Levels and actions of progesterone and its metabolites in the nervous system during physiological and pathological conditions

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ABSTRACT

Progesterone is synthesized and actively metabolized in the central and peripheral nervous system, into neuroactive steroid metabolites, such as dihydroprogesterone, allopregnanolone and isopregnanolone. Progesterone and/or its metabolites exert a variety of effects acting as physiological regulators of neuronal and glial development and plasticity, controlling reproduction, neuroendocrine events, mood and affection. In addition, these neuroactive steroids maintain neural homeostasis and exert neuroprotective actions. In agreement, metabolic pathways of progesterone are affected by modifications in the level of gonadal hormones and by pathology or injury with a regional specificity and in a sex-dimorphic way. Therefore, observations here summarized may provide a background to design sex-specific therapies based on progesterone metabolites. On this point of view, considering that one of the major limits of a therapy based on neuroactive steroids could be modifications in their plasma levels and their consequent peripheral effects, pharmacological treatments aimed to increase their levels in the nervous system could provide an interesting therapeutic option.

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Abbreviations: 3 α -HSOR, 3 α -hydroxysteroid oxidoreductase; 3 β -HSOR, 3 β -hydroxysteroid oxidoreductase; 3 α -THP, 3 α ,5 α -tetrahydroprogesterone; 3 β -HSD, 3 β -hydroxysteroid dehydrogenase; 3 β -THP, 3 β ,5 α -tetrahydroprogesterone; 5 α -R, 5 α -reductase; CNS, central nervous system; DHP, dihydroprogesterone; EAE, experimental autoimmune encephalomyelitis; Iba1, ionized calcium-binding adapter molecule 1; LC-MS/MS, liquid chromatography tandem mass spectrometry; LXR, liver X receptor; MBP, myelin basic protein; P0, glycoprotein zero; P450sc, P450 side-chain cleavage enzyme; PMP22, peripheral myelin protein 22; PNS, peripheral nervous system; PR, progesterone receptor; PREG, pregnenolone; PROG, progesterone; StAR, steroidogenic acute regulatory protein; STZ, streptozotocin; TSPO, translocator protein of 18 kDa; WT, wild type.

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1. Introduction

Neuroactive steroids are steroid molecules acting in the nervous system. These molecules are synthesized by neurons and glial cells (i.e., neurosteroids) and in peripheral glands, such as the testis, the ovary and the adrenal glands (i.e., steroid hormones) (Melcangi et al., 2008). The present review will focus on progesterone (PROG) and its metabolites, dihydroprogesterone (DHP), 3 α ,5 α -tetrahydroprogesterone (3 α -THP), also known as allopregnanolone, and 3 β ,5 α -tetrahydroprogesterone (3 β -THP), also known as isopregnanolone. Indeed, these neuroactive steroids are synthesized and metabolized in the nervous system and recent studies have shown that their levels can be affected by pathology or injury, as demonstrated in experimental models of Alzheimer's disease, Parkinson's disease, multiple sclerosis, peripheral neuropathies and diabetic encephalopathy (Caruso et al., 2008a, 2010a, 2013; Giatti et al., 2010, 2013; Labombarda et al., 2006b; Meffre et al., 2007; Melcangi et al., 2012; Melcangi and Garcia-Segura, 2010; Pesaresi et al., 2010b).

In turn, as demonstrated in experimental models and in clinical studies, PROG and its metabolites exert a variety of effects acting as physiological regulators of nervous function as well as protective agents. Therefore, a potential role of these molecules as components of therapeutic strategies for neurodegenerative and psychiatric disorders has been recently proposed.

2. Synthesis and metabolism of progesterone in the nervous system

2.1. Synthesis of progesterone

It is now well ascertained that synthesis and metabolism of neuroactive steroids occur in neurons and glial cells (i.e., astrocytes, oligodendrocytes and Schwann cells) of the central (CNS) and peripheral (PNS) nervous system (Garcia-Segura and Melcangi, 2006; Melcangi et al., 2008; Panzica and Melcangi, 2008; Pelletier, 2010). Steroidogenesis is a process highly compartmentalized in a sequence of reactions, which implies as first step the translocation of cholesterol from the cytoplasm to the inner mitochondrial membrane. This is a limiting step hormonally controlled and mediated by the steroidogenic acute regulatory protein (StAR) (Lavaque et al., 2006) and the translocator protein of 18 kDa (TSPO) (Papadopoulos et al., 2006a). In the mitochondria, cholesterol is then converted into pregnenolone (PREG) by the action of P450 side-chain cleavage enzyme (P450_{sc} also known as CYP11A1). PREG is then transformed into progesterone (PROG) by the action of the enzyme 3 β -hydroxysteroid dehydrogenase (3 β -HSD) (Melcangi et al., 2008) (Fig. 1).

2.2. Metabolism of progesterone

Enzymes involved in the metabolism of PROG are expressed, in CNS and PNS, by neurons, astrocytes, oligodendrocytes and Schwann cells (Pelletier, 2010). PROG is converted into DHP by

(5 α -R). In turn, DHP is further converted into 3 α -THP or 3 β -THP by 3 α -hydroxysteroid oxidoreductase (3 α -HSOR) and 3 β -hydroxysteroid oxidoreductase (3 β -HSOR), respectively (Melcangi et al., 2008) (Fig. 1).

The metabolism of PROG has a deep impact on the mechanism of action of this neuroactive steroid. Indeed, while DHP, like PROG, is able to interact with the classical steroid receptor, the PROG receptor (Melcangi et al., 2008), 3 α -THP is a potent ligand of a non-classical steroid receptor, such as the GABA-A receptor (Belelli and Lambert, 2005; Lambert et al., 2003). In contrast, 3 β -THP, does not bind directly to the GABA-A receptor (Bitran et al., 1991), but it antagonizes the effect of 3 α -THP on the GABA-A receptor (Wang et al., 2002). Thus, metabolic conversion of PROG into its derivatives might differently and specifically modulate the mechanism of action of the precursor molecule by recruiting CNS specific pathways.

2.3. Modulation of the synthesis and metabolism of progesterone in the nervous system by steroid hormones

The effects that modifications in the peripheral levels of steroid hormones have on the levels of PROG and its metabolites in nerve tissue have been studied in rats. Data so far obtained have

Fig. 1. Formation of progesterone and its metabolism in the nervous system. Details are provided in the text. TSPO: translocator protein of 18 kDa; StAR: steroidogenic acute regulatory protein; 1: cytochrome P450_{sc}; 2: 3 β -hydroxysteroid dehydrogenase; 3: 5 α -reductase; 4: 3 α -hydroxysteroid oxidoreductase; 5: 3 β -hydroxysteroid oxidoreductase; PREG: pregnenolone; PROG: progesterone; DHP: dihydroprogesterone; 3 α -THP: 3 α ,5 α -tetrahydroprogesterone; 3 β -THP: 3 β ,5 α -tetrahydroprogesterone.

demonstrated that after 15 days of orchidectomy, the levels of PREG, the PROG precursor, persisted in the brain (Jo et al., 1989). Further studies with the same experimental protocol have indicated that in the brain of castrated and adrenalectomized male and female rats PROG and its metabolite DHP are still present in measurable amounts (Corpechot et al., 1993). Moreover, after three weeks of gonadectomy and adrenalectomy, significant amounts of PREG and PROG still remained in the spinal cord of male rats (Coirini et al., 2002).

These observations have been confirmed by studies performed on proteins involved in steroidogenesis, such as StAR and 3β -HSD. In particular, it has been demonstrated that both in male and female rats, StAR mRNA levels present in the brain were not significantly affected after 3 weeks of gonadectomy and adrenalectomy (Kim et al., 2003). After the same period of gonadectomy and adrenalectomy, 3β -HSD mRNA and protein levels were not modified in spinal cord of male rats (Coirini et al., 2002).

We have recently analyzed, by liquid chromatography tandem mass spectrometry (LC-MS/MS), the amounts of neuroactive steroids in the sciatic nerve, in three CNS regions (i.e., cerebellum, cerebral cortex and spinal cord) and in plasma of male and female animals. The levels present in gonadally intact animals were compared with those present in short (one week) and long-term (4 months) gonadectomized animals (Caruso et al., 2010b). Data obtained indicate that the absolute concentrations of neuroactive steroids show sex and regional differences in the nervous system of control animals. As already reported by our studies (Pesaresi et al., 2010b), we confirmed that in sciatic nerve, the levels of PREG, PROG and its derivatives are higher in females. In the case of PREG and 3β -THP, this sex difference seems to be a peculiarity of the PNS. Moreover, in female animals, the concentration of 3β -THP in PNS is higher than that in the CNS. Finally, among female CNS areas, the spinal cord and the cerebellum show higher levels of DHP, associated with lower levels of its precursor PROG, in comparison to the cerebral cortex (Caruso et al., 2010b).

Another key point emerging from this study was a different modulation of neuroactive steroid levels by gonadectomy in the PNS compared to the CNS, and within different CNS regions. Concerning other regional differences, in male rats the levels of the precursor of PROG (i.e., PREG) decrease after short-term gonadectomy in the spinal cord, but remain unchanged in the sciatic nerve and the cerebellum (Caruso et al., 2010b).

Uptake from periphery partly determines the levels of neuroactive steroids in the PNS and the CNS. For instance, it is well known that PROG originating in the periphery is taken up by the nervous system in considerable amounts (Billiar et al., 1975). We observed that females have higher levels of PROG in the PNS and CNS than males, which reflect their plasma concentration. However, as also reported in previous studies (Corpechot et al., 1993; Jo et al., 1989; Labombarda et al., 2006b; Robel et al., 1995), we observed that not all the changes in neuroactive steroid levels in the nervous system after gonadectomy are correlated with changes in plasma levels (Caruso et al., 2010b). Indeed, differently from what observed in plasma, detectable amounts of neuroactive steroids are still maintained in nervous system of gonadectomized animals. In addition, we have observed that some neuroactive steroids levels increase in the nervous system after gonadectomy. For instance, at variance to what is observed in the plasma of male animals, in the cerebral cortex short-term gonadectomy increases the levels of DHP, the first derivative of PROG. Long-term gonadectomy also induced an increase of PREG in female cerebellum. These increases in neuroactive steroids do not reflect the changes in plasma, where short- or long-term gonadectomy causes a decrease or result in undetectable levels of these molecules. Therefore, our findings suggest that gonadectomy, by still unidentified mechanisms, affects the rate of local synthesis

and/or local metabolism of some specific steroids in the nervous system. Altogether these observations strongly support the idea that peripheral and central regulation of steroids levels might be partly independent (Caruso et al., 2010b).

In some cases, changes in the levels of steroids after gonadectomy within a specific brain region parallel the changes in their precursors in the same brain region. For instance, PROG content is determined by the changes in PREG; in turn 3α -THP concentration is dependent on the levels of PROG levels (Caruso et al., 2010b). However, in other cases there is not a direct match between the difference in the levels of the precursor and the metabolite. Therefore, the regional and sex specific modifications in steroid levels in the nervous system after gonadectomy might reflect multiple mechanisms, including differences in local steroidogenesis, changes in retention of steroids and/or modifications in local steroid metabolism.

Another important finding is that long-term or short-term gonadectomy differently affect levels of neuroactive steroids in the PNS and the CNS. Thus, the amounts of some steroids are reduced by short-term gonadectomy and then recover to control values after long-term gonadectomy. An example of this phenomenon is represented by the changes in the levels of PREG in the spinal cord of male rats after gonadectomy (Caruso et al., 2010b).

In conclusion, as summarized in Fig. 2, gonadectomy influences the metabolism of PROG in nervous system depending on several variables. In particular, data so far obtained indicate that: (1) changes in the levels of PROG, its precursor PREG and its metabolites DHP, 3α -THP and 3β -THP in the nervous system after gonadectomy do not necessarily reflect their changes in plasma levels; (2) long-term gonadectomy induces changes in the levels of these neuroactive steroids in both PNS and CNS that in some cases are different to those induced by short-term gonadectomy; (3) the effect of gonadectomy on the levels of these neuroactive steroids is different between the PNS and the CNS and within different CNS regions and (4) the effects of gonadectomy on the levels of these neuroactive steroids in the nervous system show sex differences. Altogether, these observations indicate that the nervous system adapts its local levels of PREG, PROG and its metabolites in response to changes in gonadal hormones. This response is sex and regional specific and depends on the duration of the peripheral modifications (Caruso et al., 2010b).

Fig. 2. Variables influencing the effect of gonadectomy on the levels of pregnenolone (PREG), progesterone (PROG) and its metabolites, such as dihydroprogesterone (DHP), $3\alpha,5\alpha$ -tetrahydroprogesterone (3α -THP), $3\beta,5\alpha$ -tetrahydroprogesterone (3β -THP) occurring in plasma and in the central (CNS) and peripheral (PNS) nervous system. Details are provided in the text.

3. Physiological actions of progesterone and its metabolites in the nervous system

3.1. Physiological actions of progesterone and its metabolites in the CNS

The best characterized physiological function of PROG is the regulation of reproduction. Acting on the hypothalamus, PROG contributes to regulate the release of gonadotrophins (Banks and Freeman, 1980; Barraclough et al., 1986) and dampens GnRH pulsatility (Skinner et al., 1998). In addition, acting on the ventromedial hypothalamic nucleus, PROG regulates estrous responsiveness (Barfield et al., 1984). The role of PROG in the control of reproduction involves both hormonal and local actions. Thus, changes in PROG levels in plasma during the ovarian cycle as well as changes in the local synthesis of PROG by hypothalamic astrocytes (Micevych and Sinchak, 2008) participate in the control of reproduction.

PROG has also developmental effects in the brain, regulating the development of some neuronal types, such as the Purkinje cells of the cerebellum (Tsutsui et al., 2011). Another important developmental action of PROG is the regulation of the differentiation and proliferation of oligodendrocytes (Ghoumari et al., 2003, 2005; Jung-Testas et al., 1996b). In the adult brain, PROG regulates cytoskeletal proteins and the morphology of neurons and astrocytes (Guerra-Araiza et al., 2007; Reyna-Neyra et al., 2002). Thus, changes in plasma levels of PROG during the estrous cycle contribute to the regulation of astroglial (Garcia-Segura et al., 1994; Luquin et al., 1993) and synaptic plasticity (Foy et al., 2008; Garcia-Segura et al., 1994; McEwen and Woolley, 1994; Murphy and Segal, 2000; Perez et al., 1993; Woolley and McEwen, 1993) in the hypothalamus and the hippocampus. PROG also regulates adult neurogenesis in the subventricular zone and the hippocampus (Giachino et al., 2003; Wang et al., 2008).

The actions of PROG during physiological conditions involve the regulation of the activity of different kinases and phosphatases (Amorim et al., 2010; Frye and Walf, 2008; Guerra-Araiza et al., 2007, 2009; Mani and Oyola, 2012) and the activation of membrane and intracellular progesterone receptors (Brinton et al., 2008; Mani and Oyola, 2012; Meffre et al., 2013). PROG and its metabolite DHP bind to intracellular PROG receptors (Melcangi et al., 2008), which are expressed in the nervous system (Guerra-Araiza et al., 2003). PROG may also act through membrane PROG receptors, which are widely expressed in the brain and the spinal cord (Brinton et al., 2008; Labombarda et al., 2010; Meffre et al., 2013; Thomas and Pang, 2012). In several cases the physiological actions of PROG are mediated by its reduced metabolite 3 α -THP (Giachino et al., 2003; Guerra-Araiza et al., 2007, 2009) and may involve the regulation of the activity of GABA-A receptors (Follesa et al., 2001; Lambert et al., 2003; Majewska et al., 1986; Wang, 2011). Indeed, 3 α -THP is an important allosteric modulator of GABA-A receptor at different life stages (Biggio et al., 2009; Turkmen et al., 2011) and also regulates GABA and glutamate release (Giuliani et al., 2013). The actions of 3 α -THP on the activity of GABA-A receptor may contribute to changes in mood and affection (Biggio et al., 2009; Eser et al., 2008; Turkmen et al., 2011; Wirth, 2011). In addition, 3 α -THP may also regulate neuronal function by acting on membrane PROG receptors (Pang et al., 2013; Thomas and Pang, 2012).

3.2. Physiological actions of progesterone and its metabolites in the PNS

Peripheral nerves and Schwann cells are also a physiological target of PROG and its metabolites. Indeed, PROG receptor

(Groyer et al., 2006; Jung-Testas et al., 1996a; Melcangi et al., 2001) as well as GABA-A receptors have been identified in peripheral nerves and Schwann cells (Magnaghi et al., 2004; Melcangi et al., 1999). PROG and its derivatives modulate the expression of myelin proteins of the PNS, such as glycoprotein zero (P0) and the peripheral myelin protein 22 (PMP22). In this regard, the expression of P0 in sciatic nerve of adult male rats, as well as that in rat Schwann cell culture, was increased by the treatment with PROG, DHP or 3 α -THP, while in case of PMP22, only 3 α -THP was effective (Melcangi et al., 2005, 1999). P0 and PMP22 play an important role for the maintenance of the multilamellar structure of PNS myelin (D'Urso et al., 1990). In agreement with the effect exerted on the proteins of peripheral myelin, PROG is also able to stimulate the myelin formation (Chan et al., 1998, 2000).

The mechanism involved in the effects of PROG and its metabolites are different depending on the myelin protein considered. Indeed, observations performed with receptor agonists or antagonists suggest that the expression of P0 is under the control of PROG receptor (PR), while a role for GABA-A receptor, may be hypothesized in case of PMP22 (Melcangi et al., 2005). Activation of PR clearly suggests that the effect of PROG derivatives on P0 expression is due to a classical steroid genomic effect. This concept was supported by the finding that putative progesterone responsive elements are present on P0 gene (Magnaghi et al., 1999) and that a coactivator, such as steroid receptor coactivator-1, participates in the regulation of P0 gene expression by DHP (Cavarretta et al., 2004). Since *in vivo* treatment with mifepristone (i.e., a PR antagonist) specifically decreased the synthesis of P0 at postnatal day 20 (Melcangi et al., 2003b), it has been proposed that PROG derivatives may be necessary for inducing P0 synthesis during the first steps of the myelination process. Differently from what observed on P0, expression of PMP22 seems to be under the control of GABA-A receptor. Indeed, treatment with antagonist (i.e., bicuculline) or agonist (i.e., muscimol) of GABA-A receptor abolished or mimicked, respectively the stimulatory effect exerted by 3 α -THP on PMP22 (Magnaghi et al., 2001). 3 α -THP also influences GABA pathways in Schwann cells by increasing the expression of glutamic acid decarboxylase and the consequent stimulation of GABA synthesis (Magnaghi et al., 2010). 3 α -THP also increases the activity of excitatory amino acid carrier 1 and therefore, glutamate uptake (Perego et al., 2011). Therefore, PROG and its metabolites also regulate myelination program by controlling the expression of its transcription factors. For instance, PROG stimulates the gene expression of Krox-20, Krox-24, Egr-3 and FosB (Guennoun et al., 2001; Mercier et al., 2001). Moreover, the expression of Krox-20 was also stimulated by DHP and 3 α -THP, while that of another transcription factor, such as Sox-10, was only stimulated by DHP (Magnaghi et al., 2007).

The control exerted by PROG and its metabolites on the expression of P0 and PMP22 is sexually dimorphic. Thus, PROG or DHP treatment induces a stimulatory effect on P0 mRNA levels in cultures of rat Schwann cells derived from male rats, while 3 α -THP was only effective in Schwann cells derived from females. In addition, PMP22 was stimulated by PROG in cultures from males and by 3 α -THP in cultures from females (Magnaghi et al., 2006). PROG and its metabolites are also able to affect Schwann cell proliferation. A stimulatory effect of PROG has been detected *in vitro* (Bartolami et al., 2003; Fex Svenningsen and Kanje, 1999) and this effect as well seems to be dependent on sex. Indeed, PROG increased Schwann cell proliferation in cultures of segments of rat sciatic nerve from females, but was ineffective in cultures from males (Fex Svenningsen and Kanje, 1999).

The axonal compartment of PNS neurons is also a target for the action of PROG and its metabolites. Thus, PROG affects the expression of neuronal genes involved in the myelination process by Schwann cells. For instance, in co-culture of Schwann cells and

Fig. 3. Physiological effects of progesterone (PROG) and its metabolites in the peripheral nervous system. Details are provided in the text. Dihydroprogesterone (DHP); 3α,5α-tetrahydroprogesterone (3α-THP); glycoprotein zero (P0); peripheral myelin protein 22 (PMP22); Ras-like GTP-binding protein (Rap1b); phosphoribosyl diphosphate synthase-associated protein (PRPP); glutamic acid decarboxylase-67 kDa (GAD); excitatory amino acid carrier-1 (EAAC1).

DRG neurons, a small Ras-like GTP-binding protein (Rap 1b) and phosphoribosyl diphosphate synthase-associated protein, which are induced in co-cultures during myelin synthesis, were also induced by PROG treatment (Chan et al., 2000; Rodriguez-Waitkus et al., 2003). Finally, the blockade of PR by mifepristone during development resulted in axonal impairment in the sciatic nerve of male rats (Melcangi et al., 2003b) (Fig. 3).

4. Levels of progesterone and its derivatives in neurodegenerative diseases

Different pathologies of the CNS and PNS show sex differences in their incidence, symptomatology and/or neurodegenerative outcome. These include Parkinson's disease, Alzheimer's disease, Huntington's disease, multiple sclerosis, traumatic brain injury, stroke, autism, schizophrenia, depression, anxiety disorders, eating disorders and peripheral neuropathy (Melcangi and Garcia-Segura, 2010). Thus, sex differences in neuroactive steroid levels may represent causative factors for sex differences in the incidence or manifestation of pathologies of the nervous system. Examples of changes in neuroactive steroid levels during neurodegenerative events are shown in Fig. 4.

Fig. 4. Examples of neurodegenerative disorders affecting the levels of pregnenolone, progesterone and its metabolites in nervous system. Details are provided in the text.

4.1. Alzheimer's disease

In a recent study (Caruso et al., 2013), the levels of neuroactive steroids have been assessed in male brains from a mouse model of Alzheimer's disease (i.e., 3xTg-AD mice). An age-related decrease in the levels of PROG, together with an increase in the levels of its metabolite DHP, was detected in the limbic region of wild type (WT) and 3xTg-AD mice. Concerning PROG metabolites in the limbic region, a significant increase in the levels of DHP and 3β-THP in 3xTg-AD compared to WT mice was reported. In addition, DHP positively correlated with 3α-THP in young 3xTg-AD mice, and negatively correlated with 3β-THP in aged 3xTg-AD mice. These correlations were not detected in WT mice. In contrast, 3xTg-AD mice did not show the positive correlations in the levels of PREG and DHP and in the levels of 3α-THP and 3β-THP that were detected in young and older WT mice, respectively.

These findings suggest that the metabolism of PROG is altered in the limbic region of 3xTg-AD mice (Caruso et al., 2013). This modification is of utmost importance considering that PROG metabolites have been shown to be neuroprotective (Brinton et al., 2008; Brinton and Wang, 2006; Sun et al., 2012). Indeed, treatment of 3xTg-AD mice with 3α-THP decreases β-amyloid accumulation (Chen et al., 2011), reverses cognitive deficits and promotes neurogenesis (Singh et al., 2011; Wang et al., 2010). The protective action of 3α-THP may be related with its ability to activate the GABA-A receptor (Melcangi et al., 2008). Moreover, in this context, it is also important to highlight that, as recently reported (Luchetti et al., 2011a,b), the expression of several GABA-A receptor subunits is significantly reduced in post-mortem brain tissue of Alzheimer's disease patients. Indeed, alterations of GABA signals may play an important role in the cognitive and behavioral alterations occurring in Alzheimer's disease (Birzniece et al., 2006; Lancot et al., 2007). Thus it has been hypothesized that brain alterations in PROG metabolites that modulate GABA-A receptor function, may contribute to the cognitive alterations of Alzheimer's disease patients.

4.2. Parkinson's disease

Altered levels of PROG and metabolites have been also observed in Parkinson's disease and corresponding animal models. Relatively high levels of PROG, and its derivatives, DHP and 3α-THP, have been detected in the human substantia nigra pars compacta and caudate nucleus, with respect to other brain regions (Bixo et al., 1997), suggesting that substantial synthesis and accumulation of neuroactive steroids may take place in the nigrostriatal system. Moreover, various studies have reported that neuroactive steroids can affect synaptic transmission at the cortico-striatal level, by modulating the striatal release of dopamine (Whittaker et al., 2008). Furthermore, as mentioned above, neuroactive steroids can act as either positive or negative modulators of the GABA-A receptor (Belelli and Lambert, 2005). This class of molecules may, therefore, intervene in the modulation of basal ganglia circuitry activity, where GABAergic transmission plays a crucial role at various points (Blandini et al., 2000).

A significant decrease of PREG in the striatum has been observed after the degeneration of nigrostriatal dopaminergic neurons obtained by the stereotaxic injection of the neurotoxin 6-hydroxydopamine (Melcangi et al., 2012). A similar effect was observed in the levels of DHP and 3β-THP in the striatum and the cerebral cortex. In this context, it is interesting to note that gene expression of 5α-R (i.e., the enzyme converting PROG into DHP) was decreased in the substantia nigra, as well as in peripheral blood mononuclear cells of Parkinson's disease patients (Luchetti et al., 2010). Decreased DHP levels have also been detected in the cerebrospinal fluid of Parkinson's disease patients (di Michele et al., 2003).

4.3. Multiple sclerosis

It is well known that the incidence, the progression and the severity of multiple sclerosis, an inflammatory, demyelinating disease of the CNS, are affected in a sex-dependent way (Orton et al., 2006; Noonan et al., 2002; Schwendimann and Alekseeva, 2007). Physiological situations characterized by changes in sex steroid plasma levels, such as menstrual cycle, menopause or pregnancy, affect the disease course (Darlington, 2002; El-Etr et al., 2005; Holmqvist et al., 2006; Tomassini and Pozzilli, 2006; Vukusic and Confavreux, 2006; Vukusic et al., 2004), suggesting that these molecules might exert a role in this pathology. Data obtained in a classical experimental model of multiple sclerosis (i.e., experimental autoimmune encephalomyelitis, EAE) have shown that, at acute phase (i.e., 14 days after immunization) the levels of PROG and its metabolites show sex and regional differences in control and EAE nervous system and that a clear difference was also observed between CNS and plasma levels (Giatti et al., 2010). In particular, in agreement with previous observations showing that multiple sclerosis may affect sex steroid plasma levels (Foster et al., 2003; Maccio et al., 2004; Safarinejad, 2008; Tomassini et al., 2005), the levels of PROG and its metabolites DHP and 3β -THP were reduced in male plasma (Giatti et al., 2010). On the contrary, the only change induced by EAE in female plasma was a decrease in the levels of 3α -THP (Giatti et al., 2010).

In addition to the changes in plasma levels, the concentrations of PROG and its metabolites are also altered by EAE in the nervous system (Giatti et al., 2010). Thus, 3α -THP levels were significantly increased with EAE in the spinal cord and the cerebral cortex, but only in males. In contrast, the amounts of PROG, DHP and 3β -THP did not show sex differences in the spinal cord and were significantly decreased with EAE in both sexes. However, it is also important to highlight that in the cerebellum and the cerebral cortex these changes were sexually dimorphic, with a decrease occurring only in EAE males. In particular, PROG and 3β -THP levels were decreased in the cerebellum, while in the cerebral cortex a decrease of PROG and DHP was detected. Altogether, these data suggest that acute phase of EAE modifies PROG and its metabolites in a sex dimorphic way and with regional specificity (Giatti et al., 2010).

An important observation is that the changes observed in plasma in the EAE model did not fully reflect those in the CNS. For instance, the decrease of PREG and the increase of 3α -THP levels observed in male CNS areas were not observed in plasma. Similarly, the amounts of PROG, DHP and 3β -THP were decreased in female CNS areas but not in plasma. Thus, plasma neuroactive steroid levels seem to represent an incomplete picture of the changes occurring in the CNS, and consequently cannot be considered accurate biomarkers of the disease.

The effect of EAE on PROG and its metabolite levels shows several dissimilarities between the chronic (i.e., 40 days after immunization) (Caruso et al., 2010a) and the acute phase of the disease (Giatti et al., 2010). Indeed, acute and chronic EAE had similar effects on PROG and DHP levels in the cerebral cortex but different effects in the spinal cord and the cerebellum. Acute and chronic EAE also had a different effect on 3α -THP and 3β -THP levels. Thus, the levels of 3α -THP were unchanged in both sexes in spinal cord and cerebral cortex after chronic EAE (Caruso et al., 2010a) while they were increased in both male CNS areas after acute EAE (Giatti et al., 2010). On the other hand, the pattern of 3α -THP levels in the cerebellum at the chronic phase was unchanged respect to the acute phase (Caruso et al., 2010a). The amount of 3β -THP in spinal cord was decreased in both sexes in the acute phase of EAE (Giatti et al., 2010) but return to control levels in the chronic phase (Caruso et al., 2010a). Differences in 3β -THP concentration

between acute and chronic EAE were also detected in the cerebellum and the cerebral cortex.

4.4. Peripheral neuropathies

As demonstrated in several experimental models, the amounts of neuroactive steroids present in peripheral nerves are affected by traumatic injury and pathologies (Melcangi et al., 2011). In particular, in the experimental model of crush injury the levels of PREG, DHP and 3α -THP present in the distal portion of injured sciatic nerve were lowered (Roglio et al., 2008). The drop of DHP was not explained by a decrease in the levels of its first precursor (i.e., PROG) that were unchanged, but by a decrease in the expression of the enzyme 5α -R (Roglio et al., 2008).

Peripheral neuropathy is the most frequent symptomatic complication of diabetes in the nervous system. It is characterized by a spectrum of functional and structural changes in peripheral nerves including axonal degeneration, paranodal demyelination and loss of myelinated fibers and decreased nerve conduction velocity (Sugimoto et al., 2000; Vinik et al., 2000).

Diabetic neuropathy is more frequent in men than in women (ratio male/female 2.9) (Basit et al., 2004; Booya et al., 2005). Moreover, males develop neuropathy earlier than females (Aaberg et al., 2008). Neuropathic pain and negative sensory symptoms are more frequent in females, whereas muscle weakness and atrophy is more frequent in male patients (Kiziltan and Benbir, 2008). Motor nerve conduction abnormalities and ulnar nerve involvement is also more frequent and severe in males (Kiziltan and Benbir, 2008; Kiziltan et al., 2007). In particular, nerve conduction studies indicated that men have lower amplitudes and conduction velocities and longer latencies than female patients (Albers et al., 1996). In streptozotocin (STZ)-treated rats (i.e., an experimental model of diabetes), the paw withdrawal threshold is reduced more in females than in males, suggesting that nociceptive threshold is differently affected in the two sexes (Joseph and Levine, 2003).

Diabetes is also known to influence plasma levels of sex steroids (Babichev et al., 1998; Durant et al., 1998; Leonelli et al., 2007; Roglio et al., 2007; Salonia et al., 2006; Tanaka et al., 2001; van Dam et al., 2003). In the STZ-model the plasma levels of PROG are significantly decreased both in female and male animals. In addition, the amounts of PREG are significantly decreased in the sciatic nerve of male rats, while those of PROG and its metabolites 3α -THP and 3β -THP are decreased in females (Pesaresi et al., 2010b).

Similar sex dimorphic changes have been obtained in other experimental model of peripheral nerve damage. For instance, in the sciatic nerve of PMP22 transgenic rats (i.e., an experimental model of Charcot-Marie-Tooth type 1A) the levels of 3β -THP, were strongly decreased only in females (Caruso et al., 2008b).

4.5. Diabetic encephalopathy

In addition to result in peripheral neuropathy, diabetes may also induce neurophysiological and structural changes at the level of the CNS (i.e., diabetic encephalopathy) (McCall, 2002), which are associated with cognitive deficits and increased risk of dementia, stroke, cerebrovascular and Alzheimer's disease and psychiatric disorders, such as depression and eating disorders (Biessels et al., 2008, 2002; Gispén and Biessels, 2000; Jacobson et al., 2002; Kodl and Seaquist, 2008). These alterations show sex differences in incidence, progression and severity (Andersen et al., 1999; Farace and Alves, 2000; Fratiglioni et al., 1997; Kaye, 2008; Marcus et al., 2008; Niemeier et al., 2007; Simonds and Whiffen, 2003).

Sex differences in the amount of PROG and its derivatives have been detected in the brains of STZ-treated animals (Pesaresi et al., 2010b). In particular, PROG levels show a sex difference in the cerebellum of diabetic rats, decreasing versus control values in

females, but not in males. The levels of PROG metabolites are impaired in a different way. Indeed, STZ causes a decrease in the levels of DHP and 3α -THP in the cerebellum and spinal cord, respectively. However, this decrease was detected in males but not in females. In contrast, 3β -THP levels were decreased by STZ in the cerebellum of females, but not in the cerebellum of males (Pesaresi et al., 2010b). These findings indicate that in the nervous system diabetes induces, in a sex dimorphic and regionally specific manner, decrease in the levels of PROG as well as in those of its metabolites.

Interestingly, levels of neuroactive steroids, including those of 3α -THP are also decreased in cerebral areas of Alzheimer's disease patients (Marx et al., 2006) and in the experimental models of this pathology (see above). A close association between diabetes mellitus and Alzheimer's disease has been proposed (Priyadarshini et al., 2012; Takeda et al., 2011). Indeed, extensive abnormalities of insulin and insulin-like growth factor signaling pathways also occur in this neurodegenerative disorder (Steen et al., 2005). Thus, alterations in neuroactive steroid levels might be interpreted as a common feature of the cognitive impairment occurring in diabetes mellitus and Alzheimer's disease.

5. Neuroprotective actions of progesterone and its metabolites

5.1. Neuroprotective effects in the CNS

Beside the observation that PROG and its metabolites exert functions relevant for the physiology of the CNS, they are also protective agents in the CNS. PROG has been shown to exert neuroprotective effects in different animal models of neurodegenerative and neurological diseases, including experimental models of Alzheimer's disease (Wang et al., 2008), Parkinson's disease (Bourque et al., 2009; Callier et al., 2001), epilepsy (Frye and Scalise, 2000; Rhodes and Frye, 2004), excitotoxicity (Ciriza et al., 2006; Frye and Walf, 2011), stroke (Chen et al., 1999; Cervantes et al., 2002; Gibson and Murphy, 2004; Sayeed et al., 2007), motoneuron degeneration (Gonzalez Deniselle et al., 2002; Yu, 1989), multiple sclerosis and demyelination (Garay et al., 2007; Giatti et al., 2012b; Ibanez et al., 2003, 2004) and traumatic brain injury (Cutler et al., 2007; Djebaili et al., 2004, 2005). In particular, neuroprotective actions of PROG for traumatic brain injury have been characterized in detail. These include the reduction of brain edema (Roof et al., 1996), mitochondrial dysfunction (Robertson et al., 2006), lipid peroxidation (Roof et al., 1997) and neuronal loss (Robertson et al., 2006; Roof et al., 1994). Furthermore, PROG facilitates cognitive recovery after traumatic brain injury (Roof et al., 1994). Clinical studies on the use of PROG for the treatment of traumatic brain injury in humans are in progress (Stein, 2011). PROG represents also a promising drug for the treatment of spinal cord trauma (Gonzalez et al., 2005; Labombarda et al., 2006a; Thomas et al., 1999).

As it has been previously mentioned for the actions of PROG in the CNS under physiological conditions, reduced PROG metabolites DHP and 3α -THP are also involved in the neuroprotective actions of PROG. Thus 3α -THP reduces seizures (Frye and Scalise, 2000) and exerts protective actions in stroke (Sayeed et al., 2006), oxygen-glucose deprivation (Ardeshiri et al., 2006) and traumatic brain injury (Djebaili et al., 2004, 2005; He et al., 2004). Furthermore, both DHP and 3α -THP mediate the protective action of PROG against excitotoxicity (Ciriza et al., 2004, 2006). Reduced PROG metabolites, and in particular 3α -THP, are also potential candidates for the treatment of mood and anxiety disorders (Biggio et al., 2009; Eser et al., 2008; Turkmen et al., 2011; Wirth, 2011).

5.1.1. Mechanisms of neuroprotection by PROG and its metabolites in the CNS

5.1.1.1. Trophic actions and activation of survival pathways. PROG and its metabolites may exert neuroprotection by directly promoting neuronal survival by the activation of signal transduction cascades associated with cell survival, such as the mitogen-activated protein (MAP) kinase and phosphoinositide-3 kinase pathways (Kaur et al., 2007; Nilsen and Brinton, 2002; Singh, 2005) and by the regulation of the expression of proapoptotic and anti-apoptotic proteins (Djebaili et al., 2005; Nilsen and Brinton, 2002; Yao et al., 2005). PROG reduces also oxidative stress (Ozacmak and Sayan, 2009) and lipid peroxidation (Roof and Hall, 2000). In addition, the PROG metabolite 3α -THP may promote neuroprotection by the inhibition of the mitochondrial permeability transition pore (Sayeed et al., 2009).

Trophic actions of PROG and its metabolites, regulating neuritic growth and synaptic plasticity (Foy et al., 2008; Reyna-Neyra et al., 2002) or promoting neurogenesis under neurodegenerative conditions (Wang et al., 2008) may also contribute to the neuroprotective mechanisms. These trophic actions may be mediated by the regulation by PROG of the synthesis of neurotrophins, such as brain-derived neurotrophic factor, which is upregulated by PROG in the spinal cord (Gonzalez Deniselle et al., 2007; Gonzalez et al., 2005) and in organotypic cultures of the cerebral cortex (Kaur et al., 2007; Singh and Su, 2013b; Su et al., 2012).

Classical intracellular PROG receptors, as well as membrane PROG receptors, are involved in the neuroprotective actions of progesterone (Pang et al., 2013; Singh and Su, 2013a; Su et al., 2012). Furthermore, as mentioned before, PROG neuroprotective actions are also mediated through potentiation of GABA-A receptor activity by its reduced metabolite 3α -THP (Ardeshiri et al., 2006).

5.1.1.2. Control of neuroinflammation and gliosis. Neuroinflammation is an important feature that precedes and develops along with several different CNS diseases. Therefore, the effect of PROG and its metabolites on neuroinflammatory pathways has been specifically analyzed (Giatti et al., 2012a). As recently reviewed by Arevalo et al. (2012) several observations have been obtained *in vitro*, utilizing cultures of microglia or astrocytes exposed to lipopolysaccharide (LPS). These same observations have been extended to animal models reproducing pathological conditions ranging from neuro-immune disease like multiple sclerosis (EAE model) to neurodegenerative conditions like traumatic brain injury and excitotoxicity. Using these models, the anti-inflammatory effects of PROG and its metabolites have been assessed on their ability to reduce the activation of cells involved in the response (astrocytes, microglia for the CNS and macrophages and lymphocytes as peripheral cells invading the CNS) and on the consequent production of inflammatory mediators.

In EAE animals, PROG was able to reduce inflammatory markers, such as microglia activation, IL-1 β , IL-2 and IL-17 (Giatti et al., 2012b). Anti-inflammatory effects have been also reported after treatment with PROG metabolites. For instance, 3α -THP treatment reduces the immunoreactivity of ionized calcium-binding adapter molecule 1 (Iba1), the monocytoid cell marker, and CD3e, a lymphocytic marker, in lumbar spinal cord in a model of EAE (Noorbakhsh et al., 2011). In particular, 3α -THP diminishes phorbol ester myristate-induced expression of pro-inflammatory genes in primary monocyte-derived macrophage cultures, while it does not affect antigen specific proliferation of CD4⁺ T-cells as well as the expression of IFN- γ and IL-17 *in vitro* in the presence of myelin oligodendrocyte glycoprotein (Noorbakhsh et al., 2011). These results together with the absence of monocytoid and lymphocytic markers in the spinal cord suggest that 3α -THP

specifically inhibits the activation of both macrophages and microglia as well as the penetration of circulating lymphocytes and macrophages towards the CNS, thus preventing the exacerbation of the immune response. Consistent with the role of a deregulated inflammatory response in the progression of CNS diseases, 3 α -THP treatment diminishes EAE severity (Noorbakhsh et al., 2011).

The anti-inflammatory effect of PROG and its metabolites also applies to CNS diseases different in nature from EAE. In experimental models of traumatic brain injury, PROG treatment reduces edema, the accumulation of astrocytes in the cerebral cortex and the hippocampus, the activation of nuclear factor kappa B (NF- κ B) and the expression of IL-1 β and TNF- α (Pettus et al., 2005). Moreover, PROG attenuates the reactivity of astrocytes and microglia/macrophages in spinal cord injury models (Garay et al., 2011; Labombarda et al., 2011) and decreases the lesion volume and the expression of IL-1 β , inducible nitric oxide synthase (Gibson et al., 2008), Iba1 and cyclooxygenase-2 in ischemic-stroke models (Jiang et al., 2011). Indeed, in ischemic stroke, 3 α -THP reduces infarct size, and the production of pro-inflammatory cytokines, such as TNF- α and IL-6, and prevents blood brain barrier disruption (Ishrat et al., 2010). Moreover, THP decreases the expression of IL-1 β , TNF- α (He et al., 2004) after traumatic brain injury and increases CD55, a potent inhibitor of the complement convertases that are activators of the inflammatory cascade (VanLandingham et al., 2007).

PROG and its metabolites DHP and 3 α -THP also reduce reactive gliosis in the hippocampus of female rats injected with kainic acid (Ciriza et al., 2004). In this animal model of excitotoxicity, the inhibition of PROG metabolizing enzymes (i.e., 5 α -R and 3 α -HSD) blocked the antigliotic effect of PROG (Ciriza et al., 2006). This is a very interesting finding in the light of the observation that the conversion of PROG into their metabolites is altered in experimental models reproducing different neurological diseases (Melcangi et al., 2012).

Although no direct evidence exists, GABA-A receptors have been evoked to explain part of the anti-inflammatory effect of 3 α -THP. Indeed, GABA-A is expressed in resident and circulating monocytoïd cells of the immune system (Bhat et al., 2010) as well as in astrocyte and microglia (Lee et al., 2011). Furthermore, GABA suppresses the reactive response of glial cells to inflammatory stimuli, such as LPS and IFN- γ , by inhibiting the activation of the inflammatory pathways mediated by NF- κ B and p38 MAP kinase (Lee et al., 2011). Nevertheless, since 3 α -THP can be reversibly converted to DHP (Melcangi et al., 2008), the recruitment of the classical nuclear PR cannot be totally excluded.

5.1.1.3. Remyelination. Examples of stimulatory effects of PROG on myelin proteins and myelin membranes have been also reported in CNS. In this regard, as demonstrated in explant cultures of cerebellar slice, PROG promotes myelination by increasing myelin basic protein (MBP) expression (Ghoumari et al., 2003). PROG also promotes remyelination by oligodendrocytes *in vivo*. Indeed, MBP expression is increased after treatments with PROG along with 17 β -estradiol in cuprizone-treated animals (i.e., a demyelinating experimental model) (Acs et al., 2009) or with PROG alone in a model of spinal cord injury (Labombarda et al., 2006a, 2009).

In the STZ model of diabetes, the mRNA levels of two isoforms of MBP, 18.5 and 21.5 kDa, are decreased in the spinal cord and the treatment with PROG, counteracts this effect (Pesaresi et al., 2010a). Interestingly, metabolism of PROG into DHP by the enzyme 5 α -R, seems to exert an important role in such an effect. Indeed, 5 α -R mRNA levels and DHP amounts are both reduced by diabetes in spinal cord, but the treatment with PROG counteracts these effects. Moreover, treatment with DHP is able to mimic the effect of PROG on MBP gene expression, suggesting that the effects of PROG are due to its enzymatic conversion into DHP (Pesaresi et al., 2010a).

5.2. Neuroprotective effects in the PNS

The protective effects of PROG and its metabolites have been also explored with success in the PNS (Melcangi et al., 2011). For instance, aging induces important biochemical and morphological changes in peripheral nerves (Azcoitia et al., 2003; Melcangi et al., 2003a). PROG or its derivatives are able to stimulate the low expression of P0 and PMP22 in the sciatic nerve of aged rats (Melcangi et al., 1998, 2003a, 2000b). Moreover, they have also clear beneficial effects on the number and shape of myelinated fibers as well as on the frequency of myelin abnormalities (Azcoitia et al., 2003; Melcangi et al., 2003a).

Protective and regenerative effects of PROG and its metabolites have been well characterized in experimental models of degeneration occurring after physical injury of peripheral nerves. For instance, (1) PROG or DHP, increase gene expression of P0 after nerve transection (Melcangi et al., 2000a); (2) PREG and PROG counteract the decrease of the amounts of myelin membranes induced by a cryolesion in the sciatic nerve of the mouse (Koenig et al., 1995); (3) In guided regeneration of facial nerve of rabbit, PROG induces an increase in the number of Schwann cell nuclei, of nonmyelinated and myelinated nerve fibers (with increase also in their diameters), as well as in the g-ratio of myelinated nerve fibers (Chavez-Delgado et al., 2005) and (4) In crush injury model, DHP and/or PROG counteract biochemical alterations (i.e., myelin proteins and Na⁺,K⁺-ATPase pump) and stimulate reelin gene expression. These two neuroactive steroids also counteract nociception impairment, and DHP treatment significantly decreases the up-regulation of myelinated fibres' density occurring in crushed nerves (Roglio et al., 2008).

Effects of PROG and its derivatives have been also evaluated in an experimental model of chemotherapy-induced peripheral neurotoxicity, such as those due to docetaxel (Roglio et al., 2009). In particular, treatment with DHP or its precursor, PROG, counteracts docetaxel-induced neuropathy, preventing nerve conduction velocity and thermal threshold changes, and degeneration of skin nerves in the footpad. PROG and DHP also counteract the changes in gene expression of several myelin proteins, such as P0, PMP22, myelin and lymphocyte-associated protein and MBP induced by docetaxel in the sciatic nerve. Most nerve abnormalities observed during the treatment with docetaxel spontaneously recovered after drug withdrawal, similarly to what occurs in patients. However, results of midterm follow-up experiments indicate that animals treated with DHP or PROG show a faster recovery (Roglio et al., 2009).

Neuropathic pain is another important consequence of peripheral nerve damage. PROG metabolites exert a beneficial effect also on this component, since both DHP and 3 α -THP suppress neuropathic symptoms (allodynia/hyperalgesia) evoked by antineoplastic drugs, such as vincristine (Meyer et al., 2010) or oxaliplatin (Meyer et al., 2011).

Another experimental model in which protective effects of PROG and its metabolites have been ascertained is diabetic neuropathy. Thus, in STZ-treated rats, PROG or its derivatives improve sciatic nerve conduction velocity, myelin protein mRNA levels (i.e., P0 and PMP22), Na⁺,K⁺-ATPase activity, thermal threshold, skin innervation density (Leonelli et al., 2007) and counteract the increase in the number of fibers with myelin infoldings (Veiga et al., 2006).

6. Pharmacological approaches to increase the levels of progesterone metabolites during neurodegeneration

The finding of altered levels of neuroactive steroids in several experimental models of neurodegeneration and the beneficial effects for the nervous system of the treatments with these molecules, suggest possible therapies based on neuroactive

steroids themselves or with synthetic ligands able to interact with their receptors. However, an alternative strategy for neurodegenerative disorders could be to increase the levels of neuroactive steroids directly in the nervous system. This strategy may avoid possible endocrine side effects exerted by the systemic treatment with neuroactive steroids. With this perspective, ligands of TSPO, with the capacity to increase the levels of PREG and consequently those of the following neuroactive steroids (Papadopoulos et al., 2006a,b), may represent a promising option. The effects of these molecules have been assessed in male diabetic animals. As observed in STZ model, Ro5-4864 (i.e., a synthetic ligand of TSPO), increases neuroactive steroid levels in sciatic nerve of male diabetic animals and exerts neuroprotective effects (Fig. 5). In particular, the treatment with the ligand was able to significantly stimulate the low levels of PREG and PROG observed in the sciatic nerves of diabetic rats and to counteract neurophysiological, biochemical and neuropathological changes occurring in this experimental model (Giatti et al., 2009). Thus, TSPO activation was effective in reducing the severity of diabetic neuropathy through a local increase of neuroactive steroid levels.

Another possible strategy could be the activation of the liver X receptor (LXR) (Fig. 5). Indeed using a synthetic ligand, such as GW3965, an increase of neuroactive steroid levels, such as PREG, PROG and DHP occurs in the sciatic nerve of male diabetic rats. In addition, GW3965 protects the nerve from diabetic neuropathy (Cermenati et al., 2010). TSPO and LXR ligands are also effective in the CNS (Fig. 5). Data obtained have indicated that the treatment with either Ro5-4864 or with GW3965 induced an increase in the levels of PROG and its metabolites in the spinal cord, the cerebellum and the cerebral cortex of STZ-male rats, but not in

the CNS of non-diabetic animals (Mitro et al., 2012). In particular, the activation of LXRs seems to be very interesting, because, at variance to Ro5-4864, GW3965 treatment did not induce significant changes in the plasma concentrations of neuroactive steroids. Therefore, GW3965 increases neuroactive steroid levels in the CNS avoiding modifications in their plasma levels and their consequent peripheral effects, one of the major limits of a neuroprotective therapy based on neuroactive steroids themselves. Moreover, GW3965 treatment, in comparison to that with Ro5-4864, seems to be more specific in increasing the amounts of PROG and its metabolites (Mitro et al., 2012). In this context, it is important to note that treatment with DHP was able to counteract the decrease of expression of MBP observed in the spinal cord of diabetic animals (Pesaresi et al., 2010a) and that GW3965 treatment induced an increase of DHP in the spinal cord of STZ-treated male rats associated with an increase of MBP expression in the same nervous structure (Mitro et al., 2012). In conclusion, these findings suggest that the pharmacological activation of LXR to induce steroidogenesis in the CNS may represent a therapeutic approach to counteract neurophysiological and structural changes induced by diabetes and may be by other pathological conditions.

7. Conclusions

The studies reviewed here indicate that PROG and its metabolites exert a wide range of actions under physiological conditions, regulating neuronal and glial development, plasticity and function. Physiological actions of PROG and its metabolites not only play a major role in the control of neuroendocrine and behavioral events associated with reproduction, but also have an impact on mood and affection. As we have seen in the previous pages, recent studies have shown that local PROG synthesis in hypothalamic astrocytes is modulated by physiological changes in the levels of the ovarian hormone estradiol during the estrous cycle. This indicates that the levels of PROG and its metabolites can be modified in the nervous system under some physiological circumstances. However, we still have a very incomplete picture on how the levels of PROG and its metabolites are regulated in the nervous system under physiological conditions and on the consequence of these changes for neural function. In particular, it is important to determine the changes in the levels of PROG and its metabolites in the nervous system at key life stages, such as perinatal development, puberty, reproductive cycles, pregnancy, postpartum period, aging and menopause. Interestingly, the levels of PROG and its metabolites in the nervous system change with regional specificity after gonadectomy, suggesting that hormonal changes associated to the above mentioned life stages may impact on the levels of these neuroactive steroids which, in turn, may cause modifications in mood, anxiety and affection.

The functional consequences of the regional-specific changes in the nervous tissue levels of PROG and its metabolites under pathological conditions should also be explored. It may be assumed that the alteration in the local levels of PROG and its metabolites in the CNS along the course of neurological and neuropsychiatric diseases might contribute to the progression of the pathology. In particular, as it has been mentioned above, a decrease in the nervous tissue levels of PROG and DHP, together with an alteration in the levels of 3 α -THP and 3 β -THP, are common observations in several experimental models of neurodegeneration. These changes may increase neural damage due to the impairment in the endogenous protective mechanisms activated by PROG and its metabolites and by an altered regulation of GABA-A receptor due to the imbalance between 3 α -THP and 3 β -THP levels. However, it can not be excluded that at least in some cases, such as the increase in the levels of 3 α -THP observed in the spinal cord and the cerebral cortex during

Fig. 5. Effect of GW3965 (green arrow) and Ro5-4864 (red arrow) on the levels of pregnenolone (PREG), progesterone (PROG) and its metabolites in sciatic nerve and central nervous system areas of male diabetic animals. Details are provided in the text. Cholesterol (Chol); translocator protein 18 kDa (TSPO); Steroidogenic acute regulatory protein (StAR); dihydroprogesterone (DHP); 3 α ,5 α -tetrahydroprogesterone (3 α -THP); 3 β ,5 α -tetrahydroprogesterone (3 β -THP); 3 β -hydroxysteroid dehydrogenase (3 β -HSD); 5 α -reductase (5 α -R); 3 α -hydroxysteroid oxidoreductase (3 α -HSOR); 3 β -hydroxysteroid oxidoreductase (3 β -HSOR).

the acute phase of EAE in male rats, the modifications may represent an adaptive endogenous neuroprotective response.

Another important consideration is that the levels of PROG and its metabolites show a sex dimorphism in the CNS and PNS and respond to modifications in peripheral levels of steroid hormones and to pathological conditions with a sex-dimorphic pattern. These sex differences in neurosteroid levels may be related with the reported sex differences in the incidence, symptomatology and/or neurodegenerative outcome of different neurological and affective and psychiatric disorders. These include Parkinson's disease, Alzheimer's disease, Huntington's disease, multiple sclerosis, traumatic brain injury, stroke, anxiety and eating disorders, autism, schizophrenia, depression, and peripheral neuropathy. Therefore, it is important to consider the sex dimorphic levels of PROG and its metabolites in the nervous system to design sex-specific therapeutic approaches. These therapeutic approaches may include pharmacological treatments aimed to increase the levels of PROG and its metabolites in the nervous system.

Finally, the importance of PROG reduced metabolites for the physiological actions and neuroprotective mechanisms of PROG call for the consideration of the possible consequences for human health of drugs that may interfere with PROG metabolism. In this regard it is important to determine whether the inhibition of PROG metabolism contributes to the side effects reported in some men that have been treated with 5 α -reductase inhibitors (Irwig, 2012a,b).

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