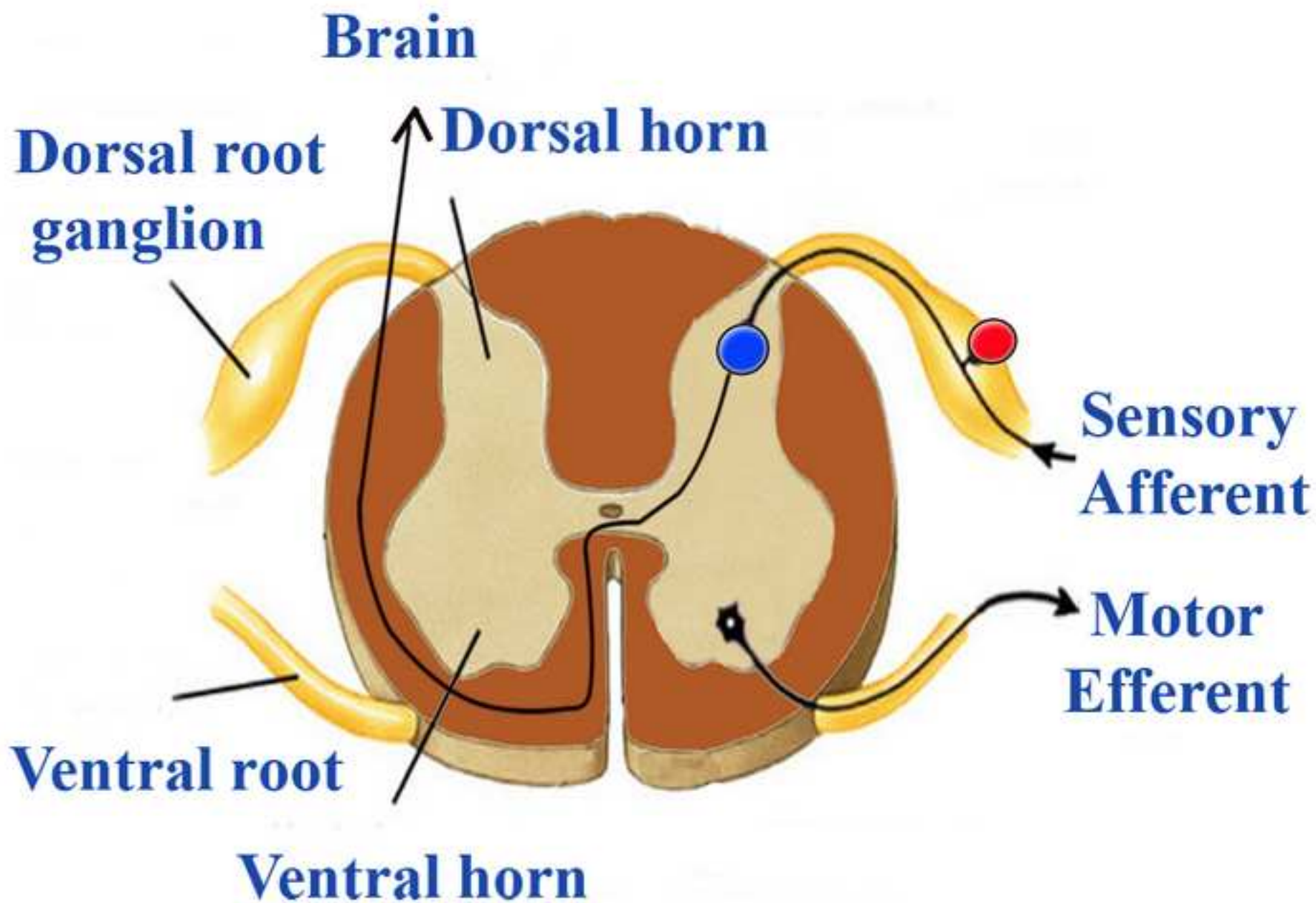
Allopregnanolone does not bind to PR and acts by modulating neurotransmitter receptors. In fact, allopregnanolone is one of the most potent endogenous positive allosteric modulators of GABA_A receptor, thus reinforcing inhibitory neurotransmission

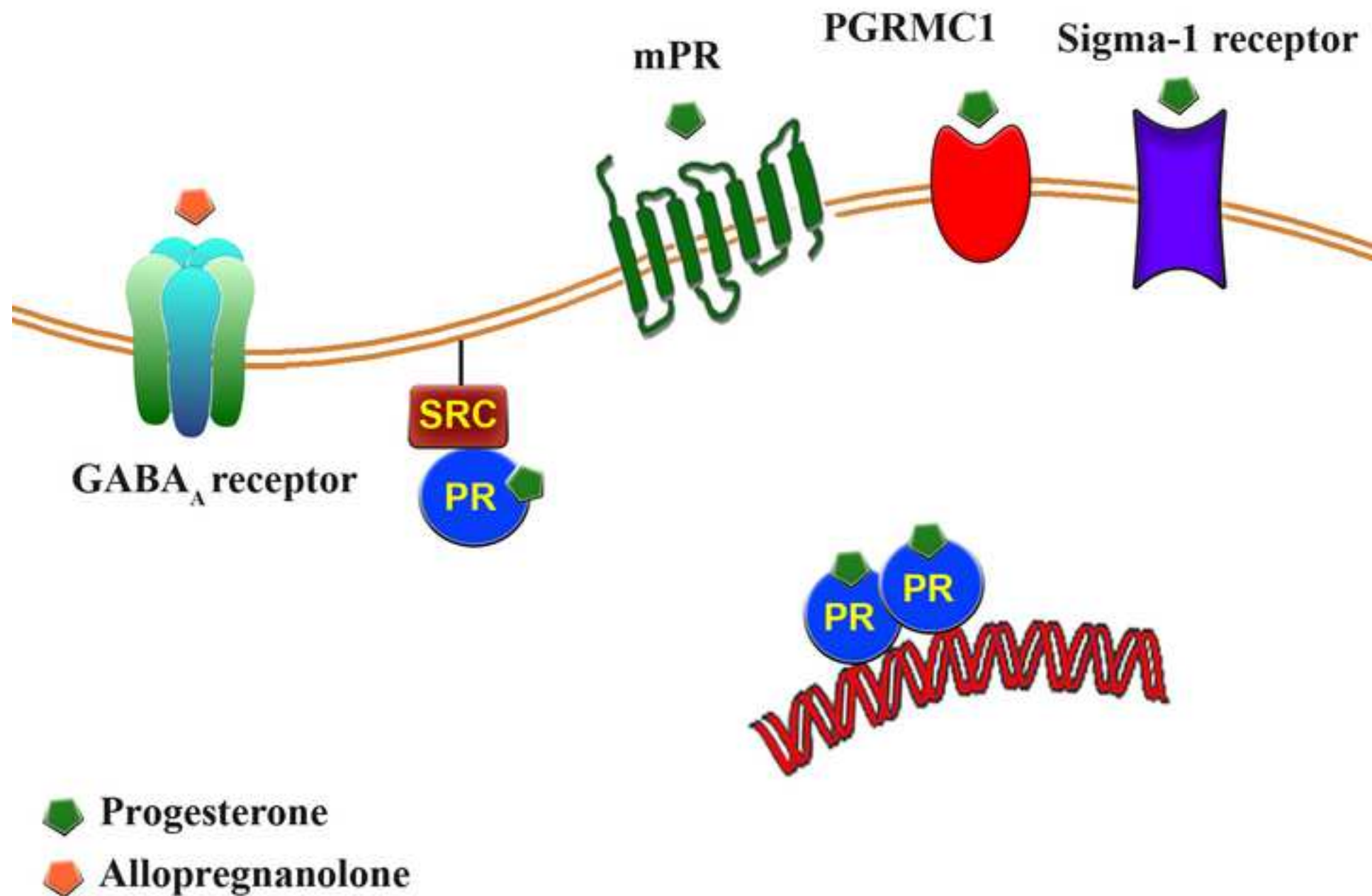
Figure 3: Biosynthetic pathway of progesterone and allopregnanolone

Cholesterol is translocated into the inner mitochondrial membrane by the 18kDa translocator protein (TSPO) and the steroidogenic acute regulatory protein, and is then converted into pregnenolone by the enzyme P450 side-chain cleavage (P450scc). The conversion of pregnenolone into progesterone is catalized by the enzyme 3 β -hydroxysteroid dehydrogenase (3 β -HSD). By the action of 5 α -reductase (5 α -R), progesterone is then reduced into 5 α -dihydroprogesterone, which is further reduced into 3 α ,5 α -tetrahydroprogesterone, also known as allopregnanolone, by 3 α -hydroxysteroid oxidoreductase (3 α -HSOR).

Table 1: Effects of exogenously administered neuroactive steroids on different experimental models of neuropathic pain

Table 2: Steroidogenic-promoting strategies used in animal models of neuropathic pain





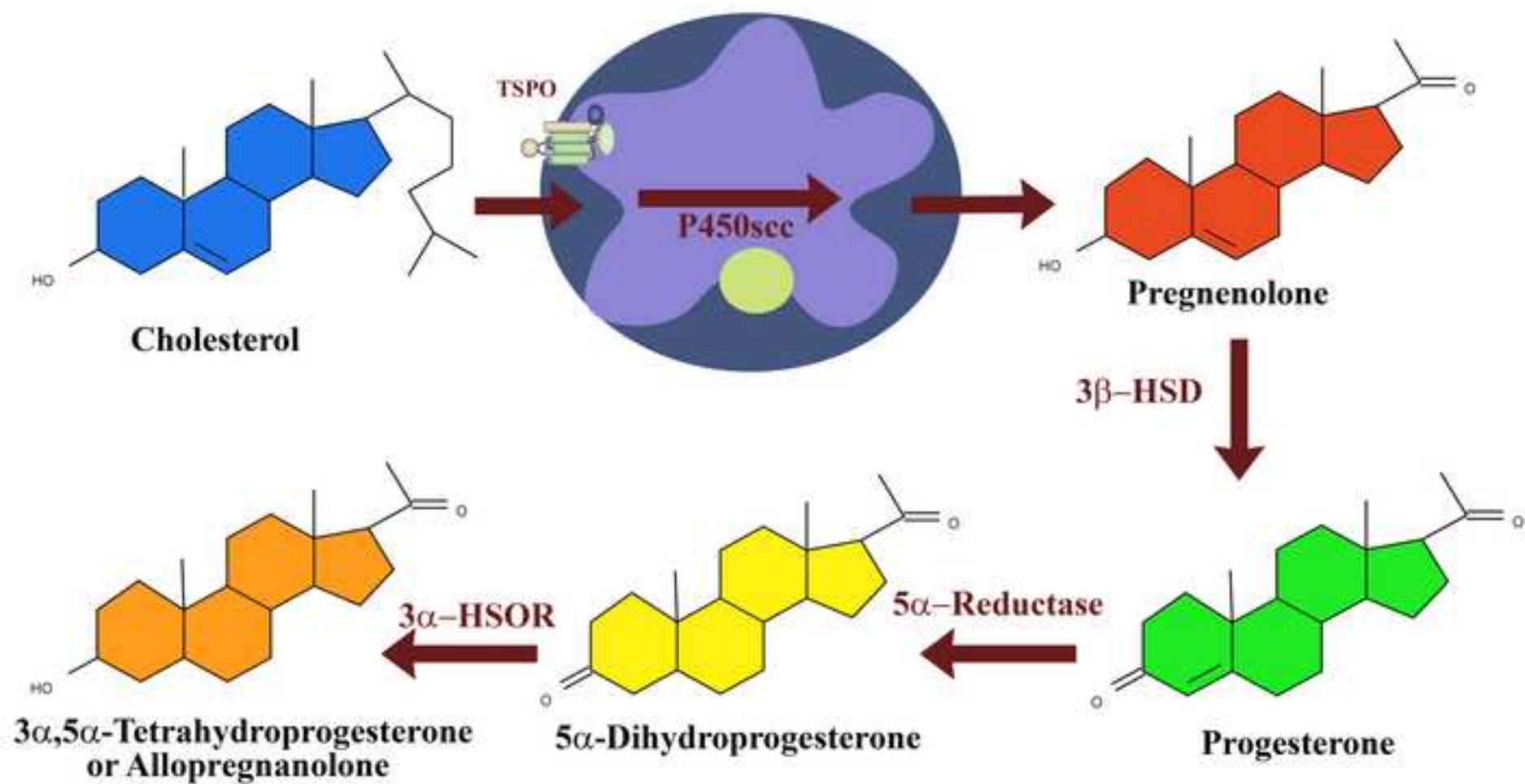


Table 1

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Neuroactive steroid	Experimental model	Effects on nociception / pain behaviors	Targets	Citation
PG	Spinal cord injury	Prevents paw mechanical and thermal allodynia	Prevents the injury- induced increase in spinal NMDA receptor subunits and PKC γ mRNA levels. Attenuates the injury-induced increase in the number of immunoreactive astrocytes and microglial cells, and in COX-2 and iNOS mRNA levels in the spinal cord. Prevents IL1 β , TNF α , IL-6 and IL1-RI spinal up-regulation. Results in lower number of dorsal horn neurons expressing IL1-RI.	Coronel et al., 2011 Coronel et al., 2014 Coronel et al., 2016
PG	Sciatic nerve constriction by single ligature	Prevents paw mechanical and thermal allodynia	Results in lower number of dorsal horn neurons expressing NR1, pNR1 and PKC γ .	Coronel et a., 2011
PG	Sciatic nerve constriction by cuffing	Prevents paw mechanical allodynia		Dableh et al., 2011
PG	Trigeminal nerve root demyelination	Prevents orofacial mechanical allodynia	Recovers P0 and PMP22 expression.	Kim et al., 2012
PG/DHP	Sciatic nerve crush	Counteracts paw nociception impairment after thermal stimulation	Counteracts injury-induced decrease in the expression of myelin proteins P0, PMP22, MAL. Improves Na ⁺ ,K ⁺ ATPase pump activity and reduces the injury-induced increase in myelinated fibers density.	Roglio et al., 2008
PG/DHP /ALLO	Streptozotocin-induced diabetic neuropathy	Counteracts paw nociception impairment after thermal stimulation	Restores skin innervation density, improves Na ⁺ ,K ⁺ ATPase pump activity and reduces the injury-induced decrease in the expression of myelin proteins P0 and PMP22. Counteracts the impairment in nerve conduction velocity.	Leonelli et al., 2007
PG/DHP /ALLO	Vincristin-induced peripheral neuropathy	Prevents and eradicates paw mechanical allodynia and hyperalgesia	Counteracts vincristine-induced alterations in peripheral nerves including 2,3cyclic nucleotide 3-phosphodiesterase, neurofilament 200 kDa and intraepidermal nerve fiber repression. Restores nerve conduction velocity.	Meyer et al., 2010
ALLO	Sciatic nerve ligation	Alleviates paw mechanical and thermal hyperalgesia		Pathirathna et al., 2005
ALLO	Streptozotocin-induced diabetic neuropathy	Ameliorates paw thermal hyperalgesia	Prevents diabetes-induced GABA _A receptor down-regulation.	Afrazi et al., 2014
ALLO	Oxaliplatin-induced peripheral neuropathy	Prevents and abolishes paw mechanical allodynia, mechanical hyperalgesia and thermal allodynia	Restores neurofilament 200 kDa expression, intraepidermal nerve fiber density and nerve conduction velocity.	Meyer et al., 2011

Table 2

ACCEPTED MANUSCRIPT

Ligands	Experimental model	Effects on nociception / pain behaviors	Targets	Citation
TSPO ligand (Ro5-4864)	Streptozotocin-induced diabetic neuropathy	Counteracts paw nociception impairment after thermal stimulation	Increases the levels of PREG and PG in the sciatic nerve. Counteracts the impairment in nerve conduction velocity. Restored skin innervation density and PO mRNA levels, and improves Na ⁺ ,K ⁺ -ATPase activity. Increases the levels of PREG, PG, DHP and ALLO in the spinal cord.	Giatti et al., 2009 Mitro et al., 2012
TSPO ligand (Ro5-4864)	L5 spinal nerve ligation	Reverts paw mechanical allodynia and thermal hyperalgesia	Inhibits activated astrocytes and reduces TNF α expression.	Wei et al., 2013
TSPO ligand (etifoxine)	Vincristin-induced peripheral neuropathy	Prevents and abolishes paw mechanical hyperalgesia and thermal allodynia	Increases the levels of 3 α -reduced neuroactive steroids such as ALLO	Aouad et al., 2009
LXR ligand (GW3965)	Streptozotocin-induced diabetic neuropathy		Increases the levels of DHP and ALLO in the spinal cord. Restores MBP expression.	Mitro et al., 2012
LXR ligand (GW3965)	Streptozotocin-induced diabetic neuropathy	Counteracts paw nociception impairment after thermal stimulation	Increases the expression of P450scc, StAR and 5 α -reductase, and the levels of PREG, PG and DHP in the sciatic nerve. Counteracts the impairment in nerve conduction velocity and Na ⁺ ,K ⁺ -ATPase activity.	Cermenati et al., 2010

Highlights

- Neuroactive steroids are involved in the modulation of pain sensitivity
- Neuroactive steroids also modulate pain messages in neuropathic conditions
- Progesterone and Allopregnanolone administration might counteract chronic pain
- Neurosteroids locally produced in the spinal cord also reduce neuropathic pain
- Promoting neurosteroidogenesis represent a useful tool to alleviate neuropathic pain