The other side of progestins: effects in the brain

Silvia Giatti, Roberto Cosimo Melcangi and Marzia Pesaresi

Department of Pharmacological and Biomolecular Sciences, Center of Excellence on Neurodegenerative Diseases, Università degli Studi di Milano, Milan, Italy

Correspondence should be addressed to R C Melcangi
Email
roberto.melcangi@unimi.it

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.

Key Words

- ▶ progesterone
- ▶ testosterone
- ▶ combined oral contraceptive
- ▶ hormone replacement therapy
- ▶ neuroprotection

Journal of Molecular Endocrinology
(2016) **57**, R109–R126

Introduction

An extensively demonstrated progesterone (PROG), produced in peripheral glands as well as directly in the nervous system (Melcangi *et al.* 2008, Porcu *et al.* 2016), exerts important effects in the neuroendocrine control of reproduction and sexual behavior (Banks & Freeman 1980, Barraclough *et al.* 1986, Skinner *et al.* 1998, Micevych & Sinchak 2008, Schumacher *et al.* 2014), in the modulation of stress responsiveness and anxiety-like behavior (Barbaccia *et al.* 2001, Schumacher *et al.* 2014) as well as in the regulation of adult neurogenesis

(Giachino *et al.* 2003, 2004, Wang *et al.* 2008), astroglial, and synaptic plasticity (Luquin *et al.* 1993, McEwen & Woolley 1993, García-Segura *et al.* 1994, Murphy & Segal 2000, Reyna-Neyra *et al.* 2002, Guerra-Araiza *et al.* 2007, Foy *et al.* 2008). Moreover, this steroid regulates the development of some neuronal types (i.e., Purkinje cells of the cerebellum) (Tsutsui *et al.* 2011), oligodendrocytes/Schwann cells (Jung-Testas *et al.* 1996, Ghoumari *et al.* 2005), as well as the myelination process (Chan *et al.* 2000,

Melcangi *et al.* 2005, Roglio *et al.* 2008, Schumacher *et al.* 2014).

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 (7TMPR α,β,γ) and the two more recently identified subtypes mPGR δ and mPGR ϵ (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 5 α -reductase. In turn, DHP is further converted into allopregnanolone or isopregnanolone by 3 α -hydroxysteroid oxidoreductase or 3 β -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 progestogens 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 spiro lactone 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, norgestrol 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, García-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 17 α -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).

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 spiro lactone 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).

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 derivatives of PROG, testosterone, and even spiro lactone, 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 3

Chemical structure of progestins structurally related to progesterone. Surrounded by a solid line is the molecule of progesterone, surrounded by a dotted line is the retroprogesterone dydrogesterone. 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 ([García-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 progestational action, also exert anti-estrogenic, antigonadotropic, and antimineralocorticoid effects (Africander *et al.* 2011). Among these, there are the third-generation 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, norgestrel acetate exhibits partial anti-androgenic activity (Lello 2010, Van Diepen *et al.* 2011) DNG, DRSP, and trimegestone have a significant anti-androgenic activity, whereas nestorone 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)-diene-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 (Δ^4 -3-ketone structure). These characteristics are the prerequisite for the progestogenic activity. Therefore, progestins can be metabolized by 5 α - or 5 β -steroid reductases and hydroxysteroid dehydrogenases in a similar manner as their parental compound. Apart from steroid 5 α -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 5 β -reductase or AKR1D1 (Di Costanzo *et al.* 2008, Chen *et al.* 2011) and AKR1C enzymes: AKR1C1 (20 α , (3 α)-hydroxysteroid dehydrogenase (HSD)) (Hara *et al.* 1996), AKR1C2 (type 3, 3 α -HSD) (Deyashiki *et al.* 1994, Dufort *et al.* 1996), AKR1C3 (type 2, 3 α -HSD and type 5, 17 β -HSD) (Khanna *et al.* 1995), and AKR1C4 (type 1, 3 α -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 5 α -reduction of NET, even if confers a relatively high binding affinity to the AR, diminishes its androgenic potency (Lemus *et al.* 1997, García-Becerra *et al.* 2002, Morali *et al.* 2002). The 3 β ,5 α -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 $3\beta,5\alpha$ -NET administration, and that the activity of 5α -NET is inhibited by the treatment with CPA, a 3β -hydroxysteroid dehydrogenase/ $\Delta 4,5$ 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 $\Delta 4$ -3-ketone structure. Reduction of the 3-keto group with preservation of the $\Delta 4$ -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 $\Delta 4$ -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 $\Delta 4$ -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 $\Delta 4$ -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-ethinylestradiol (Wiegatz *et al.* 2002). The enzymatic pathway for this latter conversion, however, has not been clearly characterized (Bodine *et al.* 2002, Wiegatz *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 $3\alpha,5\alpha$ -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 $3\beta,5\alpha$ -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 $3\alpha,5\alpha$ - and $3\beta,5\alpha$ -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 norgestrel 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 norgestrel 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 norgestrel 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 SRD5A 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 nestorone 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, Sassoè-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 $\gamma 2$ -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 $\alpha 1$ GABA-A receptor subunit in cerebral cortex, whereas LNG, alone or in combination, altered the levels of the $\gamma 2$ -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/iodide 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 (*lh β*) 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 *lh β* , and significantly those of ER β 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 (Marra *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

(Gingnell *et al.* 2013). 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 chlormadinone 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 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- κ B) inhibitor expression, while decreased astrogliosis and the expression of microglial marker lymphocyte function-associated antigen 1, TNF- α , inducible nitric oxide synthase, and NF- κ B (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 lyssolecithin-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-immunocortin-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 lyssolecithin. 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.

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 uterotrophic 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 neurotrophic factor, while MPA exerted an inhibitory effect (Jodhka *et al.* 2009). In hippocampal cultures, PROG was protective by reducing the Ca²⁺ 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 Ca²⁺ 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 and 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.

Funding

The authors acknowledge financial support from the Fondazione Cariplo to R C M (grant number 2012-0547).

References

- Africander D, Verhoog N & Hapgood JP 2011 Molecular mechanisms of steroid receptor-mediated actions by synthetic progestins used in HRT and contraception. *Steroids* **76** 636–652. (doi:10.1016/j.steroids.2011.03.001)
- Anderer P, Saletu B, Saletu-Zyhlarz G, Gruber D, Metka M, Huber J & Pascual-Marqui RD 2004 Brain regions activated during an auditory discrimination task in insomniac postmenopausal patients before and after hormone replacement therapy: low-resolution brain electromagnetic tomography applied to event-related potentials. *Neuropsychobiology* **49** 134–153. (doi:10.1159/000076722)
- Azzouni F, Godoy A, Li Y & Mohler J 2012 The 5 alpha-reductase isozyme family: a review of basic biology and their role in human diseases. *Advances in Urology* **2012** 530121. (doi:10.1155/2012/530121)
- Bäckström T, Wahlström G, Wahlström K, Zhu D & Wang MD 2005 Isoallopregnanolone an antagonist to the anaesthetic effect of allopregnanolone in male rats. *European Journal of Pharmacology* **512** 15–21. (doi:10.1016/j.ejphar.2005.01.049)
- Bamberger CM, Else T, Bamberger AM, Beil FU & Schulte HM 1999 Dissociative glucocorticoid activity of medroxyprogesterone acetate in normal human lymphocytes. *Journal of Clinical Endocrinology and Metabolism* **84** 4055–4061. (doi:10.1210/jcem.84.11.6091)
- Banks JA & Freeman ME 1980 Inhibition of the daily LH release mechanism by progesterone acting at the hypothalamus. *Biology of Reproduction* **22** 217–222. (doi:10.1095/biolreprod.22.2.217)
- Barbaccia ML, Serra M, Purdy RH & Biggio G 2001 Stress and neuroactive steroids. *International Review of Neurobiology* **46** 243–272. (doi:10.1016/S0074-7742(01)46065-X)

- Barraclough CA, Camp P, Weiland N & Akabori A 1986 Stimulatory versus inhibitory effects of progesterone on estrogen-induced phasic LH and prolactin secretion correlated with estrogen nuclear and progestin cytosol receptor concentrations in brain and pituitary gland. *Neuroendocrinology* **42** 6–14. (doi:10.1159/000124241)
- Barros LA, Tufik S & Andersen ML 2015 The role of progesterone in memory: an overview of three decades. *Neuroscience and Biobehavioral Reviews* **49** 193–204. (doi:10.1016/j.neubiorev.2014.11.015)
- Baulieu EE, Schumacher M, Koenig H, Jung-Testas I & Akwa Y 1996 Progesterone as a neurosteroid: actions within the nervous system. *Cellular and Molecular Neurobiology* **16** 143–154. (doi:10.1007/BF02088173)
- Beck KD, McLaughlin J, Bergen MT, Cominski TP, Moldow RL & Servatius RJ 2008 Facilitated acquisition of the classically conditioned eyeblink response in women taking oral contraceptives. *Behavioural Pharmacology* **19** 821–828. (doi:10.1097/FBP.0b013e32831c3b82)
- Belelli D & Lambert JJ 2005 Neurosteroids: endogenous regulators of the GABA(A) receptor. *Nature Reviews. Neuroscience* **6** 565–575. (doi:10.1038/nrn1703)
- Beranić N, Gobec S & Rižner TL 2011 Progestins as inhibitors of the human 20-ketosteroid reductases, AKR1C1 and AKR1C3. *Chemo-Biological Interactions* **191** 227–233. (doi:10.1016/j.cbi.2010.12.012)
- Beranić N, Brožič P, Brus B, Sosić I, Gobec S & Rižner TL 2012 Expression of human aldo-keto reductase 1C2 in cell lines of peritoneal endometriosis: potential implications in metabolism of progesterone and dydrogesterone and inhibition by progestins. *Journal of Steroid Biochemistry and Molecular Biology* **130** 16–25. (doi:10.1016/j.jsmb.2011.12.011)
- Bernardi F, Pluchino N, Pieri M, Begliuomini S, Lenzi E, Puccetti S, Casarosa E, Luisi M & Genazzani AR 2006 Progesterone and medroxyprogesterone acetate effects on central and peripheral allopregnanolone and beta-endorphin levels. *Neuroendocrinology* **83** 348–359. (doi:10.1159/000095400)
- Bimonte-Nelson HA, Singleton RS, Williams BJ & Granholm A-CE 2004 Ovarian hormones and cognition in the aged female rat: II. progesterone supplementation reverses the cognitive enhancing effects of ovariectomy. *Behavioral Neuroscience* **118** 707–714. (doi:10.1037/0735-7044.118.4.707)
- Bimonte-Nelson HA, Francis KR, Umphlet CD & Granholm A-C 2006 Progesterone reverses the spatial memory enhancements initiated by tonic and cyclic oestrogen therapy in middle-aged ovariectomized female rats. *European Journal of Neuroscience* **24** 229–242. (doi:10.1111/j.1460-9568.2006.04867.x)
- Bitran D, Hilvers RJ & Kellogg CK 1991 Anxiolytic effects of 3 alpha-hydroxy-5 alpha[beta]-pregnan-20-one: endogenous metabolites of progesterone that are active at the GABAA receptor. *Brain Research* **561** 157–161. (doi:10.1016/0006-8993(91)90761-J)
- Bodine PVN, Harris HA, Lyttle CR & Komm BS 2002 Estrogenic effects of 7α-methyl-17α-ethynylestradiol: a newly discovered tibolone metabolite. *Steroids* **67** 681–686. (doi:10.1016/S0039-128X(02)00021-1)
- Brinton RD, Thompson RF, Foy MR, Baudry M, Wang J, Finch CE, Morgan TE, Pike CJ, Mack WJ, Stanczyk FZ, et al. 2008 Progesterone receptors: form and function in brain. *Frontiers in Neuroendocrinology* **29** 313–339. (doi:10.1016/j.yfrne.2008.02.001)
- Campos MG, Oropeza MV, Lemus AE, García GA, Reynoso ME, Campos P & Ponce-Monter H 1999 The androgenic effect of norethisterone and 5α-pregnane-20-one on the contractile response of the rat vas deferens to methoxamine and serotonin. *Life Sciences* **64** PL227–PL233. (doi:10.1016/S0024-3205(99)00132-0)
- Chan JR, Rodriguez-Waitkus PM, Ng BK, Liang P & Glaser M 2000 Progesterone synthesized by Schwann cells during myelin formation regulates neuronal gene expression. *Molecular Biology of the Cell* **11** 2283–2295. (doi:10.1091/mbc.11.7.2283)
- Chen M, Drury JE & Penning TM 2011 Substrate specificity and inhibitor analyses of human steroid 503B2-reductase (AKR1D1). *Steroids* **76** 484–490. (doi:10.1016/j.steroids.2011.01.003)
- Choi JM, Romeo RD, Brake WG, Bethea CL, Rosenwaks Z & McEwen BS 2003 Estradiol increases pre- and post-synaptic proteins in the CA1 region of the hippocampus in female rhesus macaques (*Macaca mulatta*). *Endocrinology* **144** 4734–4738. (doi:10.1210/en.2003-0216)
- Colombo D, Ferraboschi P, Prestileo P & Toma L 2006 A comparative molecular modeling study of dydrogesterone with other progestational agents through theoretical calculations and nuclear magnetic resonance spectroscopy. *Journal of Steroid Biochemistry and Molecular Biology* **98** 56–62. (doi:10.1016/j.jsmb.2005.07.009)
- Conneely OM & Lydon JP 2000 Progesterone receptors in reproduction: functional impact of the A and B isoforms. *Steroids* **65** 571–577. (doi:10.1016/S0039-128X(00)00115-X)
- Cooke PS, Nanjappa MK, Yang Z & Wang KKW 2013 Therapeutic effects of progesterone and its metabolites in traumatic brain injury may involve non-classical signaling mechanisms. *Frontiers in Neuroscience* **7** 108. (doi:10.3389/fnins.2013.00108)
- Delyani JA 2000 Mineralocorticoid receptor antagonists: the evolution of utility and pharmacology. *Kidney International* **57** 1408–1411. (doi:10.1046/j.1523-1755.2000.00983.x)
- Deyashiki Y, Ogasawara A, Nakayama T, Nakanishi M, Miyabe Y, Sato K & Hara A 1994 Molecular cloning of two human liver 3 alpha-hydroxysteroid/dihydrodiol dehydrogenase isoenzymes that are identical with chlordecone reductase and bile-acid binder. *Biochemical Journal* **299** 545–552. (doi:10.1042/bj2990545)
- Di Costanzo L, Drury JE, Penning TM & Christianson DW 2008 Crystal structure of human liver Delta4-3-ketosteroid 5beta-reductase (AKR1D1) and implications for substrate binding and catalysis. *Journal of Biological Chemistry* **283** 16830–16839. (doi:10.1074/jbc.M801778200)
- Do Rego JL, Seong JY, Burel D, Leprince J, Luu-The V, Tsutsui K, Tonon M-C, Pelletier G & Vaudry H 2009 Neurosteroid biosynthesis: enzymatic pathways and neuroendocrine regulation by neurotransmitters and neuropeptides. *Frontiers in Neuroendocrinology* **30** 259–301. (doi:10.1016/j.yfrne.2009.05.006)
- Dröge MJ, Oostebing F, Oosting E, Verheul HAM & Kloosterboer HJ 2007 7α-methyl-ethinyl estradiol is not a metabolite of tibolone but a chemical stress artifact. *Menopause* **14** 474–480. (doi:10.1097/01.gme.0000247015.63877.d4)
- Dufort I, Soucy P, Labrie F & Luu-The V 1996 Molecular cloning of human type 3 3 alpha-hydroxysteroid dehydrogenase that differs from 20 alpha-hydroxysteroid dehydrogenase by seven amino acids. *Biochemical and Biophysical Research Communications* **228** 474–479. (doi:10.1006/bbrc.1996.1684)
- Edgren RA & Stanczyk FZ 1999 Nomenclature of the gonane progestins. *Contraception* **60** 313. (doi:10.1016/S0010-7824(99)00101-8)
- El-Etr M, Rame M, Boucher C, Ghomari AM, Kumar N, Liere P, Pianos A, Schumacher M & Sitruk-Ware R 2015 Progesterone and nestorone promote myelin regeneration in chronic demyelinating lesions of corpus callosum and cerebral cortex. *Glia* **63** 104–117. (doi:10.1002/glia.22736)
- Enriquez J, Lemus AE, Chimal-Monroy J, Arzate H, García GA, Herrero B, Larrea F & Pérez-Palacios G 2007 The effects of synthetic 19-noprogestins on osteoblastic cell function are mediated by their non-phenolic reduced metabolites. *Journal of Endocrinology* **193** 493–504. (doi:10.1677/JOE-06-0038)
- Falkenstein E, Heck M, Gerdes D, Grube D, Christ M, Weigel M, Buddhikot M, Meisel S & Wehling M 1999 Specific progesterone binding to a membrane protein and related nongenomic effects on Ca²⁺-fluxes in sperm. *Endocrinology* **140** 5999–6002. (doi:10.1210/endo.140.12.7304)
- Follesa P, Porcu P, Sogliano C, Cinus M, Biggio F, Mancuso L, Mostallino MC, Paoletti AM, Purdy RH, Biggio G, et al. 2002 Changes in GABAA receptor gamma 2 subunit gene expression induced by long-term administration of oral contraceptives in rats. *Neuropharmacology* **42** 325–336. (doi:10.1016/S0028-3908(01)00187-3)

- Fotherby K 1990 Interactions with oral contraceptives. *American Journal of Obstetrics and Gynecology* **163** 2153–2159. (doi:10.1016/0002-9378(90)90556-M)
- Foy MR, Akopian G & Thompson RF 2008 Progesterone regulation of synaptic transmission and plasticity in rodent hippocampus. *Learning & Memory* **15** 820–822. (doi:10.1101/lm.1124708)
- Frye CA, Koonce CJ & Walf AA 2013 Pregnane xenobiotic receptors and membrane progestin receptors: role in neurosteroid-mediated motivated behaviours. *Journal of Neuroendocrinology* **25** 1002–1011. (doi:10.1111/jne.12105)
- Garay L, Gonzalez Denisse MC, Sitruk-Ware R, Guennoun R, Schumacher M & De Nicola AF 2014 Efficacy of the selective progesterone receptor agonist Nestorone for chronic experimental autoimmune encephalomyelitis. *Journal of Neuroimmunology* **276** 89–97. (doi:10.1016/j.jneuroim.2014.08.619)
- García-Becerra R, Borja-Cacho E, Cooney AJ, Jackson KJ, Lemus AE, Pérez-Palacios G & Larrea F 2002 The intrinsic transcriptional estrogenic activity of a non-phenolic derivative of levonorgestrel is mediated via the estrogen receptor-03B1. *Journal of Steroid Biochemistry and Molecular Biology* **82** 333–341. (doi:10.1016/S0960-0760(02)00192-9)
- García-Becerra R, Cooney AJ, Borja-Cacho E, Lemus AE, Pérez-Palacios G & Larrea F 2004 Comparative evaluation of androgen and progesterone receptor transcription selectivity indices of 19-nortestosterone-derived progestins. *Journal of Steroid Biochemistry and Molecular Biology* **91** 21–27. (doi:10.1016/j.jsbmb.2004.02.003)
- García-Segura LM, Chowen JA, P00E1rduc A & Naftolin F 1994 Gonadal hormones as promoters of structural synaptic plasticity: cellular mechanisms. *Progress in Neurobiology* **44** 279–307. (doi:10.1016/0301-0082(94)90042-6)
- Genazzani AR, Pluchino N, Begliuomini S, Pieri M, Centofanti M, Freschi L, Casarosa E & Luisi M 2007 Drospirenone increases central and peripheral beta-endorphin in ovariectomized female rats. *Menopause* **14** 63–73. (doi:10.1097/01.gme.0000230348.05745.7d)
- Germain P, Staels B, Dacquet C, Spedding M & Laudet V 2006 Overview of nomenclature of nuclear receptors. *Pharmacological Reviews* **58** 685–704. (doi:10.1124/pr.58.4.2)
- Ghoumari AM, Baulieu EE & Schumacher M 2005 Progesterone increases oligodendroglial cell proliferation in rat cerebellar slice cultures. *Neuroscience* **135** 47–58. (doi:10.1016/j.neuroscience.2005.05.023)
- Giachino C, Galbiati M, Fasolo A, Peretto P & Melcangi R 2003 Neurogenesis in the subependymal layer of the adult rat: a role for neuroactive derivatives of progesterone. *Annals of the New York Academy of Sciences* **1007** 335–339. (doi:10.1196/annals.1286.032)
- Giachino C, Galbiati M, Fasolo A, Peretto P & Melcangi RC 2004 Effects of progesterone derivatives, dihydroprogesterone and tetrahydroprogesterone, on the subependymal layer of the adult rat. *Journal of Neurobiology* **58** 493–502. (doi:10.1002/neu.10290)
- Giatti S, García-Segura LM & Melcangi RC 2015 New steps forward in the neuroactive steroid field. *Journal of Steroid Biochemistry and Molecular Biology* **153** 127–134. (doi:10.1016/j.jsbmb.2015.03.002)
- Gingnell M, Engman J, Frick A, Moby L, Wikström J, Fredrikson M & Sundström-Poromaa I 2013 Oral contraceptive use changes brain activity and mood in women with previous negative affect on the pill—a double-blinded, placebo-controlled randomized trial of a levonorgestrel-containing combined oral contraceptive. *Psychoneuroendocrinology* **38** 1133–1144. (doi:10.1016/j.psyneuen.2012.11.006)
- Gingnell M, Bannbers E, Engman J, Frick A, Moby L, Wikström J & Sundström-Poromaa I 2016 The effect of combined hormonal contraceptives use on brain reactivity during response inhibition. *European Journal of Contraception & Reproductive Health Care* **21** 150–157. (doi:10.3109/13625187.2015.1077381)
- Gogos A 2013 Natural and synthetic sex hormones: effects on higher-order cognitive function and prepulse inhibition. *Biological Psychology* **93** 17–23. (doi:10.1016/j.biopsycho.2013.02.001)
- Gogos A, Wu YC, Williams AS & Byrne LK 2014 The effects of ethinylestradiol and progestins (2018the pill2019) on cognitive function in pre-menopausal women. *Neurochemical Research* **39** 2288–2300. (doi:10.1007/s11064-014-1444-6)
- Griksiene R & Ruksenas O 2011 Effects of hormonal contraceptives on mental rotation and verbal fluency. *Psychoneuroendocrinology* **36** 1239–1248. (doi:10.1016/j.psyneuen.2011.03.001)
- Gruber CJ & Huber JC 2003 Differential effects of progestins on the brain. *Maturitas* **46** S71–S75. (doi:10.1016/j.maturitas.2003.09.021)
- Guerra-Araiza C, Amorim MAR, Camacho-Arroyo I & García-Segura LM 2007 Effects of progesterone and its reduced metabolites, dihydroprogesterone and tetrahydroprogesterone, on the expression and phosphorylation of glycogen synthase kinase-3 and the microtubule-associated protein tau in the rat cerebellum. *Developmental Neurobiology* **67** 510–520. (doi:10.1002/dneu.20383)
- Hammond GL 2002 Access of reproductive steroids to target tissues. *Obstetrics and Gynecology Clinics of North America* **29** 411–423. (doi:10.1016/S0889-8545(02)00008-6)
- Hara A, Matsuura K, Tamada Y, Sato K, Miyabe Y, Deyashiki Y & Ishida N 1996 Relationship of human liver dihydrodiol dehydrogenases to hepatic bile-acid-binding protein and an oxidoreductase of human colon cells. *Biochemical Journal* **313** 373–376. (doi:10.1042/bj3130373)
- Henderson JA & Shively CA 2004 Triphasic oral contraceptive treatment alters the behavior and neurobiology of female cynomolgus monkeys. *Psychoneuroendocrinology* **29** 21–34. (doi:10.1016/S0306-4530(02)00132-4)
- Henderson LP, Penatti CAA, Jones BL, Yang P & Clark AS 2006 Anabolic androgenic steroids and forebrain GABAergic transmission. *Neuroscience* **138** 793–799. (doi:10.1016/j.neuroscience.2005.08.039)
- Holloway JL, Beck KD & Servatius RJ 2011 Facilitated acquisition of the classically conditioned eyeblink response in females is augmented in those taking oral contraceptives. *Behavioural Brain Research* **216** 301–307. (doi:10.1016/j.bbr.2010.08.008)
- Honjo H, Iwasa K, Kawata M, Fushiki S, Hosoda T, Tatsumi H, Oida N, Mihara M, Hirasugi Y, Yamamoto H, et al. 2005 Progestins and estrogens and Alzheimer2019s disease. *Journal of Steroid Biochemistry and Molecular Biology* **93** 305–308. (doi:10.1016/j.jsbmb.2004.12.001)
- Hossain MB, Oshima T, Hirose S, Wang J & Tokumoto T 2015 Expression and purification of human membrane progestin receptor 03B1 (mPR03B1). *PLoS ONE* **10** 1–16. (doi:10.1371/journal.pone.0138739)
- Huber JC, Heskamp M-LS & Schramm GAK 2008 Effect of an oral contraceptive with chlormadinone acetate on depressive mood202F: analysis of data from four observational studies. *Clinical Drug Investigation* **28** 783–791. (doi:10.2165/0044011-200828120-00006)
- Hussain R, El-Etr M, Gaci O, Rakotomamonjy J, Macklin WB, Kumar N, Sitruk-Ware R, Schumacher M & Ghoumari AM 2011 Progesterone and Nestorone facilitate axon remyelination: a role for progesterone receptors. *Endocrinology* **152** 3820–3831. (doi:10.1210/en.2011-1219)
- Inhoffen HH & Hohlweg W 1938 Neue per os-wirksame weibliche Keimdr00FCsenhormon-Derivate: 17-Aethinyl-oestradiol und Pregnen-in-on-3-ol-17. *Die Naturwissenschaften* **26** 96. (doi:10.1007/BF01681040)
- Irwin RW, Yao J, Hamilton RT, Cadenas E, Brinton RD & Nilsen J 2008 Progesterone and estrogen regulate oxidative metabolism in brain mitochondria. *Endocrinology* **149** 3167–3175. (doi:10.1210/en.2007-1227)
- Irwin RW, Yao J, Ahmed SS, Hamilton RT, Cadenas E & Brinton RD 2011 Medroxyprogesterone acetate antagonizes estrogen up-regulation of brain mitochondrial function. *Endocrinology* **152** 556–567. (doi:10.1210/en.2010-1061)
- Islam F, Sparkes C, Roodenrys S & Astheimer L 2008 Short-term changes in endogenous estrogen levels and consumption of soy isoflavones affect working and verbal memory in young adult females. *Nutritional Neuroscience* **11** 251–262. (doi:10.1179/147683008X301612)

- Jin Y, Duan L, Chen M, Penning TM & Kloosterboer HJ 2012 Metabolism of the synthetic progestogen norethynodrel by human ketosteroid reductases of the aldo-keto reductase superfamily. *Journal of Steroid Biochemistry and Molecular Biology* **129** 139–144. (doi:10.1016/j.jsmb.2011.12.002)
- Jodhka PK, Kaur P, Underwood W, Lydon JP & Singh M 2009 The differences in neuroprotective efficacy of progesterone and medroxyprogesterone acetate correlate with their effects on brain-derived neurotrophic factor expression. *Endocrinology* **150** 3162–3168. (doi:10.1210/en.2008-1247)
- Jones BL, Whiting PJ & Henderson LP 2006 Mechanisms of anabolic androgenic steroid inhibition of mammalian epsilon-subunit-containing GABAA receptors. *Journal of Physiology* **573** 571–593. (doi:10.1113/jphysiol.2006.106534)
- Jung-Testas I, Schumacher M, Robel P & Baulieu EE 1996 Demonstration of progesterone receptors in rat Schwann cells. *Journal of Steroid Biochemistry and Molecular Biology* **58** 77–82. (doi:10.1016/0960-0760(96)00009-X)
- Khanim F, Davies N, Veliça P, Hayden R, Ride J, Pararasa C, Chong MG, Gunther U, Veerapen N, Winn P, et al. 2014 Selective AKR1C3 inhibitors do not recapitulate the anti-leukaemic activities of the pan-AKR1C inhibitor medroxyprogesterone acetate. *British Journal of Cancer* **110** 1506–1516. (doi:10.1038/bjc.2014.83)
- Khanna M, Qin KN, Wang RW & Cheng KC 1995 Substrate specificity, gene structure, and tissue-specific distribution of multiple human 3 alpha-hydroxysteroid dehydrogenases. *Journal of Biological Chemistry* **270** 20162–20168. (doi:10.1074/jbc.270.34.20162)
- King SR 2013 *Neurosteroids and the Nervous System*. New York, NY: Springer New York. (doi:10.1007/978-1-4614-5559-2)
- Koubovec D, Ronacher K, Stubrud E, Louw A & Hapgood JP 2005 Synthetic progestins used in HRT have different glucocorticoid agonist properties. *Molecular and Cellular Endocrinology* **242** 23–32. (doi:10.1016/j.mce.2005.07.001)
- Krattenmacher R 2000 Drospirenone: pharmacology and pharmacokinetics of a unique progestogen. *Contraception* **62** 29–38. (doi:10.1016/S0010-7824(00)00133-5)
- Kroupova HK, Trubiroha A, Lorenz C, Contardo-Jara V, Lutz I, Grabic R, Kocour M & Kloas W 2014 The progestin levonorgestrel disrupts gonadotropin expression and sex steroid levels in pubertal roach (*Rutilus rutilus*). *Aquatic Toxicology* **154** 154–162. (doi:10.1016/j.aquatox.2014.05.008)
- Kuhl H 2005 Pharmacology of estrogens and progestogens: influence of different routes of administration. *Climacteric* **8** 3–63. (doi:10.1080/13697130500148875)
- Lambert JJ, Belelli D, Peden DR, Vardy AW & Peters JA 2003 Neurosteroid modulation of GABAA receptors. *Progress in Neurobiology* **71** 67–80. (doi:10.1016/j.pneurobio.2003.09.001)
- Larrea F, Vilchis F, Chávez B, Pérez AE, Garza-Flores J & Pérez-Palacios G 1987 The metabolism of 19-nor contraceptive progestins modulates their biological activity at the neuroendocrine level. *Journal of Steroid Biochemistry* **27** 657–663. (doi:10.1016/0022-4731(87)90134-8)
- Larrea F, García-Becerra R, Lemus AE, García GA, Pérez-Palacios G, Jackson KJ, Coleman KM, Dace R, Smith CL & Cooney AJ 2001 A-ring reduced metabolites of 19-nor synthetic progestins as subtype selective agonists for ER alpha. *Endocrinology* **142** 3791–3799. (doi:10.1210/en.142.9.3791)
- LeBlanc ES & Laws A 1999 Benefits and risks of third-generation oral contraceptives. *Journal of General Internal Medicine* **14** 625–632. (doi:10.1046/j.1525-1497.1999.08108.x)
- Lello S 2010 Norgestrel acetate: pharmacology, safety profile and therapeutic efficacy. *Drugs* **70** 541–559. (doi:10.2165/11532130-000000000-00000)
- Lemus AE, Enríquez J, García GA, Grillasca I & Pérez-Palacios G 1997 5α-Reduction of norethisterone enhances its binding affinity for androgen receptors but diminishes its androgenic potency. *Journal of Steroid Biochemistry and Molecular Biology* **60** 121–129. (doi:10.1016/S0960-0760(96)00172-0)
- Lenzi E, Pluchino N, Begliuomini S, Ninni F, Freschi L, Centofanti M, Casarosa E, Luisi S, Valentino V, Luisi M, et al. 2008 Effects of norgestrel acetate administration on central and peripheral beta-endorphin and allopregnanolone in ovx rats. *Journal of Steroid Biochemistry and Molecular Biology* **110** 67–75. (doi:10.1016/j.jsmb.2008.02.002)
- Lenzi E, Pluchino N, Begliuomini S, Casarosa E, Merlini S, Giannini A, Luisi M, Kumar N, Sitruk-Ware R & Genazzani AR 2009 Central modifications of allopregnanolone and beta-endorphin following subcutaneous administration of Nestorone. *Journal of Steroid Biochemistry and Molecular Biology* **116** 15–20. (doi:10.1016/j.jsmb.2009.04.004)
- Liang Y-Q, Huang G-Y, Liu S-S, Zhao J-L, Yang Y-Y, Chen X-W, Tian F, Jiang Y-X & Ying G-G 2015a Long-term exposure to environmentally relevant concentrations of progesterone and norgestrel affects sex differentiation in zebrafish (*Danio rerio*). *Aquatic Toxicology* **160** 172–179. (doi:10.1016/j.aquatox.2015.01.006)
- Liang Y-Q, Huang G-Y, Ying G-G, Liu S-S, Jiang Y-X, Liu S & Peng F-J 2015b A time-course transcriptional kinetics of the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axes in zebrafish eleutheroembryos after exposure to norgestrel. *Environmental Toxicology and Chemistry* **34** 112–119. (doi:10.1002/etc.2766)
- Liang Y-Q, Huang G-Y, Ying G-G, Liu S-S, Jiang Y-X & Liu S 2015c Progesterone and norgestrel alter transcriptional expression of genes along the hypothalamic-pituitary-thyroid axis in zebrafish embryos-larvae. *Comparative Biochemistry and Physiology. Toxicology & Pharmacology* **167** 101–107. (doi:10.1016/j.cbpc.2014.09.007)
- Liu L, Zhao L, She H, Chen S, Wang JM, Wong C, McClure K, Sitruk-Ware R & Brinton RD 2010 Clinically relevant progestins regulate neurogenic and neuroprotective responses in vitro and in vivo. *Endocrinology* **151** 5782–5794. (doi:10.1210/en.2010-0005)
- Liu A, Margail I, Zhang S, Labombarda F, Coqueran B, Deslespierre B, Liere P, Marchand-Leroux C, O'Malley BW, Lydon JP, et al. 2012 Progesterone receptors: a key for neuroprotection in experimental stroke. *Endocrinology* **153** 3747–3757. (doi:10.1210/en.2012-1138)
- Lorenz C, Contardo-Jara V, Trubiroha A, Krüger A, Viehmann V, Wiegand C, Pflugmacher S, Nützmann G, Lutz I & Kloas W 2011a The synthetic gestagen Levonorgestrel disrupts sexual development in *Xenopus laevis* by affecting gene expression of pituitary gonadotropins and gonadal steroidogenic enzymes. *Toxicological Sciences* **124** 311–319. (doi:10.1093/toxsci/kfr241)
- Lorenz C, Contardo-Jara V, Pflugmacher S, Wiegand C, Nützmann G, Lutz I & Kloas W 2011b The synthetic gestagen levonorgestrel impairs metamorphosis in *Xenopus laevis* by disruption of the thyroid system. *Toxicological Sciences* **123** 94–102. (doi:10.1093/toxsci/kfr159)
- Luquin S, Naftolin F & Garcia-Segura LM 1993 Natural fluctuation and gonadal hormone regulation of astrocyte immunoreactivity in dentate gyrus. *Journal of Neurobiology* **24** 913–924. (doi:10.1002/neu.480240705)
- Maki PM & Sundermann E 2009 Hormone therapy and cognitive function. *Human Reproduction Update* **15** 667–681. (doi:10.1093/humupd/dmp022)
- Markov G, Bonneton F & Laudet V 2010 Nuclear receptors. In *Nuclear Receptors Current Concepts and Future Challenges*, pp 15–29. Eds CM Bunce & MJ Campbell. Dordrecht: Springer Netherlands.
- Marra C, Gainotti G, Fadda L, Perri R, Lacidogna G, Scaricamazza E, Piccininni C & Quaranta D 2015 Usefulness of an integrated analysis of different memory tasks to predict the progression from mild cognitive impairment to Alzheimer's disease: the Episodic Memory Score (EMS). *Journal of Alzheimer's Disease* **50** 61–70. (doi:10.3233/JAD-150613)
- McEwen BS & Woolley CS 1993 Estradiol and progesterone regulate neuronal structure and synaptic connectivity in adult as well as

- developing brain. *Experimental Gerontology* **29** 431–436. (doi:10.1016/0531-5565(94)90022-1)
- McIntyre KL, Porter DM & Henderson LP 2002 Anabolic androgenic steroids induce age-, sex-, and dose-dependent changes in GABA(A) receptor subunit mRNAs in the mouse forebrain. *Neuropharmacology* **43** 634–645. (doi:10.1016/S0028-3908(02)00154-5)
- McLeod DG 1993 Antiandrogenic drugs. *Cancer* **71** 1046–1049. (doi:10.1002/1097-0142(19930201)71:3<1046::aid-cncr2820711424>3.0.co;2-m)
- Melcangi RC, Cavarretta ITR, Ballabio M, Leonelli E, Schenone A, Azcoitia I, Miguel Garcia-Segura L & Magnaghi V 2005 Peripheral nerves: a target for the action of neuroactive steroids. *Brain Research. Brain Research Reviews* **48** 328–338. (doi:10.1016/j.brainresrev.2004.12.021)
- Melcangi RC & Garcia-Segura LM 2010 Sex-specific therapeutic strategies based on neuroactive steroids: in search for innovative tools for neuroprotection. *Hormones and Behavior* **57** 2–11. (doi:10.1016/j.yhbeh.2009.06.001)
- Melcangi RC, Garcia-Segura LM & Mensah-Nyagan AG 2008 Neuroactive steroids: state of the art and new perspectives. *Cellular and Molecular Life Sciences* **65** 777–797. (doi:10.1007/s00018-007-7403-5)
- Melcangi RC, Giatti S, Calabrese D, Pesaresi M, Cermenati G, Mitro N, Viviani B, Garcia-Segura LM & Caruso D 2014 Levels and actions of progesterone and its metabolites in the nervous system during physiological and pathological conditions. *Progress in Neurobiology* **113** 56–69. (doi:10.1016/j.pneurobio.2013.07.006)
- Melcangi RC, Giatti S & Garcia-Segura LM 2015 Levels and actions of neuroactive steroids in the nervous system under physiological and pathological conditions: sex-specific features. *Neuroscience and Biobehavioral Reviews* **67** 25–40. (doi:10.1016/j.newbiorev.2015.09.023)
- Meyer M, Gonzalez Denisselle MC, Garay L, Sitruk-Ware R, Guennoun R, Schumacher M & De Nicola AF 2015 The progesterone receptor agonist Nestorone holds back proinflammatory mediators and neuropathology in the wobbler mouse model of motoneuron degeneration. *Neuroscience* **308** 51–63. (doi:10.1016/j.neuroscience.2015.09.007)
- Micevych P & Sinchak K 2008 Synthesis and function of hypothalamic neuroprogesterone in reproduction. *Endocrinology* **149** 2739–2742. (doi:10.1210/en.2008-0011)
- Mihalik JP, Ondrak KS, Guskiewicz KM & McMurray RG 2009 The effects of menstrual cycle phase on clinical measures of concussion in healthy college-aged females. *Journal of Science and Medicine in Sport* **12** 383–387. (doi:10.1016/j.jsams.2008.05.003)
- Morali G, Lemus AE, Munguia R, Garcia GA, Grillasca I & Pérez-Palacios G 2002 Hormone-like behavioral effects of levonorgestrel and its metabolites in the male rat. *Pharmacology Biochemistry and Behavior* **73** 951–961. (doi:10.1016/S0091-3057(02)00946-2)
- Mordecai KL, Rubin LH & Maki PM 2008 Effects of menstrual cycle phase and oral contraceptive use on verbal memory. *Hormones and Behavior* **54** 286–293. (doi:10.1016/j.yhbeh.2008.03.006)
- Muhn P, Fuhrmann U, Fritzemeier KH, Krattenmacher R & Schillinger E 1995 Drospirenone: a novel progestogen with antimineralocorticoid and antiandrogenic activity. *Annals of the New York Academy of Sciences* **761** 311–335. (doi:10.1111/j.1749-6632.1995.tb31386.x)
- Murphy DD & Segal M 2000 Progesterone prevents estradiol-induced dendritic spine formation in cultured hippocampal neurons. *Neuroendocrinology* **72** 133–143. (doi:10.1159/000054580)
- Nielsen SE, Ertman N, Lakhani YS & Cahill L 2011 Hormonal contraception usage is associated with altered memory for an emotional story. *Neurobiology of Learning and Memory* **96** 378–384. (doi:10.1016/j.nlm.2011.06.013)
- Nilsen J & Brinton RD 2002 Impact of progestins on estradiol potentiation of the glutamate calcium response. *Neuroreport* **13** 825–830. (doi:10.1097/00001756-200205070-00018)
- Nilsen J & Brinton RD 2003 Divergent impact of progesterone and medroxyprogesterone acetate (Provera) on nuclear mitogen-activated protein kinase signaling. *PNAS* **100** 10506–10511. (doi:10.1073/pnas.1334098100)
- Nilsson B & von Schoultz B 1989 Binding of levonorgestrel, norethisterone and desogestrel to human sex hormone binding globulin and influence on free testosterone levels. *Gynecologic and Obstetric Investigation* **27** 151–154. (doi:10.1159/000293644)
- Oelkers W 2004 Drospirenone, a progestogen with antimineralocorticoid properties: a short review. *Molecular and Cellular Endocrinology* **217** 255–261. (doi:10.1016/j.mce.2003.10.030)
- Oelkers W, Berger V, Bolik A, Bähr V, Hazard B, Beier S, Elger W & Heithecker A 1991 Dihydrospirorenone, a new progestogen with antimineralocorticoid activity: effects on ovulation, electrolyte excretion, and the renin-aldosterone system in normal women. *Journal of Clinical Endocrinology and Metabolism* **73** 837–842. (doi:10.1210/jcem-73-4-837)
- Olbrich M, Weigl K, Kahler E & Mihara K 2016 Dydrogesterone metabolism in human liver by aldo-keto reductases and cytochrome P450 enzymes. *Xenobiotica* **8254** 1–7. (doi:10.3109/00498254.2015.1134852)
- Pang Y, Dong J & Thomas P 2013 Characterization, neurosteroid binding and brain distribution of human membrane progesterone receptors δ and ϵ (mPR δ and mPR ϵ) and mPR δ involvement in neurosteroid inhibition of apoptosis. *Endocrinology* **154** 283–295. (doi:10.1210/en.2012-1772)
- Paoletti AM, Lello S, Fratta S, Orrù M, Ranuzzi F, Sogliano C, Concas A, Biggio G & Melis GB 2004 Psychological effect of the oral contraceptive formulation containing 3 mg of drospirenone plus 30 microg of ethinyl estradiol. *Fertility and Sterility* **81** 645–651. (doi:10.1016/j.fertnstert.2003.08.030)
- Pasapera AM, Gutierrez-Sagal R, Herrera J, Galicia-Canales N, Garcia de la Mora G & Ulloa-Aguirre A 2002 Norethisterone is bioconverted to oestrogenic compounds that activate both the oestrogen receptor alpha and oestrogen receptor beta in vitro. *European Journal of Pharmacology* **452** 347–355. (doi:10.1016/S0014-2999(02)02337-3)
- Pasqualini JR 1996 Progestins: present and future. *Journal of Steroid Biochemistry and Molecular Biology* **5-6** 357–363. (doi:10.1016/S0960-0760(96)00183-5)
- Pawlak M, Lefebvre P & Staels B 2012 General molecular biology and architecture of nuclear receptors. *Current Topics in Medicinal Chemistry* **12** 486–504. (doi:10.2174/156802612799436641)
- Penning TM, Burczynski ME, Jez JM, Hung CF, Lin HK, Ma H, Moore M, Palackal N & Ratnam K 2000 Human 3 α ph-hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. *Biochemical Journal* **351** 67–77. (doi:10.1042/bj3510067)
- Penning TM, Chen M & Jin Y 2015 Promiscuity and diversity in 3-ketosteroid reductases. *Journal of Steroid Biochemistry and Molecular Biology* **151** 93–101. (doi:10.1016/j.jsbmb.2014.12.003)
- Petersen LH, Hala D, Carty D, Cantu M, Martinović D & Huggett DB 2015 Effects of progesterone and norethindrone on female fathead minnow (*Pimephales promelas*) steroidogenesis. *Environmental Toxicology and Chemistry* **34** 379–390. (doi:10.1002/etc.2816)
- Philibert D, Bouchoux E, Degryse M, Lecaque D, Petit F & Gaillard M 1999 The pharmacological profile of a novel norpregnane progestin (trimegestone). *Gynecological Endocrinology* **13** 316–326. (doi:10.3109/09513599909167574)
- Pletzer BA & Kerschbaum HH 2014 50 years of hormonal contraception-time to find out, what it does to our brain. *Frontiers in Neuroscience* **8** 256. (doi:10.3389/fnins.2014.00256)
- Pletzer B, Kronbichler M & Kerschbaum H 2015 Differential effects of androgenic and anti-androgenic progestins on fusiform and frontal

- gray matter volume and face recognition performance. *Brain Research* **1596** 108–115. (doi:10.1016/j.brainres.2014.11.025)
- Pluchino N, Lenzi E, Casarosa E, Cela V, Begliuomini S, Ninni F, Freschi L, Luisi S & Genazzani AR 2008 Dydrogesterone increases allopregnanolone in selected brain areas and in serum of female rats. *Fertility and Sterility* **89** 1384–1389. (doi:10.1016/j.fertnstert.2007.03.077)
- Pluchino N, Lenzi E, Merlini S, Giannini A, Cubeddu A, Casarosa E, Begliuomini S, Luisi M, Cela V & Genazzani AR 2009 Selective effect of chlormadinone acetate on brain allopregnanolone and opioids content. *Contraception* **80** 53–62. (doi:10.1016/j.contraception.2009.01.005)
- Porcu P, Mostallino MC, Sogliano C, Santoru F, Berretti R & Concas A 2012 Long-term administration with levonorgestrel decreases allopregnanolone levels and alters GABA(A) receptor subunit expression and anxiety-like behavior. *Pharmacology, Biochemistry, and Behavior* **102** 366–372. (doi:10.1016/j.pbb.2012.05.011)
- Porcu P, Barron AM, Frye CA, Walf AA, Yang S-Y, He X-Y, Morrow AL, Panzica GC & Melcangi RC 2016 Neurosteroidogenesis today: novel targets for neuroactive steroid synthesis and action and their relevance for translational research. *Journal of Neuroendocrinology* **28** 12351. (doi:10.1111/jne.12351)
- Rabe T, Luxembourg B, Ludwig M, Dinger J, Bauersachs R, Rott H, Mueck AO & Albring C 2012 Kontrazeption & Thrombophilie – Eine Stellungnahme der deutschen gesellschaft für gynäkologische endokrinologie und fortpflanzungsmedizin (DGGEF) e.V. und des berufsverbands für frauenärzte (BVF) e.V. *Journal Fur Reproduktionsmedizin Und Endokrinologie* **9** 20–46.
- Rapkin AJ, Morgan M, Sogliano C, Biggio G & Concas A 2006 Decreased neuroactive steroids induced by combined oral contraceptive pills are not associated with mood changes. *Fertility and Sterility* **85** 1371–1378. (doi:10.1016/j.fertnstert.2005.10.031)
- Raudrant D & Rabe T 2003 Progestogens with antiandrogenic properties. *Drugs* **63** 463–492. (doi:10.2165/00003495-200363050-00003)
- Rettberg JR, Yao J & Brinton RD 2014 Estrogen: a master regulator of bioenergetic systems in the brain and body. *Frontiers in Neuroendocrinology* **35** 8–30. (doi:10.1016/j.yfrne.2013.08.001)
- Reyna-Neyra A, Camacho-Arroyo I, Ferrera P & Arias C 2002 Estradiol and progesterone modify microtubule associated protein 2 content in the rat hippocampus. *Brain Research Bulletin* **58** 607–612. (doi:10.1016/S0361-9230(02)00829-8)
- Rižner TL & Penning TM 2014 Role of aldo-keto reductase family 1 (AKR1) enzymes in human steroid metabolism. *Steroids* **79** 49–63. (doi:10.1016/j.steroids.2013.10.012)
- Rižner TL, Brožič P, Doucette C, Turek-Etienne T, Müller-Vieira U, Sonneveld E, van der Burg B, Böcker C & Husen B 2011 Selectivity and potency of the retroprogesterone dydrogesterone in vitro. *Steroids* **76** 607–615. (doi:10.1016/j.steroids.2011.02.043)
- Roglio I, Giatti S, Pesaresi M, Bianchi R, Cavaletti G, Lauria G, Garcia-Segura LM & Melcangi RC 2008 Neuroactive steroids and peripheral neuropathy. *Brain Research Reviews* **57** 460–469. (doi:10.1016/j.brainresrev.2007.04.010)
- Rosenberg L & Park S 2002 Verbal and spatial functions across the menstrual cycle in healthy young women. *Psychoneuroendocrinology* **27** 835–841. (doi:10.1016/S0306-4530(01)00083-X)
- Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SAA, Howard B V, Johnson KC, et al. 2002 Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA* **288** 321–333. (doi:10.1001/jama.288.3.321)
- Saletu B, Anderer P, Saletu-Zyhlarz GM, Gruber D, Metka M & Huber J 2005 Identifying target regions for vigilance improvement under hormone replacement therapy in postmenopausal syndrome patients by means of electroencephalographic tomography (LORETA). *Psychopharmacology* **178** 389–399. (doi:10.1007/s00213-004-2029-x)
- Santillán R, Pérez-Palacios G, Reyes M, Damián-Matsumura P, García GA, Grillasas I & Lemus AE 2001 Assessment of the oestrogenic activity of the contraceptive progestin levonorgestrel and its non-phenolic metabolites. *European Journal of Pharmacology* **427** 167–174. (doi:10.1016/S0014-2999(01)01263-8)
- Santoru F, Berretti R, Locci A, Porcu P & Concas A 2014 Decreased allopregnanolone induced by hormonal contraceptives is associated with a reduction in social behavior and sexual motivation in female rats. *Psychopharmacology* **231** 3351–3364. (doi:10.1007/s00213-014-3539-9)
- Sassoè-Pognetto M, Follesa P, Panzanelli P, Perazzini A-Z, Porcu P, Sogliano C, Cherchi C & Concas A 2007 Fluctuations in brain concentrations of neurosteroids are not associated to changes in gephyrin levels. *Brain Research* **1169** 1–8. (doi:10.1016/j.brainres.2007.06.057)
- Sator MO, Wieser F, Gruber DM, Joura EA & Huber JC 1999 Trends in the prescription of psychotropic drugs and hormone substitutes in Austria. *Wiener Klinische Wochenschrift* **111** 402–405. (doi:10.1016/j.brainres.2007.06.057)
- Schindler AE, Campagnoli C, Druckmann R, Huber J, Pasqualini JR, Schweppe KW & Thijssen JHH 2003 Classification and pharmacology of progestins. *Maturitas* **46** S7–S16. (doi:10.1016/j.maturitas.2003.09.014)
- Schneider LS & Farlow M 1997 Combined tacrine and estrogen replacement therapy in patients with Alzheimer's disease. *Annals of the New York Academy of Sciences* **826** 317–322. (doi:10.1111/j.1749-6632.1997.tb48482.x)
- Schumacher M, Mattern C, Ghoumari A, Oudinet JP, Liere P, Labombarda F, Sitruk-Ware R, De Nicola AF & Guennoun R 2014 Revisiting the roles of progesterone and allopregnanolone in the nervous system: resurgence of the progesterone receptors. *Progress in Neurobiology* **113** 6–39. (doi:10.1016/j.pneurobio.2013.09.004)
- Schumacher M, Denier C, Oudinet J-P, Adams D & Guennoun R 2016 Progesterone neuroprotection: the background of clinical trial failure. *Journal of Steroid Biochemistry and Molecular Biology* **160** 53–66. (doi:10.1016/j.jsmb.2015.11.010)
- Shetty KT & Gaitonde BB 1980 Effect of the chronic administration of ethinyl estradiol and norgestrel on biogenic amine(s) level and monoamine oxidase enzyme activity in rat brain. *Biochemical Pharmacology* **29** 821–825. (doi:10.1016/0006-2952(80)90563-8)
- Shumaker SA, Legault C, Rapp SR, Thal L, Wallace RB, Ockene JK, Hendrix SL, Jones BN, Assaf AR, Jackson RD, et al. 2003 Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. *JAMA* **289** 2651–2662. (doi:10.1001/jama.289.20.2663)
- Simone J, Bogue EA, Bhatti DL, Day LE, Farr NA, Grossman AM & Holmes P V 2015 Ethinyl estradiol and levonorgestrel alter cognition and anxiety in rats concurrent with a decrease in tyrosine hydroxylase expression in the locus coeruleus and brain-derived neurotrophic factor expression in the hippocampus. *Psychoneuroendocrinology* **62** 265–278. (doi:10.1016/j.psyneuen.2015.08.015)
- Singh M 2007 Progestins and neuroprotection: are all progestins created equal? *Minerva Endocrinologica* **32** 95–102.
- Singh M & Su C 2013 Progesterone and neuroprotection. *Hormones and Behavior* **63** 284–290. (doi:10.1016/j.yhbeh.2012.06.003)
- Singh M, Su C & Ng S 2013 Non-genomic mechanisms of progesterone action in the brain. *Frontiers in Neuroscience* **7** 1–7.
- Sitruk-Ware L 2000 *Progestins and Antiprogestins in Clinical Practice*. New York: Marcel Dekker.
- Sitruk-Ware R 2002 Progestogens in hormonal replacement therapy: new molecules, risks, and benefits. *Menopause* **9** 6–15. (doi:10.1097/00042192-200201000-00003)

- Sitruk-Ware R 2004 Pharmacological profile of progestins. *Maturitas* **47** 277–283. (doi:10.1016/j.maturitas.2004.01.001)
- Sitruk-Ware R & Nath A 2010 The use of newer progestins for contraception. *Contraception* **82** 410–417. (doi:10.1016/j.contraception.2010.04.004)
- Skinner DC, Evans NP, Delaleu B, Goodman RL, Bouchard P & Caraty A 1998 The negative feedback actions of progesterone on gonadotropin-releasing hormone secretion are transduced by the classical progesterone receptor. *PNAS* **95** 10978–10983. (doi:10.1073/pnas.95.18.10978)
- Smith YR, Love T, Persad CC, Tkaczyk A, Nichols TE & Zubieta J-K 2006 Impact of combined estradiol and norethindrone therapy on visuospatial working memory assessed by functional magnetic resonance imaging. *Journal of Clinical Endocrinology and Metabolism* **91** 4476–4481. (doi:10.1210/jc.2006-0907)
- Sobey W 2008 The handbook of contraception: a guide for practical management. *Journal of Midwifery* **53** 99–100. (doi:10.1016/j.jmwh.2007.08.021)
- Stanczyk FZ 2003 All progestins are not created equal. *Steroids* **68** 879–890. (doi:10.1016/j.steroids.2003.08.003)
- Stanczyk FZ & Bhavnani BR 2014 Use of medroxyprogesterone acetate for hormone therapy in postmenopausal women: is it safe? *Journal of Steroid Biochemistry and Molecular Biology* **142** 30–38. (doi:10.1016/j.jsbmb.2013.11.011)
- Stanczyk FZ & Bhavnani BR 2015 Reprint of 'Use of medroxyprogesterone acetate for hormone therapy in postmenopausal women: is it safe?'. *Journal of Steroid Biochemistry and Molecular Biology* **153** 151–159. (doi:10.1016/j.jsbmb.2015.08.013)
- Stanczyk FZ, Hapgood JP, Winer S & Mishell DR 2013 Progestogens used in postmenopausal hormone therapy: differences in their pharmacological properties, intracellular actions, and clinical effects. *Endocrine Reviews* **34** 171–208. (doi:10.1210/er.2012-1008)
- Steckelbroeck S, Jin Y, Oyesanmi B, Kloosterboer HJ & Penning TM 2004 Tibolone is metabolized by the 3 α /3 β -hydroxysteroid dehydrogenase activities of the four human isozymes of the aldo-keto reductase 1C subfamily: inversion of stereospecificity with a delta5(10)-3-ketosteroid. *Molecular Pharmacology* **66** 1702–1711. (doi:10.1124/mol.104.004515)
- Stuenkel CA, Davis SR, Gompel A, Lumsden MA, Murad MH, Pinkerton JV & Santen RJ 2015 Treatment of symptoms of the menopause: an Endocrine Society Clinical Practice Guideline. *Journal of Clinical Endocrinology and Metabolism* **100** 3975–4011. (doi:10.1210/jc.2015-2236)
- Szot P, Wilkinson CW, White SS, Leverenz JB, Greenup JL, Colasurdo EA, Peskind ER & Raskind MA 2004 Chronic cortisol suppresses pituitary and hypothalamic peptide message expression in pigtailed macaques. *Neuroscience* **126** 241–246. (doi:10.1016/j.neuroscience.2004.03.030)
- Tepper R, Neri A, Kaufman H, Schoenfeld A & Ovadia J 1987 Menopausal hot flushes and plasma beta-endorphins. *Obstetrics and Gynecology* **70** 150–152.
- Thomas P 2008 Characteristics of membrane progesterin receptor alpha (mPR α) and progesterone membrane receptor component 1 (PGMRC1) and their roles in mediating rapid progesterin actions. *Frontiers in Neuroendocrinology* **29** 292–312. (doi:10.1016/j.yfrne.2008.01.001)
- Thomas P, Pang Y, Dong J, Groenen P, Kelder J, De Vlieg J, Zhu Y & Tubbs C 2007 Steroid and G protein binding characteristics of the seatrout and human progesterin membrane receptor α subtypes and their evolutionary origins. *Endocrinology* **148** 705–718. (doi:10.1210/en.2006-0974)
- Toffoletto S, Lanzenberger R, Gingnell M, Sundström-Poromaa I & Comasco E 2014 Emotional and cognitive functional imaging of estrogen and progesterone effects in the female human brain: a systematic review. *Psychoneuroendocrinology* **50** 28–52. (doi:10.1016/j.psyneuen.2014.07.025)
- Tsutsui K 2008 Progesterone biosynthesis and action in the developing neuron. *Endocrinology* **149** 2757–2761. (doi:10.1210/en.2007-1592)
- Tsutsui K, Ukena K, Sakamoto H, Okuyama S-I & Haraguchi S 2011 Biosynthesis, mode of action, and functional significance of neurosteroids in the purkinje cell. *Frontiers in Endocrinology* **2** 61. (doi:10.3389/fendo.2011.00061)
- Van Diepen HA, Lam TW & Kuil CW 2011 Nomegestrol acetate: steroid receptor transactivation profile in Chinese hamster ovary cells and ovulation inhibition in rat and monkey. *Contraception* **84** 199–204. (doi:10.1016/j.contraception.2010.11.017)
- Wagner KD & Berenson AB 1994 Norplant-associated major depression and panic disorder. *Journal of Clinical Psychiatry* **55** 478–480.
- Walf AA, Rhodes ME & Frye CA 2006 Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormone-primed rats. *Neurobiology of Learning and Memory* **86** 35–46. (doi:10.1016/j.nlm.2006.01.004)
- Wang M, He Y, Eisenman LN, Fields C, Zeng C-M, Mathews J, Benz A, Fu T, Zorumski E, Steinbach JH, et al. 2002 3 β -hydroxypregnane steroids are pregnenolone sulfate-like GABA(A) receptor antagonists. *Journal of Neuroscience* **22** 3366–3375.
- Wang JM, Liu L, Irwin RW, Chen S & Brinton RD 2008 Regenerative potential of allopregnanolone. *Brain Research Reviews* **57** 398–409. (doi:10.1016/j.brainresrev.2007.08.010)
- Wharton W, Hirshman E, Merritt P, Doyle L, Paris S & Gleason C 2008 Oral contraceptives and androgenicity: influences on visuospatial task performance in younger individuals. *Experimental and Clinical Psychopharmacology* **16** 156–164. (doi:10.1037/1064-1297.16.2.156)
- Wiegatz I, Sängster N & Kuhl H 2002 Formation of 7 α -methyl-ethinyl estradiol during treatment with tibolone. *Menopause* **9** 293–295. (doi:10.1097/00042192-200207000-00011)
- Yao J, Irwin R, Chen S, Hamilton R, Cadenas E & Brinton RD 2012 Ovarian hormone loss induces bioenergetic deficits and mitochondrial β -amyloid. *Neurobiology of Aging* **33** 1507–1521. (doi:10.1016/j.neurobiolaging.2011.03.001)
- Zacharia LC, Jackson EK, Kloosterboer HJ, Imthurn B & Dubey RK 2007 Conversion of tibolone to 7 α -methyl-ethinyl estradiol using gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry: interpretation and clinical implications. *Menopause* **13** 926–934. (doi:10.1097/01.gme.0000227331.49081.d7)
- Zhao L, Morgan TE, Mao Z, Lin S, Cadenas E, Finch CE, Pike CJ, Mack WJ & Brinton RD 2012 Continuous versus cyclic progesterone exposure differentially regulates hippocampal gene expression and functional profiles. *PLoS One* **7** e31267. (doi:10.1371/journal.pone.0031267)
- Zhao Y, Castiglioni S & Fent K 2015a Synthetic progestins medroxyprogesterone acetate and dydrogesterone and their binary mixtures adversely affect reproduction and lead to histological and transcriptional alterations in zebrafish (*Danio rerio*). *Environmental Science & Technology* **49** 4636–4645. (doi:10.1021/es505575v)
- Zhao Y, Castiglioni S & Fent K 2015b Environmental progestins progesterone and drospirenone alter the circadian rhythm network in zebrafish (*Danio rerio*). *Environmental Science & Technology* **49** 10155–10164. (doi:10.1021/acs.est.5b02226)
- Zhu Y, Bond J & Thomas P 2003 Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progesterin receptor. *PNAS* **100** 2237–2242. (doi:10.1073/pnas.0436133100)
- Zucchi S, Mirbahai L, Castiglioni S & Fent K 2014 Transcriptional and physiological responses induced by binary mixtures of drospirenone and progesterone in zebrafish (*Danio rerio*). *Environmental Science & Technology* **48** 3523–3531. (doi:10.1021/es405584f)

Received in final form 17 May 2016

Accepted 22 June 2016

Accepted Preprint published online 23 June 2016