The other side of progestins: effects in the brain

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Abstract
Progestins are a broad class of progestational agents widely differing in their chemical structures and pharmacological properties. Despite emerging data suggest that progestins, besides their action as endometrial protection, can also have multiple nonreproductive functions, much remains to be discovered regarding the actions exerted by these molecules in the nervous system. Here, we report the role exerted by different progestins, currently used for contraception or in postmenopausal hormone replacement therapies, in regulating cognitive functions as well as social behavior and mood. We provide evidence that the effects and mechanisms underlying their actions are still confusing due to the use of different estrogens and progestins as well as different doses, duration of exposure, route of administration, baseline hormonal status and age of treated women. We also discuss the emerging issue concerning the relevant increase of these substances in the environment, able to deeply affect aquatic wildlife as well as to exert a possible influence in humans, which may be exposed to these compounds via contaminated drinking water and seafood. Finally, we report literature data showing the neurobiological action of progestins and in particular their importance during neurodegenerative events. This is extremely interesting, since some of the progestins currently used in clinical practice exert neuroprotective and anti-inflammatory effects in the nervous system, opening new promising opportunities for the use of these molecules as therapeutic agents for trauma and neurodegenerative disorders.

Introduction

These effects are mediated by an array of PROG receptors that include the classic nuclear progesterone receptors (PGR) and membrane-bound PROG receptors (Fig. 1). Two major isoforms of PGR are known: PGR-A and PGR-B, which are defined as ligand-activated transcription factors. Binding of PROG to these receptors produces multiple effects by regulating gene transcription (Brinton et al. 2008). PROG-induced nongenomic activity is mainly mediated by two different membrane-bound PROG receptor types: the seven-transmembrane progesterone adiponectin Q receptors (PAQR) and the single-transmembrane PGRs (Thomas 2008, Singh et al. 2013). The former are G protein-coupled membrane receptors and include three subtypes of receptors: PROG membrane receptors (mPGR) α, β, and γ, also referred to as transmembrane PGRs α, β, or γ (7TMPRa,β,γ) and the two more recently identified subtypes mPGRδ and mPGRe (Pang et al. 2013, Hossain et al. 2015). When bound to its ligand, these receptors block adenylate cyclase activity to reduce excitation in the CNS (Brinton et al. 2008, Thomas 2008, Do Rego et al. 2009, King 2013). The single-transmembrane PGRs are progesterone receptor membrane component one (PGMRC1, also termed as 25-Dx in rat and Hpr6 in human) and the closely related PGMRC2, which displays moderately high binding affinity for PROG. It is also worth mentioning that the classical PGR can also mediate the effects of PROG on signaling pathways through rapid nongenomic extranuclear mechanisms (Singh et al. 2013).

Besides PROG itself, its metabolites also exert important effects in the nervous system through the involvement of multiple receptors and signaling pathways. Indeed, PROG is converted into dihydroprogesterone (DHP) by the enzyme 5α-reductase. In turn, DHP is further converted into allopregnanolone or isopregnanolone by 3α-hydroxysteroid oxidoreductase or 3β-hydroxysteroid oxidoreductase, respectively (Melcangi et al. 2008). These enzymatic conversions have a deep impact on the mechanism of action of PROG. Indeed, while DHP, like its precursor, is able to interact with the classical steroid receptor, the PGR (Melcangi et al. 2008), allopregnanolone is a potent ligand of a nonclassical steroid receptor, such as the gamma-aminobutyric acid type A (GABA-A) receptor (Lambert et al. 2003, Belelli & Lambert 2005) (Fig. 1). Furthermore, several effects of allopregnanolone appear to be mediated by PAQR and by the pregnane xenobiotic receptor (PXR) (Cooke et al. 2013, Frye et al. 2013). In contrast, isopregnanolone does not bind directly to the GABA-A receptor (Bäckström et al. 2005, Bitran et al. 1991), but it antagonizes the effect of allopregnanolone on the GABA-A receptor (Wang et al. 2002). Many reviews have recently discussed the effects of PROG and its metabolites in the nervous system (Melcangi et al. 2014, 2015, Schumacher et al. 2014, 2016, Barros et al. 2015). On the contrary, little attention was addressed on the actions exerted by synthetic progestins on the nervous functions. In this study, data so far obtained will be reviewed.

Figure 1
Schematic representation of the different mechanisms of action of progesterone, its metabolites, and progestins. As reported in the text, progesterone and progestins interact with the intracellular steroid receptor (SR), which is coupled to heat shock proteins (HSP) in the cytoplasm. After binding with its ligand, SR interacts into the nucleus as dimer with the hormone responsive elements (HRE), thus activating transcription of target genes. SR includes different kinds of receptors, such as progesterone receptor, androgen receptor, estrogen receptor, glucocorticoid receptor, and mineralocorticoid receptor. Moreover, progesterone, allopregnanolone, and progestins (see text for details) may interact with members of membrane receptor families, such as the seven-transmembrane progesterone adiponectin Q receptors (PAQR) and progesterone receptor membrane components (PGMRC), promoting intracellular signaling cascades. Furthermore, allopregnanolone interacts with GABA-A receptor, promoting a chloride (Cl–) influx.
Chemical structure and mechanism of action

In recent years, several papers have tried to clarify the terminology used to describe synthetic steroids structurally distinct to PROG but designed to target the PGR. However, there is still confusion about which terminology should be used.

The term progestogens is a functional definition and refers to the broad class of progestational agents. These include both the natural hormone PROG and the synthetic progestational compounds. The latter are referred to as progestins (Stanczyk 2003).

Although progestins are generally classified as compounds with progestational activity, meaning the capacity to induce secretory endometrium and support gestation, in the human only PROG is capable of maintaining pregnancy. Synthetic progestins commonly used in clinical practice, indeed, have been selected on other characteristics, like for instance greater bioavailability, half-life, and activity after oral administration or inhibition of ovulation (Pasqualini 1996, Sitruk-Ware 2000).

From the first progestin, synthesized by Hans H. Inhoffen and Walter Hohlweg in 1938 (Inhoffen & Hohlweg 1938), several synthetic progestins, which differ in their chemical structures and biological effect, have been synthesized.

These molecules are generally classified into two groups based on the structural similarities with testosterone or PROG.

Progestins structurally related to testosterone

Progestins structurally related to testosterone are subdivided into those containing an ethinyl group at carbon 17 and those nonethinylated (Fig. 2). The substitution of a hydrogen on C17 with an ethinyl group into the testosterone molecule increases the oral potency of these compounds (Sobey 2008). This led to the synthesis of the first oral progestin ethisterone. Subsequently, removal of the carbon-19 from ethisterone to form norethisterone changed the major hormonal effect from that of an androgen to that of a progestogen without destroying the oral activity. This characteristic is present in most of the progestins related to testosterone available now for therapeutic use (19-nortestosterone derivatives), conferring them progestational activity. Progestins belonging to the ethinylated group are further divided into those related to the parent steroid with 18 carbon (estrane), with a methyl group at carbon 13 and those with an ethyl group at carbon 13 (13-ethylgonanes, also known as gonanes) (Edgren & Stanczyk 1999).

The nonethinylated group of progestins includes dienogest (DNG) and the spirolactone derivative drospirenone (DRSP), whose structure is similar to that of the aldosterone antagonist spironolactone. DRSP, compared with both estrane and 13-ethylgonanes, is the only exception in which the methyl group at carbon 10 has not been removed.

Progestins structurally related to progesterone

Progestins structurally related to PROG (Fig. 3) can be subdivided into 17-hydroxyprogesterone derivatives (or pregnanes 21 carbons) and 19-norpregnane derivatives (20 carbons) on the basis of whether or not they contain a methyl group on carbon 10, respectively (Stanczyk 2003). The deletion of the CH3 radical in position C19 of the hydroxyprogesterone skeleton confers to the 19-norpregnane derivatives higher progestational potency when compared with pregnane, making these molecules to bind more selectively to the PGR. In particular, nomegestrol acetate, 19-norprogesterone and promegestone (R5020) are the most selective agonists of the PGR, with little or no activity for other steroid receptors (Sitruk-Ware 2002, Schindler et al. 2003, Garcia-Becerra et al. 2004).

Pregnanes and 19-norpregnanes are further separated into classes of compounds with and without an acetyl group. Among the acetylated pregnane, the 17α-hydroxyprogesterone derivative medroxyprogesterone acetate (MPA), is one of the best-known and most widely used progestins in the USA (Stanczyk & Bhavnani 2014).

Finally, the retroprogesterone derivative dydrogesterone is a stereoisomer of natural PROG. Compared with other progestogens, whose molecular structure (with the four rings in a plane) is almost flat, retroprogesterone molecule is bent. This unique conformation is due to the presence of the methyl group at carbon 10 in α-position, the hydrogen at C9 in β-position and an additional double bond between carbon 6 and 7.

Unlike other progestogens, dydrogesterone is a highly selective progestin, binding almost exclusively PGR. This is due to the rigid conformation of its retrostructure, which is suitable for its interaction with PGR, but not with the other steroid hormone receptors (Colombo et al. 2006). In particular, dydrogesterone is inactive as agonists of human mineralocorticoid receptor (MR) and shows negligible androgenic, estrogenic or mineralocorticoid activities (Kuhl 2005, Rižner et al. 2011).
Interaction of progestins with steroid receptors

Small structural changes of progestins may account for considerable differences in their pharmacological properties and actions. The chemical structure of progestins influences their binding affinity with receptors and, in turn, the transcriptional activity of the dimer complex. Thus, considering that progestins can be derivates of PROG, testosterone, and even spirolactone, it is not surprising that they can also bind with other members of the steroid receptor family, exhibiting additional nonprogestagenic biological effects.

Most progestins, indeed, also interact with the androgen receptor (AR), estrogen receptor (ER), glucocorticoid receptor (GR), and MR (Sitruk-Ware 2004). Progestins present clear differences in their binding affinities for AR, ER, GR, and MR based on the chemical structure of both the progestin and the ligand-binding domain of the steroid receptor. The interaction of progestins with one or more receptors, in turn, may be associated with an agonistic, antagonistic, or no effect according to the co-activators or co-repressors involved in the receptor interaction (Conneely & Lydon 2000, Stanczyk et al. 2013).

Figure 2
Chemical structure of progestins structurally related to testosterone. Surrounded by a solid line is the molecule of testosterone, surrounded by a dotted line is the spirolactone derivative drospirenone. In the gray panels are reported prodrugs. These latter molecules require in vivo biochemical transformation to active metabolites. Chemical structures of clinically relevant progestins are identified with an asterisk (*) before the name. Data from Liu et al. (2010) and Stuenkel et al. (2015).
Among 17-hydroxyprogesterone derivatives, cyproterone acetate (CPA), DNG, and chlormadinone acetate (CMA) are potent anti-androgenic compounds, whereas MPA transactivates both AR and GR (McLeod 1993, Bamberger et al. 1999, Raudrant & Rabe 2003).

The 19-norpregnane derivatives like, for example, promegestone, trimegestone (TMG), nestorone, and nomogesterol acetate, are the most selective agonists of the PGR with very little or no activity for other steroid receptors (Sitruk-Ware & Nath 2010, Stanczyk et al. 2013). On the other hand, it is quite common to find androgenic properties among progestins structurally related to testosterone like levonorgestrel (LNG), norethisterone (NET), and tibolone (TIB). Moreover, some reduced metabolites of progestins structurally related to testosterone, besides having some androgenic effects, also show a slight estrogenic activity (Garcia-Becerra et al. 2002). DRSP, since it is a derivative of the well-known MR antagonist spironolactone, has antimineralocorticoid properties (Oelkers et al. 1991, Delyani 2000, Oelkers 2004) and anti-androgenic effects (Muhn et al. 1995, Krattenmacher 2000).
The finding that progestins can also interact with other steroid receptors (i.e., AR, ER, GR, MR) is indeed not surprising. All these proteins belong to the nuclear receptor superfamily and have structural similarity exhibiting a highly conserved overall domain structure (Germain et al. 2006, Pawlak et al. 2012). Progestins, which exhibit relatively high affinity to the AR, generally belong to the first-generation synthetic progestins (Stanczyk et al. 2013). Among these, there are MPA and norethynodrel, one of the first progestin synthesized (Sitruk-Ware 2000). NET (also known as norethindrone) and LNG are examples of progestins of second generation. They also have high binding affinity for the AR conferring them some undesirable androgenic effects (Nilsson & von Schoultz 1989, Campos et al. 1999).

Over the more recent decades, newer progestins were synthesized with the goal of finding a molecule without any androgenic or glucocorticoid effect. These new progestins, beside having a strong progesterational action, also exert anti-estrogenic, antigonadotropic, and antimineralocorticoid effects (Africander et al. 2011). Among these, there are the third-generation synthetic progestins (desogestrel or gestodene), derived from LNG group, developed to decrease androgenic activity (LeBlanc & Laws 1999), and the fourth-generation progestins. In this latter group, nomegestrol acetate exhibits partial anti-androgenic activity (Lello 2010, Van Diepen et al. 2011) DNG, DRSP, and trimgestone have a significant anti-androgenic activity, whereas norethisterone has no activity via the AR (Fotherby 1990, Philibert et al. 1999, Schindler et al. 2003).

In contrast to PROG, which also shows an affinity for membrane PGRs (Falkenstein et al. 1999, Zhu et al. 2003), very little is known concerning the possible binding of progestins to this receptor. As showed by Thomas et al. (2007), among the 30 steroidal compounds tested, only norprogesterone and pregna-4,9(11)-dione-3,20-dione displayed a relative binding affinity (RBA) of 51.8 and 50.9% compared with that of PROG, whereas the RBAs of the remaining progestins tested were less than 50% (Thomas et al. 2007).

However, no conclusive data about affinity of progestins for membrane PGRs are present in literature, so we cannot exclude that some of the effects of these synthetic compounds in the CNS could be mediated by these receptors. It is worthwhile to note that, since the relative levels of different receptors can vary greatly in different tissues, the physiological effects of a particular progestin may be influenced by the different expression of steroid receptors in different cell types (Markov et al. 2010). The specific action of different progestins may also be influenced by other factors such as the bioavailable (free) fraction of both the circulating progestins and endogenous competing steroids. This, in turn, depends on the concentration and affinity of plasma binding proteins that bind steroids (i.e., albumin, corticosteroid-binding globulin (CBG), and sex hormone binding globulin (SHBG)) (Hammond 2002, Schindler et al. 2003) as well as by the metabolism of progestins, which can result in compounds that have different activities (Stanczyk 2003).

**Metabolism of progestins**

Most of progestins, like their related steroids (i.e., PROG and testosterone), contain a ketone group at carbon 3 and a double bond between carbons 4 and 5 (Δ4-3-ketone structure). These characteristics are the prerequisite for the progestogenic activity. Therefore, progestins can be metabolized by 5α- or 5β-steroid reductases and hydroxysteroid dehydrogenases in a similar manner as their parental compound. Apart from steroid 5α-reductase isozyme family (e.g., SRD5A1, SRD5A2, and SRD5A3) (Azzouni et al. 2012), all the remaining enzymes involved in the two-step reduction process in humans belong to the aldo-keto reductase (AKR) superfamily. This includes steroid 5β-reductase or AKR1D1 (Di Costanzo et al. 2008, Chen et al. 2011) and AKR1C enzymes: AKR1C1 (20α,(3α)-hydroxysteroid dehydrogenase (HSD)) (Hara et al. 1996), AKR1C2 (type 3, 3α-HSD) (Deyashiki et al. 1994, Dufort et al. 1996), AKR1C3 (type 2, 3α-HSD and type 5, 17β-HSD) (Khanna et al. 1995), and AKR1C4 (type 1, 3α-HSD) (Penning et al. 2000). These enzymes are promiscuous since they can catalyze 3-keto- and also 20-keto- and 17-keto-steroid reduction and are generally expressed in different tissues with the exception of AKR1C4, which is highly liver specific (Penning et al. 2000). The metabolism of progestins by the AKR1C enzymes may influence their availability for their cognate steroid hormone receptors and in turn their tissue-specific effects.

Reduction at a double bond between carbons 4 and 5 and at the carbonyl group at carbon 3, of NET, LNG, and gestodene, results in the formation of dihydro- and tetrahydro-derivatives (Stanczyk 2003). The 5α-reduction of NET, even if confers a relatively high binding affinity to the AR, diminishes its androgenic potency (Lemus et al. 1997, Garcia-Becerra et al. 2002, Morali et al. 2002). The 3β,5α-reduced metabolite of this compound is able to
activate gene transcription via ER, although its binding affinity is lower than estradiol (Larrea et al. 2001, Santillán et al. 2001, Pasapera et al. 2002, Enríquez et al. 2007). This finding is in accordance with further results, showing that tamoxifen (i.e., an estrogen binding site competitor) reduced the effect of 3β,5α-NET administration, and that the activity of 5α-NET is inhibited by the treatment with CPA, a 3β-hydroxysteroid dehydrogenase/Δ4,5 isomerase inhibitor (Larrea et al. 1987).

The 17-hydroxyprogesterone derivative CMA, together with MPA, is one of the first PROG derivatives. Like PROG, it has a Δ4-3-ketone structure. Reduction of the 3-keto group with preservation of the Δ4-double bond results in the formation of 3α-hydroxy-CMA, which exhibits strong anti-androgenic activity (Rabe et al. 2012). On the other hand, although MPA also possesses a Δ4-3-ketone structure, is not metabolized in the same way as the related steroid PROG, probably because of the steric hindrance due to the acetate group at carbon 17 (Stanczyk 2003). Indeed, MPA undergoes ring A reduction and hydroxylation at carbons 6 (Stanczyk et al. 2013).

The hydroxylation of the 19-norpregnane derivatives TMG into 1β- and 6β-hydroxy-TMG metabolites confers progestogenic potency to these compounds.

The retroprogesterone derivative dydrogesterone, after its oral administration, is rapidly absorbed and metabolized into its active 20α-dihydro metabolite (20α-DHD) by hydrogenation of the 20-ketogroup (Beranič et al. 2011). Both human AKR1C isoforms, AKR1C1 and AKR1C2 can metabolize dydrogesterone with high efficacy, whereas AKR1C3 is less active (Beranič et al. 2011, 2012). Interestingly, although dydrogesterone is close in structure to progesterone, it is metabolized by AKR enzymes only to 20α-DHD, whereas progesterone is transformed to several products including 5α-pregnane, 5β-pregnane and 4-pregnene metabolites (Rižner & Penning 2014). Recently, Olbrich et al. (2016) showed an involvement of cytochrome P450 isozymes in the metabolism of dydrogesterone.

Some of the progestins lack Δ4-3-ketone structure. Thus, they require in vivo biochemical transformation to active metabolites in order to exert progestational activity. Among these, norethynodrel, lynestrenol, and ethynodiol diacetate are prodrugs of NET. TIB is a derivative of norethynodrel, however, in contrast to its precursor, it is not converted to the progestin NET but it is transformed to other active metabolites. These metabolites are Δ4-isomer, such as 7α-methyl-NET, as well as the 3α- and 3β-hydroxy metabolites. The former is generated by the enzyme 3β-hydroxysteroid dehydrogenase-isomerase, and exhibits a moderate androgenic and progestogenic activity. Conversion of TIB into 3α- or 3β-hydroxytibolone depends on the tissue where its metabolism occurs. In peripheral tissues, AKR1C1 and AKR1C2 form 3β-hydroxytibolone which exhibits estrogenic activity, whereas in liver, TIB is reduced to 3α-hydroxytibolone by AKR1C4 which functions predominantly as 3α-hydroxysteroid dehydrogenase (Steckelbroeck et al. 2004). This explains why 3α-hydroxytibolone is the major circulating metabolite, whereas 3β-hydroxytibolone is the major metabolite in target tissues (Penning et al. 2014).

Interestingly, AKR1C2 exhibits different stereochemical preference depending on the ketosteroid substrate. Indeed, it produces 3α-products with the potent androgen 5α-dihydrotestosterone (5α-DHT) behaving as an efficient 3α-hydroxysteroid dehydrogenase, whereas it inverts its stereospecificity with TIB acting as a 3β-hydroxysteroid dehydrogenase (Steckelbroeck et al. 2004, Rižner & Penning 2014). Finally, TIB can also be metabolized, in less amount, to the potent estrogen 7α-methyl-ethinyloestradiol (Wiegratz et al. 2002). The enzymatic pathway for this latter conversion, however, has not been clearly characterized (Bodine et al. 2002, Wiegratz et al. 2002, Dröge et al. 2007, Zacharia et al. 2007, Rabe et al. 2012). Norethynodrel which is identical to TIB except for lacking a methyl group at carbon 7, follows the same stereochemical outcome as observed for TIB suggesting that the reduction of the 3-ketosteroid by AKR1C isoforms is not influenced by the 7-methyl group (Jin et al. 2012).

Desogestrel and norgestimate (NGM) are also prodrugs converted in the progestationally active metabolites, such as etonogestrel (also called 3-ketodesogestrel) and LNG, respectively (Stanczyk et al. 2013).

It has been well established that PROG can exert multiple effects on neurons and glial cells by local metabolism to allopregnanolone (Singh 2007, Tsutsui 2008). This 3α,5α-reduced metabolite, due to a hydroxyl group at C3, does not interact with classical intracellular steroid receptors, but it is a potent positive modulator of GABA-A receptor and results in the potentiation of GABA-induced chloride conductance (Lambert et al. 2003, Belelli & Lambert 2005). On the other hand, the conversion to 3β,5α-reduced metabolite (i.e., isopregnanolone), can antagonize the effects of allopregnanolone at GABA-A receptor (Bäckström et al. 2005, Bitran et al. 1991). As mentioned above, progestins are also converted in both 3α,5α- and 3β,5α-reduced metabolites, but the action exerted by these metabolites on GABA-A receptor needs to be clarified.
Effect of progestins in the nervous system

During lifetime, healthy women may assume progestin compounds in order to control ovary functions at younger ages, or to limit complications of menopause. Thus, the progestin compound assumed is related to the peripheral function that a woman wants to control. As previously described, a plethora of progestin molecules have been synthesized, with different characteristics. However, little information is available about their effects on brain functions. Here, we discuss some data concerning their neural effects in animal models and in women having combined oral contraceptive (COC) or hormone replacement therapy (HRT).

Observations in animal models

Ovariectomy is a surgical procedure widely used in experimental models to mimic the hormone drop occurring in menopause. Indeed, this technique has been used to explore the effects of different progestin compounds, alone or in combination with estrogens, on allopregnanolone and β-endorphin levels. As mentioned above, the PROG metabolite allopregnanolone interacts with GABA-A receptor, and the reduction of its levels in menopause are thought to negatively influence women’s psychosocial behavior (Sator et al. 1999). Clinical evidence, indeed, suggested that the administration of PROG to peri-menopausal women promotes the length and quality of sleep (Gruber & Huber 2003). Also alterations of the levels of β-endorphins (i.e., ligands of the opioid receptor exerting analgesic effects) have been reported in menopausal women, and their drop is significantly correlated to hot flushes (Tepper et al. 1987). Ovariectomy in Wistar rats reduces levels of β-endorphins and allopregnanolone in several brain regions, such as frontal and parietal lobes, hippocampus, hypothalamus, as well as in anterior pituitary and serum, whereas it increases allopregnanolone content in the adrenal gland (Genazzani et al. 2007). The progestin DRSP, when orally administered to ovariectomized rats, was able to increase the levels of β-endorphin (Genazzani et al. 2007), without affecting the reduced content of allopregnanolone. The concomitant administration of DRSP and estradiol valerate (E2-V) was able to increase β-endorphin and allopregnanolone levels. The same effect was also obtained by the administration of E2-V alone (Genazzani et al. 2007). Also CMA was able to increase allopregnanolone content in hippocampus, hypothalamus and anterior pituitary, but only if administered in combination with E2-V (Pluchino et al. 2009). In contrast, dydrogesterone alone increased allopregnanolone levels in frontal lobe, hippocampus, hypothalamus, and serum. This treatment was ineffective on β-endorphin levels that were increased only when dydrogesterone was administered in combination with E2-V (Pluchino et al. 2008). Nestorone administration was able to increase β-endorphin levels in hippocampus, while, when administered in combination with E2-V, possessed a synergistic effect on allopregnanolone levels in hippocampus and anterior pituitary (Lenzi et al. 2009). Also treatment with nomegestrol acetate influences β-endorphin and allopregnanolone levels in ovariectomized rats. In particular, when this progestin was administered alone, it induced an increase of allopregnanolone levels in hippocampus. However, when nomegestrol acetate was administered with E2-V, it induced an increase of allopregnanolone levels in both the hypothalamus and anterior pituitary (Lenzi et al. 2008). This pattern of effects was a peculiarity of the nervous system. Indeed, administration of this progestin, alone or in combination with E2-V, was able to reduce allopregnanolone levels in adrenal gland (Lenzi et al. 2008). Administration of high doses of nomegestrol acetate (1 mg/kg/day) also increased hippocampal and hypothalamic levels of β-endorphin, while only when combined with E2-V, β-endorphin levels were increased also in the anterior pituitary and plasma (Lenzi et al. 2008). Finally, also the first-generation molecule MPA was able to increase allopregnanolone levels in the frontal and parietal lobes, hypothalamus, hippocampus, and anterior pituitary and, when in combination with E2-V, to a greater degree respect to estrogen alone (Bernardi et al. 2006). The authors proposed that the increased levels of allopregnanolone may be a consequence of SRDSA and ARK1C modulation mediated by the combined treatment (Bernardi et al. 2006). On the other hand, the effect on β-endorphin levels could be supposed based on progestin structure. For example, MPA has a glucocorticoid activity that could inhibit the release of β-endorphins (Szot et al. 2004). In contrast, DRSP, through a combination of progestin, antimineralcorticoid, and anti-androgenic effects, is able to positively regulate β-endorphin levels (Genazzani et al. 2007).

Estrogens exert beneficial effects on the bioenergetics system of the brain, acting also on mitochondrial functions (Rettberg et al. 2014), which is important to sustain the energy demand of the nervous system for neurotransmission and plasticity. The drop in estrogens levels during menopause is thought to deeply affect
mitochondrial respiration and ATP generation. In agreement, OVX mice displayed decreased mitochondrial bioenergetics, a condition that could be reversed by estrogen treatment, when administered immediately after surgical procedure (Yao et al. 2012). Also PROG can modulate mitochondrial functions. Indeed, PROG and E2 are able to increase the respiratory activity of brain mitochondria in OVX rats. These effects are coupled to a reduced free radical leak and lipid peroxidation, suggesting that these compound could promote efficient and balanced bioenergetics (Irwin et al. 2008). Also other progestins may impact mitochondrial functions. In particular, as PROG, LNG, and noretosterone significantly increased ATP synthase-α subunit (complex V) expression, exerting also protective effects. In contrast, MPA did not exert similar effects. However, both MPA and LNG produced an increased Bax/Bcl-2 ratio, suggesting apoptosis (Liu et al. 2010). Overall, these data suggest that hormonal replacement may affect brain mitochondrial efficiency.

Also the effect of COC has been explored in animal models. For example, a study in female rats treated with a contraception pill containing ethinyl estradiol (EE) and norgestrel revealed that the progestin component of the pill produces a depletion of biogenic amines particularly in the hypothalamus and pons/medulla oblongata (Shetty & Gaitonde 1980). COC administration (EE + LNG) to female rats produced a decline in learning and memory (Simone et al. 2015). In addition, the same combination reduced allopregnanolone, PROG, and pregnenolone levels in cerebral cortex, hippocampus, and plasma, resulting in an impairment in social behavior and sexual motivation (Follesa et al. 2002, Sassoe-Pognetto et al. 2007, Santoru et al. 2014). An impaired social behavior was also observed in monkeys (Macaca fascicularis), after 2 years of treatment with EE and LNG (Henderson & Shively 2004). Moreover, long-term administration of this combination was also able to alter GABA-A receptor composition, increasing the expression of the γ2-subunit and thus affecting anxiety behavior in female rats (Follesa et al. 2002). A recent study addressed the question which component of the pill would be responsible for such alterations. Thus, female rats were treated with a combination of EE and LNG, or with the single molecules. The results obtained suggest that either EE or LNG alone or their combination were able to decrease pregnenolone, PROG, and allopregnanolone in hippocampus and cerebral cortex. However, only the progestin affected the expression of the α1 GABA-A receptor subunit in cerebral cortex, whereas LNG, alone or in combination, altered the levels of the γ2-subunit, increasing anxiety behavior in the elevated plus maze (Porcu et al. 2012). These results may suggest that changes in GABA-A receptor expression were not due to the reduced content of neuroactive steroids, but rather due to LNG component. Since androgens are able to affect GABA-A receptor composition (McIntyre et al. 2002, Henderson et al. 2006, Jones et al. 2006), a possible explanation of this effect could be ascribed to the androgenic activity of LNG.

Environmental effects in aquatic life

An emerging issue, concerning the role of these compounds on brain functions, is the effect of environmental relevant concentrations of PROG or progestins. Observations so far obtained underline the risk for the aquatic wildlife. For instance, decreased fecundity was observed in zebrafish (Danio rerio) after the exposition to MPA or dydrogesterone (Zhao et al. 2015a). Moreover, the effect of norgestrel exposure was evaluated in the hypothalamic–pituitary–gonadal (HPG) and hypothalamic–pituitary–adrenal (HPA) axes of juvenile zebrafish. An influence on sex differentiation was observed, with a shift in the sex ratio toward males or females depending on the dose. This shift was probably due to altered sex hormone levels, as a consequence of changes in the transcription of genes encoding enzymes of steroidogenesis (Liang et al. 2015a). Moreover, also the exposition to norgestrel in zebrafish eleuthero embryos produced alterations in many genes related to production of steroids (e.g., star, cyp19a1a, cyp11b) or their actions (Pgr and Ar) (Liang et al. 2015b). This progestin, affecting the first step of the synthesis of thyroid hormones, seems also to exert an effect in the hypothalamic–pituitary–thyroid axis. Indeed, norgestrel induced the gene expression of the sodium/iiodide symporter that is responsible for the intake of iodide anion into the follicular cells (Liang et al. 2015c). DRSP affects central functions in zebrafish brain, disturbing the circadian rhythm network (Zucchi et al. 2014, Zhao et al. 2015b). Environmental relevant doses of LNG (31 ng/L) affects the reproductive system of male pubertal roach (Rutilus rutilus), acting on the pituitary expression of gonadotrophins and on the sex steroid levels. Females were also affected by this progestin, even if at higher doses (Kroupova et al. 2014). LNG was able to disrupt the sexual development also in Xenopus laevis. In particular, the expression of follicle-stimulating hormone β-subunit was affected in a specific way in relation to the sex, LNG concentration, and stage of development, whereas the expression of luteinizing hormone β-subunit (lhβ) was reduced in both sexes (Lorenz et al. 2011a).
Moreover, LNG impaired thyroid activity during the metamorphosis of X. laevis, producing a developmental arrest (Lorenz et al. 2011b). NET (168 ± 7.5 ng/L) was able to reduce, even if not significantly, the levels of lhβ, and significantly those of ERβ in brain of female fathead minnow (Pimephales promelas) (Petersen et al. 2015).

The reported observations highlight that progestins concentration found in water could affect brain functions. This consideration may suggest potential actions on humans, possibly exposed to these compounds via contaminated drinking water and seafood, with possible additional effects in women already assuming a progestin compound.

Clinical observations

As depicted above, progestins may alter brain function in experimental models. However, whether these effects may also occur in brain of women on HRT or assuming COC, need to be elucidated. In particular, it is difficult to establish the impact of pharmacological regimens in healthy women, owing to the different preparations, doses, routes of administration, as well as the different study designs.

Progestins as components of HRT The Women’s Health Initiative (WHI) Memory Study reported a decline of cognitive functions in postmenopausal women treated with conjugated equine estrogen (CEE) and MPA (Shumaker et al. 2003). The same combination produced, in postmenopausal women independently of their age, a decline in verbal memory, which is considered a predictor of Alzheimer’s disease (Matra et al. 2015). In contrast, estrogen treatment alone was beneficial, but only in younger postmenopausal women (Maki & Sundermann, 2009). However, DNG, when combined with E2-V, was effective in improving the vigilance decrement, accounting for daytime fatigue. As detected by low-resolution brain electromagnetic tomography (LORETA), this combination, but not E2-V alone, increased activity in the right prefrontal, temporal, and superior parietal cortices and in the anterior cingulate gyrus (Saletu et al. 2005). In another study, insomniac postmenopausal women were tested with LORETA during a two-tone oddball paradigm (i.e., an auditory discrimination task). Data indicated that E2-V+DNG combination was able to increase the stimulus-induced cortical arousal in the primary and higher-order auditory cortex; an effect that was not achieved by E2-V treatment alone (Anderer et al. 2004). Moreover, the combination of EE and NET acetate produced, during a spatial working memory task, a higher activation of prefrontal cortex compared with placebo (Smith et al. 2006). Furthermore, a direct influence of estrogen and PROG on memory has been widely demonstrated (Schneider & Farlow 1997, Choi et al. 2003, Bimonte-Nelson et al. 2004, Walf et al. 2006). However, the mechanisms involved in these processes have not been yet fully identified. In particular, contradictory results have been so far obtained about PROG. For example, while estrogen seems to exert a positive action on memory, PROG administration to women with Alzheimer’s disease (Honjo et al. 2005) and in animal models (Bimonte-Nelson et al. 2006) seems to disrupt this effect. However, recent observations (Barros et al. 2015) suggest that the effect of PROG on memory is dependent on the time of administration (e.g., chronic exposure before learning vs acute administration after training). Moreover, as suggested by animal models, also the schedule of PROG treatment could be relevant. Indeed, continuous vs cyclic administration may produce different effects. For example, in hippocampus of OVX rats, no differences between vehicle-treated animals and rats administered with E2 and continuous PROG were observed. In contrast, in the same model, E2 and cyclic PROG treatment improved the expression of gene involved in mitochondrial energy, redox homeostasis, insulin signaling and cholesterol trafficking (Zhao et al. 2012). These results are also relevant in the context of HRT. Since the WHI study reported that combined estrogen–progestin regimens could increase the risk to develop dementia, the data here discussed may help the comprehension of the leading causes (Barros et al. 2015).

Progestins as components of oral contraceptives In women assuming COC, the situation seems to be more complicated. For instance, only marginal effects in the response inhibition have been observed in women who underwent EE and LNG combination (Gingnell et al. 2016). In addition, in healthy COC-naïve women, a low-dose combination was ineffective on mood (Rapkin et al. 2006). On the other hand, in another study enrolling women with previous history of COC-induced adverse mood, it was reported that the administration of EE and LNG produced depressed mood, mood swings, fatigue and lower emotional-induced reactivity in brain (i.e., in left insula, left middle frontal gyrus, and bilateral inferior frontal gyri) of COC users vs placebo controls.
Progestins in the brain

Moreover, healthy women, with no previous history of depression, reported increased anxiety and mood disorders after a long-acting subdermal implant of LNG (Wagner & Berenson 1994). Furthermore, different combinations of COC decreased plasma levels of allopregnanolone (Follesa et al. 2002, Paoletti et al. 2004, Rapkin et al. 2006). On the contrary, DRSP or chloromadinone were able to reduce anxiety and mood swings (Paoletti et al. 2004, Huber et al. 2008). Possible differences in cognitive performances have also been evaluated between COC users and nonusers. In particular, a better performance in verbal memory task has been identified in COC users vs nonusers (Gogos et al. 2013), while other observations reported no changes (Islam et al. 2008, Mihalik et al. 2009). COC users outperform in associative learning (Beck et al. 2008, Holloway et al. 2011), and better results were obtained also in verbal fluency and mental rotation (Wharton et al. 2008, Griksiene & Ruksenas 2011) when compared with nonusers. On the contrary, other observations reported no differences in spatial ability, verbal fluency, and attention (Rosenberg & Park 2002, Islam et al. 2008, Mordecai et al. 2008, Nielsen et al. 2011, Gogos 2013).

As recently emerged, discordant results on the effects of COC in human brain have also been obtained by imaging studies (Gogos et al. 2014, Pletzer & Kerschbaum 2014, Toffoletto et al. 2014). A possible explanation may be due to the molecular structure and specific generation of progestin component into COC, an issue that received little attention in the studies performed so far (Toffoletto et al. 2014). For instance, the ‘androgenicity’ of progestin compound into the pill may affect brain anatomy and consequent cognitive skills. Indeed, Pletzer et al. (2015) recently reported that frontal gray matter volume and face recognition performance are differentially affected in COC users depending on the androgenic vs nonandrogenic potency of the progestin compound. An increased volume in the parahippocampal and fusiform gyri and in cerebellum of women assuming a nonandrogenic progestin was also reported. This was accompanied by a better performance in the face recognition test with respect to the COC users with an androgenic progestin component in the pill and non-COC users (Pletzer et al. 2015).

Protective effects of progestins in the brain

Recently, it has been proposed that some of the protective effects exerted by PROG in models of neurodegeneration and trauma (Melcangi & Garcia-Segura 2010, Giatti et al. 2015, Melcangi et al. 2015) could also be achieved through the administration of a progestin compound, such as nestorone. In particular, this progestin (i.e., a potent PGR agonist) was studied in different experimental models to test possible neuroprotective effects. In the wobbler mutant mice, a model for progressive motoneuron degeneration, nestorone administration restored the spinal cord abnormalities and inverted the curved digits in forelimbs, typical of the pathology. Moreover, it increased choline-acetyltransferase, glutamine synthase, and nuclear factor of kappa light polypeptide gene enhancer in B-cells (NF-κB) inhibitor expression, while decreased astrogliosis and the expression of microglial marker lymphocyte function-associated antigen 1, TNF-α, inducible nitric oxide synthase, and NF-κB (Meyer et al. 2015). Neuroprotective and anti-inflammatory effects were also obtained in three different models of multiple sclerosis, such as the EAE mice, the cuprizone feeding, and the lysolecithin-induced demyelination models. In particular, in EAE mice, nestorone was able to decrease the neurological disability and increased the motor behavior in affected animals. In this experimental model, this progestin also decreased microglial reactivity in hippocampus, and it was able to promote cell proliferation and double-immunocytin-positive neuroblast in the same brain region, increasing also GABAergic interneurons (Garay et al. 2014). Female mice fed with cuprizone and injected with PROG or nestorone presented, to nearly the same extent, less astrocyte and microglial activation. An increased expression of two important myelin proteins (i.e., myelin basic protein and proteolipid protein), and increased density of both precursor and mature oligodendrocytes was also observed in this experimental model (El-Etr et al. 2015). Finally, the myelin-promoting effects of nestorone were evaluated in organotypic cultures of cerebellar slices from postnatal rodents, treated with lysolecitin. The synthetic progestin was able to promote the remyelination of neurons by oligodendrocytes, through a mechanism involving recruitment and maturation of oligodendrocytes precursor cells. The effects exerted by nestorone are probably due to its interaction with the intracellular PGR, because these effects were not observed in slices from PGR knockout animals (Hussain et al. 2011).

Also in a model of ischemia (i.e., the middle cerebral artery occlusion mice), nestorone exerted neuroprotective effects similar to PROG, reducing the total infarct volume and increasing the time spent by lesioned mice on the rotarod, compared with vehicle animals (Liu et al. 2012).

In contrast to what is observed with nestorone, MPA, one of the most prescribed progestin compound in HRT regiment, does not exert protective effects.

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WHI results reported an increased risk for breast cancer, coronary heart disease, stroke, and pulmonary embolism in the CEE+MPA arm of the study (Rossouw et al. 2002). In addition, many studies report conflicting results of PROG vs MPA administration in different animal models and HRT patients (Singh & Su 2013, Stanczyk & Bhavnani 2015). Observations so far obtained suggest that, even if PROG and MPA are nearly identical in preventing the uterine effects of unopposed estrogen treatment, they are profoundly different in their mechanism of action on brain. Indeed, both steroids can bind to PGR, but MPA, unlike PROG, is also able to bind AR and GR (Schindler et al. 2003) with a 300-fold higher affinity (Koubovec et al. 2005). In experimental neurodegenerative models, PROG, but not MPA, exerts protective effects. For instance, in cerebral cortical explants, PROG increased expression and protein levels of the brain-derived neutrophic factor, while MPA exerted an inhibitory effect (Jodhka et al. 2009). In hippocampal cultures, PROG was protective by reducing the Ca\(^{2+}\) influx mediated by glutamate, through the activation of extracellular signal-regulated kinase signaling and the promotion of the transcription of anti-apoptotic factors, such as B-cell lymphoma 2 (BCL2). In contrast, MPA was ineffective in contrasting the Ca\(^{2+}\) influx, did not promote the transcription of BCL2 and inhibited the protective action of estradiol, when co-administered (Nilsen & Brinton 2002, 2003). Similarly, when tested for glycolysis, oxidative stress, and mitochondrial function in brain, OVX rats treated with MPA showed a decline in glycolytic and oxidative phosphorylation protein activity, suggesting mitochondrial impairment (Irwin et al. 2011). Many neuroprotective effects of PROG seem to be mediated by its conversion into neuroactive metabolites, such as DHP and allopregnanolone (Melcangi et al. 2014, Giatti et al. 2015). Interestingly, it has been reported that MPA could antagonize the conversion of PROG into allopregnanolone, through the inhibition of ARK1C enzyme (Khanim et al. 2014), possibly blocking progesterone neuroprotective effects (Stanczyk & Bhavnani 2015).

Conclusions
Since the synthesis of the first progestin in 1938, the increasing interest and request for efficient compounds stimulated the production of new molecules, possibly with high affinity for PGR. However, despite the large clinical use of these molecules, their possible effects on brain functions have been little explored. In recent years, preclinical and clinical studies tried to fill this gap of knowledge, even though very different study designs and few attention of the molecules assumed may have produced confounding results, in particular in human studies. Moreover, all the combinations in terms of dose, duration of exposure, and route of administration, baseline hormonal status, and age of treated women, just to cite some, increased the complexity of this field. Furthermore, another level of complication, highly ignored, is represented by their mechanism of action, such as the interaction with different receptors as well as by their metabolism. In particular, this latter aspect should be further explored because the effects of PROG in brain are deeply linked to its metabolism, and, possibly, also progestin compounds are exposed to the same fate. For these reasons, rigorous guidelines should be proposed, and new animal and human studies performed, in order to dissect this intriguing and fascinating field of research.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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