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Physiological Relevance of Pregnanolone Isomers and Their Polar Conjugates with Respect to the Gender, Menstrual Cycle and Pregnancy

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1. Introduction

5α/β-Reduced progesterone metabolites (PM) including pregnanolone isomers (PI) and their polar conjugates (PIC), are efficient neuromodulators operating in a number of physiological and pathological processes including psychiatric diseases or problems connected with pregnancy, parturition and postpartum period. The substances mostly belong to the group of neuroactive steroids.

The neuroactive steroids (including PI) originating directly in the central and peripheral nervous system are called neurosteroids. They have a wide variety of functions. Some neuroactive steroids are able to easily pass through the blood-brain barrier (Bixo, Andersson, Winblad, Purdy, & Backstrom, 1997; Kancheva, et al., 2010; M. D. Wang, Wahlstrom, & Backstrom, 1997). Disorders in their biosynthesis or malfunctions in interactions with the target sites can be the cause of many pathologies, including psychiatric illnesses (Backstrom, et al., 2003; Backstrom, Carstensen, & Sodergard, 1976). In contrast with the most common steroid hormones acting, neuroactive steroids largely affect non-genomic mechanisms and influence nerve excitability in both directions. Some neuroactive steroids, such as allopregnanolone and its derivates, also show neuro-protective effects (Ciriza, Azcoitia, & Garcia-Segura, 2004; Morfin & Starka, 2001; Shi, Schulze, & Lardy, 2000).

The enzymes involved in the neurosteroidogenesis can be classified in two main groups: the cytochrome P450 and the non-P450 group. Experiments proved the direct biosynthesis of steroids in the brain independent of the periphery (Corpechot, Leclerc, Baulieu, & Brazeau, 1985; Corpechot, Robel, Axelson, Sjovall, & Baulieu, 1981). Three years later, Harrison and Simmonds published their work on the anesthetic effect of the synthetic pregnane steroid ganaxalone through modulation of the stimulation of the γ-aminobutyric acid receptor (GABA-r) (Harrison & Simmonds, 1984). The following year Majewska and coworkers published the first study on the modulation effect of endogenous steroids on GABA-r, and thereby initiated an intense research effort focused on the mechanisms of action of neuroactive steroids (Majewska, Bisserbe, & Eskay, 1985).

This chapter is focused on the origin and the physiological impact of endogenous neuroactive PI and PIC in humans, respecting the status of sex, menstrual cycle and pregnancy. Particular attention is paid on the role of PI in pregnancy and parturition respecting their extensive production in this period.

2. The neuromodulating effects of 5α/β-reduced pregnanes

2.1 Effects of 5α/β-reduced pregnanes on γ-aminobutyric acid receptors

Pregnanolone isomers are known to modulate ionotropic receptors on neuronal membranes (Pisu & Serra, 2004). 3α-PI shorten the paradoxical sleeping, reduce the acetylcholine in neocortex and hippocampus, suppress the neurogenesis and deteriorate the spatial memory (Mayo, et al., 2003). From the membrane receptors influenced by PI, the most familiar are the type-A γ–aminobutyric acid receptors ($GABA_A$-r) that control the influx of chloride ions into the neuronal cells. 5α/β-Reduced pregnane (and androstane) steroids with a hydroxy-group in the 3α-position positively modulate the $GABA_A$-r. The maximum sensitivity to 3α-PI and namely to pregnanolone (3α-hydroxy-5β-pregnane-20-one, 3α,5β-THP) was observed in the receptor subtype $\alpha_4\beta_3\delta$ (A. J. Smith, et al., 2001). Alternatively, inactive 3β-PI compete with the 3α-PI on $GABA_A$-r (Lundgren, Stromberg, Backstrom, & Wang, 2003; Prince & Simmonds, 1992; M. Wang, et al., 2002). While the 3α-PI are potent endogenous neuroinhibitory substances, the PIC are their antagonists. Conjugation counteracts the effect of 3α-PI, and further amplifies the antagonistic effect of the 3β-PI on $GABA_A$-r (Park-Chung, Malayev, Purdy, Gibbs, & Farb, 1999).

2.2 Effects of 5α/β-reduced pregnanes on N-methyl-D-aspartate receptors

Further receptors, which are influenced by steroid polar conjugates, are the N-methyl-D-aspartate receptors (NMDA-r). The NMDA-r are responsible for Ca^{2+} influx into the neurons inducing their rapid activation. 5α-PIC operate as activators of NMDA-r like the sulfates of 3β-hydroxy-5-en-steroids but the 5β-PIC are the antagonists of 5α-PIC. Besides the effects on neuronal membranes, some PI bind to progesterone intracellular receptors and influence also the gene expression of $GABA_A$-r subunits (Dubrovsky, 2005). Apart from the central nervous system (CNS), both NMDA-r and GABA-r are present in the peripheral neurons (Leung, et al., 2002; Majewska, Falkay, & Baulieu, 1989).

2.3 Effects of 5α/β-reduced pregnanes on T-type calcium channels

5β-PM block calcium channels of T-type in the rat peripheral neurons playing a significant role in pain perception and transmission (Todorovic, et al., 2004). The aforementioned mechanism indicates an antinociceptive action of 5β-PM on the peripheral level.

2.4 Effects of 5α/β-reduced pregnanes on L-type calcium channels

Factors regulating intracellular calcium concentration are known to play a critical role in the brain function and neural development, including neural plasticity and neurogenesis. 3α,5α-THP-induced intracellular calcium concentration increase may serve as the initiation mechanism whereby 3α,5α-THP promotes neurogenesis. 3α,5α-THP induces a rapid, dose-dependent, stereo-specific, and developmentally regulated increase in intracellular calcium in (rat embryonic) hippocampal neurons via a mechanism that requires both the $GABA_A$-r and L-type calcium channels (J. M. Wang & Brinton, 2008).

3. 5α/β-Reduced pregnanes in non-pregnant subjects

3.1 Sources of 5α/β-reduced pregnanes in non-pregnant subjects

The results in the literature show the necessity to differentiate between men, women in follicular phase of the menstrual cycle (FP), women in luteal phase of the menstrual cycle (LP), and pregnant women when evaluating changes of circulating PI that are linked to various pathologies. The neuroexcitatory PIC (acting via $GABA_A$-r) strikingly prevail over the neuroinhibitory unconjugated 3α-PI irrespectively of the subject status. On the other hand, the proportions in the circulating levels of neuroactive steroids do not necessarily have to reflect steroid ratios at the sites where they have an effect. It is likely that the pronounced excess of polar PI conjugates in the circulation is principally connected to their higher solubility in comparison with their non-polar free analogs. However, the chances of overcoming the blood-brain barrier generally increase with the decreasing polarity of the substance (Oren, Fleishman, Kessel, & Ben-Tal, 2004). This means that the transport of free PI would be preferred over that of the conjugates, despite their striking excess as reported in the model focused on the transport of free and conjugated pregnenolone from circulation into the brain in rats (M. D. Wang, et al., 1997). The conjugation of PI is also important for regulating the proportion between neuroexcitatory and neuroinhibitory pregnane steroids or, at least as a key metabolic step responsible for the elimination of neuroactive PI. Figure 1 demonstrates a simplified scheme of the biosynthesis and catabolism of 5α/β-reduced pregnanes.

3.1.1 Biosynthesis of neuroactive steroids in the cells of neuronal system

The 3α,5α-THP is present in human post-mortem brain tissue at considerably higher concentrations than typically observed in blood (Marx, et al., 2006). These neurosteroids are synthesized in brain, peripheral glial cells and neurons (Schumacher, et al., 2000). As demonstrated on rats, the enzymes that are necessary for synthesis of neuroactive 5α/β-PM as type 2 3β-hydroxysteroid dehydrogenase (HSD3B2) and 5α-reductase of types 1 (SRD5A1) and 2 (SRD5A2) are present in the CNS. While the SRD5A1 was identified for the most part in glial cells of white matter, SRD5A2 was found in oligodendrocytes, neurons and astrocytes of the grey matter. The enzyme isoforms, which are effective as the 3α-hydroxysteroid dehydrogenase are present in oligodendrocytes, neurons and astrocytes of white and grey matter (Patte-Mensah, Penning, & Mensah-Nyagan, 2004; Schumacher, et al., 2004; Stoffel-Wagner, et al., 2000; Tsuruo, 2005). The important system mediating changes or even reversion of neuromodulating activity involves a steroid sulfatase (STS) and sulfotransferases controlling the balance between neuroinhibitory 3α-PI and PIC that exert an opposite effect. However, relatively high STS activity but very low sulfotransferase activity were detected in the brain (Compagnone, Salido, Shapiro, & Mellon, 1997; Kriz, Bicikova, Hill, & Hampl, 2005).

The GABAergic steroids can be inactivated by their 3α-oxidation to yield 5α-dihydroprogesterone (5α-DHP). It was found that 5α-DHP levels in HEK293 cells expressing type 10 17β-hydroxysteroid dehydrogenase (HSD17B10) increased as 3α,5α-THP was added to culture media. Brain astrocytes contain a moderate level of HSD17B10, which is elevated in activated astrocytes of brains with Alzheimer type pathology. Cerebral cortex has the lowest level of HSD17B10; whereas the hippocampus, hypothalamus, and amygdala possess relatively higher levels of this enzyme. The catalysis of HSD17B10 appears to be essential for maintaining normal functions of GABAergic neurons (He, Wegiel, & Yang, 2005).

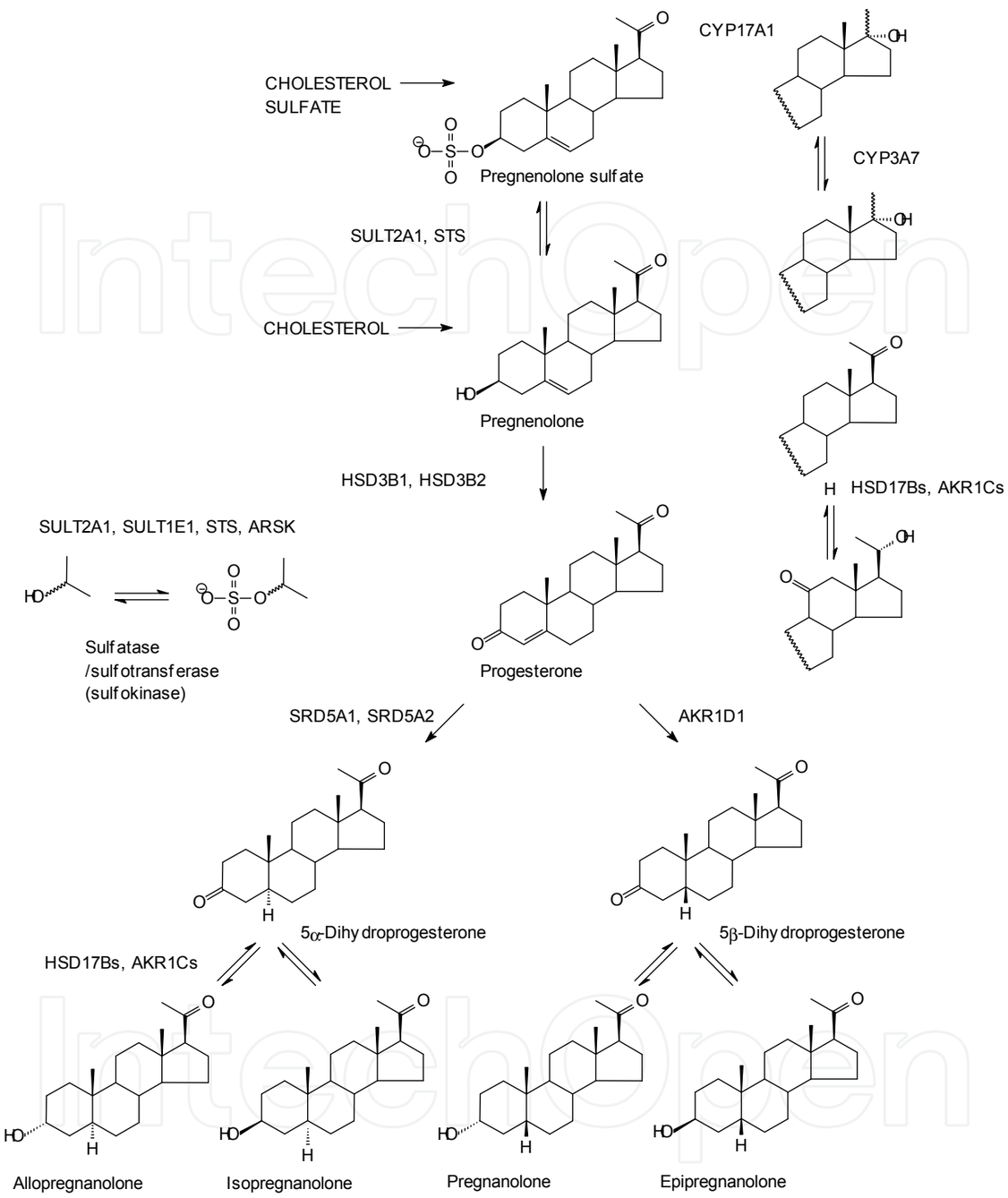

Fig. 1. Simplified scheme of the biosynthesis and catabolism of 5α/β-reduced pregnanes

3.1.2 Gonadal function and neuroactive steroids

The most part of neuroactive steroids in women in the luteal phase of menstrual cycle (LP) consists of metabolites of progesterone, which is formed in *corpus luteum* (Ottander, et al., 2005). The levels of PI and PIC strongly depend on the menstrual cycle, reflecting changes in progesterone formation. The mRNA of 5α-reductase (SRD5A), 5β-reductase (AKR1D1) and 3α-hydroxysteroid oxidoreductase (3α-HSOR) mRNA are all expressed in human *corpus*

luteum and the release of 3α,5α-THP and 3α,5β-THP herein is stimulated by a trophic hormone. The dominant PI is the 3α,5α-THP (Havlikova, et al., 2006; M. Hill, et al., 2005). In women, progesterone and its reduced metabolites exhibit a decrease with increasing age and a qualitative change after menopause (Genazzani, et al., 1998). It is likely that the gonadal 5α/β-PM easily overcome the blood-brain-barrier (Bixo, et al., 1997; Kancheva, et al., 2010).

3.1.3 The role of adrenals in the biosynthesis of neuroactive steroids

Zona glomerulosa controlled by the renin-angiotensin axis produces deoxycorticosterone (DOC), the metabolites of which are neuroactive like the 3α,5α-tetrahydro-DOC (3α,5α-THDOC) and its isomers. However, DOC is produced in substantially greater quantities in *zona fasciculata*, which is controlled by the CRH-ACTH system. In contrast to the 3α,5α-THP reaching about 10% of progesterone concentration, the basal levels of DOC and 3α,5α-THDOC are almost comparable (<0.5 nmol/L) (Reddy, 2006).

Zona fasciculata primarily produces cortisol in relatively high amounts. 3α-5α/β-Reduced metabolites of cortisol are GABAergic such as the 3α-PI. The 3α,5α-tetrahydrocortisol and 3α,5α-THP posses a comparable activity on $GABA_A$-r (Stromberg, Backstrom, & Lundgren, 2005). In addition, adrenal *zona fasciculata* produces relatively abundantly pregnenolone sulfate (PregS) (20-400 nmol/l), which, like the cortisol, readily reacts to adrenocorticotropin (ACTH) stimulation (de Peretti, et al., 1986). PregS appears to be the most important precursor of progesterone and DOC of adrenal origin. Growing formation of PregS in adrenals that is further metabolized up to neuroactive PI may explain the increased levels of brain 5α/β-PM in patients with diagnoses associated with stress (Higashi, Takido, & Shimada, 2005). Increasing peripheral production of pregnane steroids and their precursors and their subsequent transport across the blood-brain-barrier could contribute to the physiological compensation of stress. Most probably, the peripheral levels of pregnane steroids primarily depend on adrenal activity in women in FP (Havlikova, et al., 2006; M. Hill, et al., 2005), postmenopausal women, children and men (Fig. 2A,B). However, the proportion of 5α/β-PM derived from the adrenal activity is pronouncedly lower compared to the quantity originating in the *corpus luteum* (Meczekalski, et al., 2000) (Fig. 2C).

3.2 Human CNS-related pathologies that are linked to the 5α/β-reduced pregnanes

3.2.1 Premenstrual syndrome

Changes in progesterone levels and respective changes in its neuroactive metabolites are apparently the cause of premenstrual dysphoric disorder in women (PMDD). Withdrawal effect in case of abrupt drop of steroid positive modulators rapidly supervenes like the addiction effect while increasing the steroid levels. Changing 5α/β-PI concentrations induce a decreased affinity of $GABA_A$-r for these steroids due to the changed expression of the receptor subunits and/or as a result of the changed phosphorylation status of the specific sites on the $GABA_A$-r (Brussaard, Wossink, Lodder, & Kits, 2000; Koksma, et al., 2003; Leng & Russell, 1999; Maguire & Mody, 2009). The aforementioned mechanism requires synchronization, the disturbances of which could have significant neuropsychiatric consequences in physiological and pathological situations like pregnancy, parturition, onset of menopause, traumas, endocrine diseases, and stress. Several $GABA_A$-r modulators, including 3α,5α-THP, exert biphasic effect. The low concentrations induce an adverse, anxiogenic effect whereas the higher concentrations decrease this effect and show calming properties (Andreen, et al., 2009). The severity of these mood symptoms is related to the

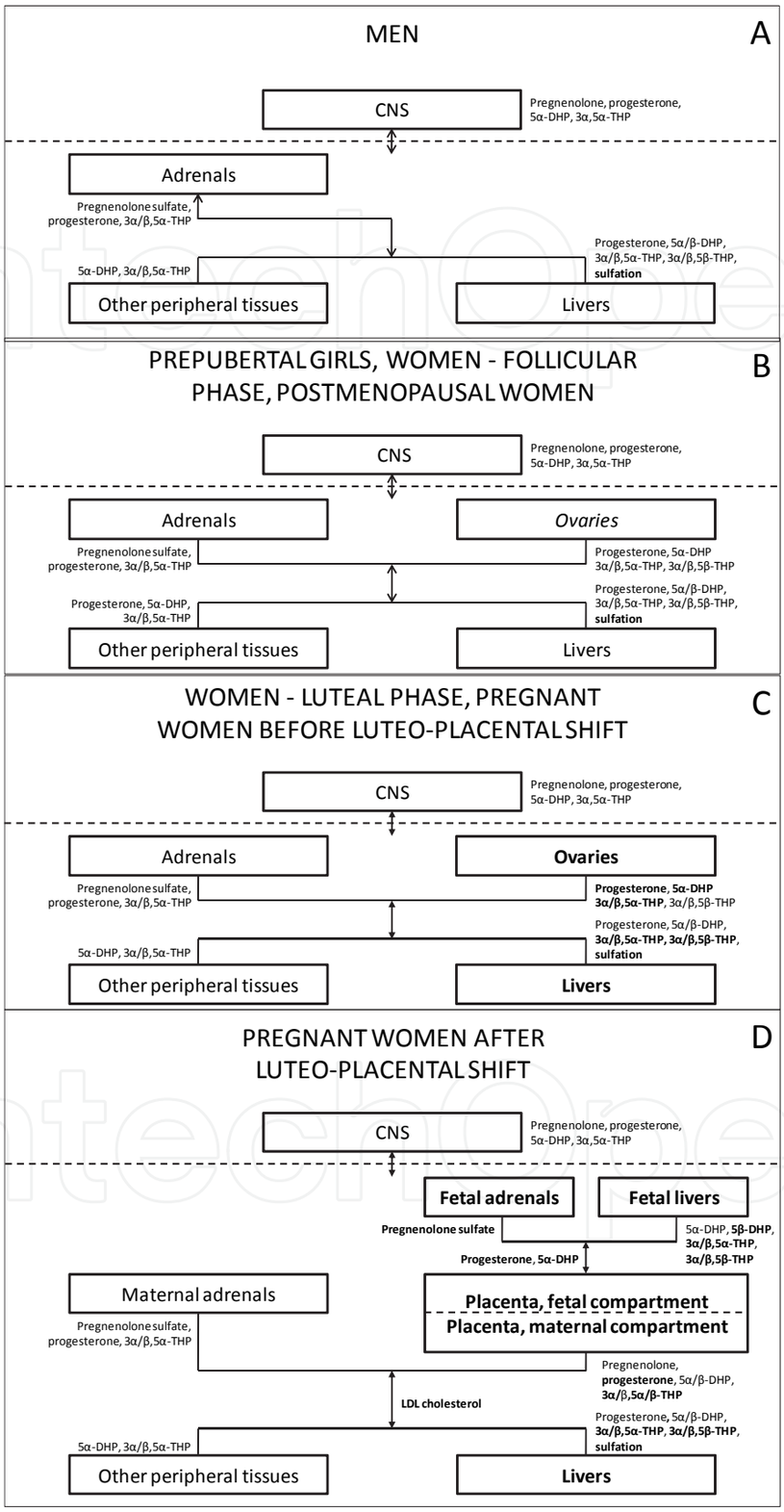

Fig. 2. Origin of pregnanolone isomers

3α,5α-THP serum concentrations in a manner similar to a bell-shaped curve. Negative mood symptoms occur when the serum concentration of 3α,5α-THP is similar to the endogenous LP levels, while low and high concentrations have no such effect. Progesterone/3α,5α-THP treatment in women increases the activity in the amygdala in a similar manner as the changes seen during anxiety reactions. Women with PMDD in LP show changes in $GABA_A$-r sensitivity and GABA concentrations that are related to the severity of the condition (Andreen, et al., 2009).

3.2.2 Chronic fatigue syndrome

Increased inhibition through $GABA_A$-r due to the accumulation of neuroinhibitory steroids may represent an important pathophysiological mechanism of fatigue in chronic liver diseases. The levels of 3α,5α-THP and 3β,5α-THP are increased in plasma of patients with chronic fatigue syndrome (Ahboucha, et al., 2008).

3.2.3 Depression

Antidepressants elevate 3α-PI levels in rodent brain (Uzunova, et al., 1998). However, recent studies suggest that changes in plasma neuroactive steroid levels may not be a general mandatory component of clinically effective antidepressant treatment *per se*, but may reflect distinct properties of pharmacotherapy only (Uzunova, Sampson, & Uzunov, 2006).

3.2.4 Epilepsy

Women with epilepsy show reduced progesterone levels in the LP. The progesterone deficit results in the debit of neuroinhibitory 3α-hydroxy- 5α/β-reduced progesterone metabolites (Stoffel-Wagner, 2001). Rat models of catamenial epilepsy exhibit an abstinence effect at lowered 3α,5α-THP concentrations, which however results in higher sensitivity after restitution of its original levels. Various authors demonstrate that catamenial epilepsy is linked to the disturbed biosynthesis of progesterone and its reduced metabolites (Backstrom, et al., 2003), particularly in the LP (Bonuccelli, et al., 1989).

3.2.5 Schizophrenia

GABAergic steroids may be candidate modulators for the pathophysiology of schizophrenia and bipolar disorder, and relevant to the treatment of these disorders. 3α,5α-THP levels tend to be decreased in parietal cortex in subjects with schizophrenia compared to control subjects (Marx, et al., 2006).

3.2.6 Neurodegenerative disorders

3α,5α-THP is reduced in prefrontal cortex in male patients with Alzheimer disease (AD) compared to male cognitively intact control subjects, and inversely correlated with neuropathological disease stage. 3α,5α-THP levels are reduced in temporal cortex in patients with AD compared to control subjects and inversely correlated with neuropathological disease stage. Patients carrying an APOE4 allele demonstrate reduced 3α,5α-THP levels in temporal cortex (Naylor, et al., 2010).

3.2.7 Eating disorders

Compared with healthy women, the patients with eating disorders exhibit increased plasma levels of 3α,5α-THP. However, the relevance of such hormonal alteration to the

pathophysiology of eating disorders remains to be elucidated (Monteleone, et al., 2001) (Monteleone, et al., 2003).

4. 5α/β-Reduced pregnanes in human pregnancy

4.1 Fetal adrenal is the primary source of pregnancy steroids

4.1.1 Placental CRH controls the steroid biosynthesis in the fetal adrenal

The machinery regulating production of pregnancy steroids (including pregnanolone isomers and their polar conjugates) is based on the excessive placental production of corticoliberin (CRH) (Goland, Wardlaw, Stark, Brown, & Frantz, 1986; Rainey, Rehman, & Carr, 2004; R. Smith, et al., 2009). CRH in non-pregnant subjects is a hypothalamic hormone controlling the pituitary secretion of adrenocorticotropic hormone (ACTH) and, in turn, the corticosteroid production in adult adrenal. The hypothalamic-pituitary-adrenal axis in these subjects is based on a negative feedback loop between the final active hormone, ACTH and CRH. Alternatively, the pregnant women after luteo-placental shift produce CRH primarily in placenta and instead of the negative feedback loop cortisol-ACTH-CRH; there is a positive one between cortisol and CRH, while the ACTH production stagnates. The rising CRH levels in the last four weeks of pregnancy stimulate the synthesis of conjugated Δ^5 steroids (Sirianni, Mayhew, Carr, Parker, & Rainey, 2005; R. Smith, Mesiano, Chan, Brown, & Jaffe, 1998) in the fetal zone of the fetal adrenal (FZ), which is a specific transient tissue gradually converting to *zona reticularis* after labor. The excessive production of placental CRH is unique for primates and the boosting CRH production near term is exclusive for humans and great apes (Power & Schulkin, 2006).

The sulfated Δ^5 steroids, originating in the FZ represent the largest fraction of steroids in pregnancy (Ingelman-Sundberg, Rane, & Gustafasson, 1975; Lacroix, Sonnier, Moncion, Cheron, & Cresteil, 1997; Leeder, et al., 2005; Moghrabi, Head, & Andersson, 1997) (Fig. 2D). Sulfotransferase 2A1 (SULT2A1) transcript shows even 13-fold higher levels in the fetal adrenal. Alternatively, HSD3B2 mRNA expression in midgestation is 127-fold lower than the one in the adult adrenal due to preferential synthesis of the Δ^5 C-21 steroids over corticoids. The FZ is similar to the adult *zona reticularis* but unlike the adult *zona reticularis*, the FZ produces excessive amounts of sulfated C-21 Δ^5 steroids, including pregnenolone sulfate (PregS) (M. Hill, Parizek, Cibula, et al., 2010; M. Hill, Parizek, Jirasek, et al., 2010; Rainey, et al., 2004). The Δ^5 steroid sulfates (originating in the FZ) serve as precursors for the placental production of estradiol (Sirianni, et al., 2005; R. Smith, et al., 1998) and progesterone (M. Hill, Parizek, Jirasek, et al., 2010; Jaffe & Ledger, 1966; Komatsuzaki, et al., 1987; Walsh, 1988).

4.2 Steroid metabolism in the fetal and maternal liver

4.2.1 C-3, C-17 and C-20 oxidoreductive conversions

Human liver contains various isoforms of pluripotent aldoketo reductases (AKR1C1, AKR1C2, AKR1C3, and AKR1C4) with 20α-, 17β-, 3α- or 3β-hydroxysteroid dehydrogenase-like activity (Jin, et al., 2009; Penning, et al., 2001; Shiraishi, et al., 1998). The enzyme activities could control the occupancy of $GABA_A$-receptors (Penning, 1999) via reduction of oxo-groups in the steroid C3 position. *In vivo*, all AKR1Cs preferentially work as reductases (Steckelbroeck, Jin, Gopishetty, Oyesanmi, & Penning, 2004) and are capable of reducing estrone, progesterone, and 3-oxo- pregnane (androstane) steroids to estradiol, 20α-dihydroprogesterone, and GABAergic 3α-hydroxy-5α/β- pregnane (androstane) steroids,

respectively. On the other hand, AKR1Cs may decrease the neurosteroid concentrations by inactivating 3α,5α-THP and eliminating the precursors like progesterone from the synthetic pathways via reduction of the 20-oxo-steroid group (Penning, et al., 2000; Usami, et al., 2002). The AKR1C2 preferring 3α-reduction over the 3β-reduction may catalyze 3α-, 17β-, and 20α-HSD reactions (Jin, et al., 2009; Jin, et al., 2001; Penning, et al., 2000; Usami, et al., 2002). From the family of short chain dehydrogenases (SDRs), type 7 17β-hydroxysteroid dehydrogenase (HSD17B7), preferring the reduction of the oxo-groups in 20-, 17- or 3-position to the corresponding 20α-hydroxy-, 17β-hydroxy- or 3α-hydroxy-counterparts, is also significantly expressed in the liver (Krazeisen, et al., 1999; Torn, et al., 2003).
Instead, other SDRs like type 2 HSD17B (HSD17B2), type 10 17β-HSD (HSD17B10) and type 11 17β-HSD (HSD17B11), which are also highly expressed in the liver, prefer the oxidative direction. HSD17B2 may contribute to the formation of 20-oxo- and 17-oxo-steroids from their 20α- and 17β- counterparts (Moghrabi, et al., 1997). Type 6 17β-HSD (HSD17B6) prefers oxidoreductase and 3(α-->β)-hydroxysteroid epimerase activities and acts on both C-19 and C-21 3α-hydroxysteroids (Huang & Luu-The, 2000). HSD17B10 being abundantly expressed in the liver, is capable of catalyzing the oxidation of steroid modulators of $GABA_A$-r (He, et al., 2001). HSD17B10 catalyzes the conversion of 3α,5α-THP and 3α,5α-THDOC to the corresponding inactive 3-oxo steroids (He, et al., 2003). The catalysis of HSD17B10 appears to be essential for maintaining normal functions of GABAergic neurons (Shafqat, et al., 2003).

4.2.2 5α/β-Reductases

The liver has also high activity of SRD5A and AKR1D1 (Charbonneau & The, 2001; Meikle, Stringham, Wilson, & Dolman, 1979). From the two isoforms of SRD5A, SRD5A1 is widely distributed in the body, with the highest levels in the liver and converts testosterone into 5α-dihydrotestosterone and progesterone, and corticosterone into their corresponding 3-oxo-5α-reduced steroids. In the androgen-dependent structures, 5α-DHT is almost exclusively formed by SRD5A2 (Poletti, et al., 1998). In the peripheral tissues, including the liver, SRD5A1 and 3α-HSD reductive AKR1Cs and HSD17Bs work consecutively eliminating the androgens, protecting against the hormone excess (Jin & Penning, 2001) and producing GABAergic steroids, which are, however, extensively sulfated in the liver.
Liver AKR1D1 efficiently catalyzes the reduction of both C-19 and C-21 3-oxo-Δ^4 steroids to the corresponding 5β-PM (Kochakian, 1983; Okuda & Okuda, 1984). The higher levels of 5β-PM in the fetus than in maternal compartment as well as the arteriovenous differences in the fetus indicate that steroid 5β-reduction in the fetal liver (but not in the placenta) is important for production of 5β-PM in both maternal and fetal compartment (M. Hill, Parizek, Cibula, et al., 2010).

4.2.3 Balance between polar conjugates and unconjugated steroids

The sulfotransferase SULT2A1 is highly expressed in human liver (Comer & Falany, 1992; Geese & Raftogianis, 2001; Meloche & Falany, 2001; Zhang, Varlamova, Vargas, Falany, & Leyh, 1998). However, the liver also strongly expresses the STS (Selcer, Difrancesca, Chandra, & Li, 2007) like the placenta. The formation of sulfated steroids with a 3α-hydroxy-5α configuration may account for 50% of the metabolism of progesterone in late pregnancy (Anderson, et al., 1990). The sulfation of 3β,5α-THP is an important metabolic step contributing to progesterone catabolism and significantly affecting the balance between

neuroinhibitory steroids and their antagonists in pregnant women. The further major pathway of progesterone catabolism in the maternal compartment proceeds in the sequence progesterone → 5β-DHP → 3α,5β-THP→ conjugated 3α,5β-THP (Kancheva, et al., 2007), which is analogous to the situation out of pregnancy (Havlikova, et al., 2006). In this pathway, the 5β-reduction and the reduction of the 3-oxo-group in 5β-DHP, resulting in the synthesis of 3α,5β-THP, appear to be critical metabolic steps (Kancheva, et al., 2007). Humans with low progesterone production exhibit very low concentrations of unconjugated 3α,5β-THP (men, women in the FP). In these subjects, the 3α,5β-THP is rapidly conjugated. Alternatively, in woman in the LP and so much the more in pregnant women, the conjugation capacity for 3α,5β-THP may be limited. The increased conjugation of 5α-PI probably further diminishes the difference between the 3α,5α-THP and 3α,5β-THP levels in pregnant women and may also regulate the proportions between neuroinhibitory 3α,5α-THP and antagonistic conjugated 5α-PI (Kancheva, et al., 2007).

4.3 5α/β-Reductases in the liver, placenta and fetal membranes

Placental SRD5A1 and SRD5A1 may provide precursors for 3α,5α-THP synthesis in fetal brain (Vu, et al., 2009). Milewich, et al. reported *in vitro* synthesis of 5α-reduced pregnanes [3H]5α-DHP and [3H]3β,5α-THP from [3H]progesterone by a placental tissue (Milewich, Gant, Schwarz, Chen, & Macdonald, 1978). Although AKR1D1, catalyzing the 5β-reduction is primarily expressed in the liver, its activity was also detected in other tissues including placenta (Sheehan, Rice, Moses, & Brennecke, 2005). However, AKR1D1 activity in extrahepatic tissues appears to be minor in comparison with that found in the liver (M. Hill, Parizek, Cibula, et al., 2010; Milewich, Gant, Schwarz, Chen, & MacDonald, 1979).

4.4 Steroid metabolism in placenta

4.4.1 Steroid sulfatases and placental production of sex hormones

The principal metabolic step that is indispensable for placental metabolism of sulfated Δ^5 steroids originating in FZ is their desulfation, which is catalyzed by the placental STS. Placental STS activity is independent of substrate concentration (Watanabe, et al., 1990) and of gestational age (GA) (Fukuda, Okuyama, & Furuya, 1986; Ishida, et al., 1985; Leslie, et al., 1994). The placental STS expression in pregnancy explicitly outweighs the production in other tissues (Miki, et al., 2002) and allows access of Δ^5 steroids to the HSD3B1 and CYP19A1 within the syncytiotrophoblast layer and their conversion to estrogens (Siiteri, 2005) and progestogens (M. Hill, Parizek, Cibula, et al., 2010; M. Hill, Parizek, Jirasek, et al., 2010). The latter substances are subsequently converted to 5α/β-PM by placental and liver enzymes.

4.4.2 Reversible C-3, C-17 and C-20 oxidoreductive inter-conversions in placenta and fetal membranes

The cytoplasmic type 1 HSD17B (HSD17B1) is highly expressed in syncytiotrophoblast (Moghrabi, et al., 1997). Besides catalyzing the conversion of estrone and progesterone to estradiol and 20α-dihydroprogesterone, respectively, HSD17B1 may also catalyze the formation of 5-androstene-3β,17β-diol from dehydroepiandrosterone (Lin, et al., 2006; Peltoketo, Nokelainen, Piao, Vihko, & Vihko, 1999). Syncytiotrophoblast, coming directly into contact with maternal blood, converts estrone to estradiol. In contrast to HSD17B1 mRNA, HSD17B2 mRNA is not detectable in cell cultures of human cytotrophoblast nor

syncytiotrophoblast (Bonenfant, Provost, Drolet, & Tremblay, 2000). Besides HSD17B1, the AKR1C3, HSD17B7 and type 12 HSD17B (HSD17B12) may also catalyze progesterone deactivation to 20α-dihydroprogesterone and conversion of inactive estrone to bioactive estradiol (Li, et al., 2005; Peltoketo, et al., 1999; Penning, et al., 2001; Sakurai, et al., 2006). AKR1C3 functions as a bi-directional 3α-, 17β- and 20α-HSD and can interconvert active androgens, estrogens, progestins and their 5α/β reduced metabolites with their cognate inactive metabolites. However, like other AKR1Cs *in vivo*, AKR1C3 preferentially works as a reductase (Matsuura, et al., 1998; Penning, et al., 2001; Steckelbroeck, et al., 2004). Although this enzyme is expressed in placenta, its importance appears to be secondary to the HSD17B1.

HSD17B2 (preferring the oxidative direction) converts inactive 20α-dihydroprogesterone to bioactive progesterone as well as the bioactive estradiol to inactive estrone (Moghrabi, et al., 1997). HSD17B2 may also convert the GABAergic 3α-hydroxy-5α/β-PM to inactive and antagonistic substances but, on the other hand, may transform the less active GABAergic 3α,20α-dihydroxy-5α/β-isomers to the more active 3α-hydroxy-5α/β-20-oxo-isomers. The site of expression of HSD17B2 was identified in endothelial cells of fetal capillaries and some stem villous vessels (Moghrabi, et al., 1997; Takeyama, et al., 1998) and in endothelial cells of villous arteries and arterioles (Bonenfant, Blomquist, et al., 2000) in the close proximity of the fetal circulation. The reversible oxido-reductive interconversion of GABAergic C21 and C19 3α-hydroxy-5α/β-reduced metabolites to the corresponding inactive 3-oxo-metabolites and antagonistic 3β-hydroxy-metabolites (catalyzed by HSD17Bs an AKR1C1s) may also influence the balance between neuroinhibitory and neuroexcitatory steroids (Lundgren, et al., 2003). While the reductive conversion in the C3 position produce GABAergic steroids, the conversion of 20-oxo- to 20α-hydroxy-group or a modification of the C17,20 side chain in the 3α-hydroxy-5α/β C21 steroids result in subtype dependent reduction of positive allosteric modulation of $GABA_A$-r (Belelli, Lambert, Peters, Gee, & Lan, 1996).

In all probability, the distribution of placental oxidoreductase isoforms controls the reductive and oxidative status of steroid inter-conversions in maternal and fetal compartment, respectively (Fig. 3). Therefore the difference between oxidative fetal- and reductive maternal steroid metabolomic status is the most apparent when comparing umbilical venous blood, containing placental steroids before their further metabolism in other fetal tissues and maternal venous blood. The umbilical venous blood contains higher proportions of 20-oxo-steroids including progesterone, 17-oxo steroids (e.g. estrone and dehydroepiandrosterone), 3-oxo-steroids like 5α/β-DHP, and 3β-hydroxy-steroids (3β,5α-THP and 3β,5β-THP), while maternal venous blood contains higher proportions of 20α-hydroxy-steroids (like 20α-dihydroprogesterone), 17β-hydroxy-steroids (such as estradiol and androstenediol) and 3α-hydroxy-5α/β-reduced steroids (like GABAergic 3α,5α-THP and 3α,5β-THP). Even the levels of conjugated 3α-hydroxy-5α/β-reduced-17-oxo C-19 steroids in maternal venous blood are pronouncedly higher than in the fetal circulation, while the 3β-isomers does not significantly differ (M. Hill, Parizek, Cibula, et al., 2010) (Fig. 3).

Some authors report that the metabolism of placental sex steroids in the reductive direction increases as pregnancy advances and significantly rises during human parturition (Diaz-Zagoya, Wiest, & Arias, 1979; Milewich, et al., 1978). This phenomenon may be of an importance in the mechanism of initiation and continuation of labor and might indicate a mechanism of progesterone withdrawal and estradiol rise in association with the onset of human parturition.

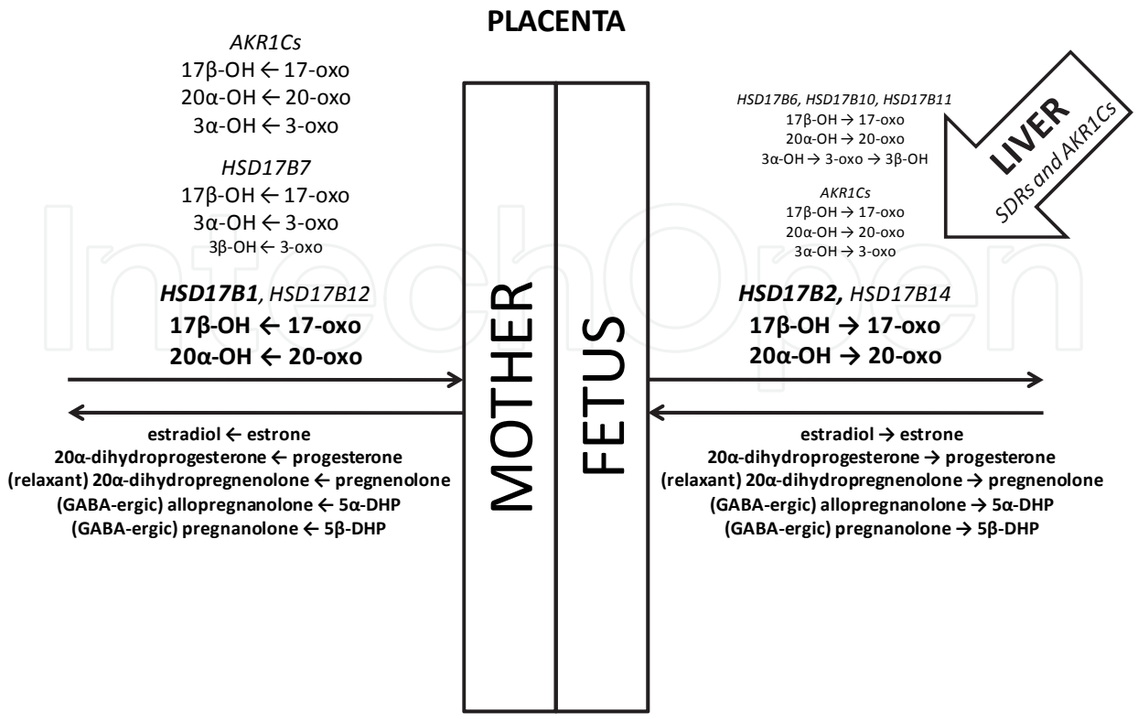

Fig. 3. Steroid conversion during the transplacental passage

4.5 Levels of 5α/β-reduced pregnanes in pregnant women and fetuses

4.5.1 5α/β-Reduced pregnanes in pregnant and non-pregnant women

In pregnant women, the levels of 5α/β-PM including the GABAergic 3α-PI are persistently elevated (M. Hill, et al., 2007; M. Hill, Parizek, Kancheva, et al., 2010; Kancheva, et al., 2007; Luisi, et al., 2000; Mickan & Zander, 1979; Parizek, et al., 2005; Pearson Murphy, Steinberg, Hu, & Allison, 2001). Their concentrations in women after luteo-placental shift reach values about two orders of magnitude higher than the concentrations detected in the FP (Parizek, et al., 2005). Pearson Murphy et al. show that the levels of C21 steroids including 5α/β-PM rise greatly during pregnancy, being the highest for progesterone (562-fold the FP level), 5α-DHP (161-fold), 3β,5α-THP (56-fold), 3α,5α-THP (37-fold), pregnenolone (30-fold), 5β-DHP (16-fold) and 3β,5β-THP (16-fold) at 37th week of gestation (Pearson Murphy, et al., 2001). As already mentioned, these conditions induce a decreased affinity of $GABA_A$-r for the 5α/β-PI.

4.5.2 5α/β-reduced progesterone metabolites around parturition

Pearson Murphy et al. demonstrate that during the period 2-7 day postpartum, the level of progesterone fall precipitously, whereas those of pregnenolone and the metabolites decrease more slowly and their levels are still elevated compared with FP levels 2 weeks after delivery. By the 7th week postpartum only 3α,5α-THP and 3β,5β-THP remains slightly elevated (Pearson Murphy, et al., 2001). Our recent report (M. Hill, Parizek, Cibula, et al., 2010; M. Hill, Parizek, Kancheva, et al., 2010) as well as our previous data for PI around parturition display significantly lower PIC/PI ratios in the umbilical venous plasma than in the maternal plasma (M. Hill, et al., 2001; Klak, et al., 2003). Changes in concentrations of PI in the maternal serum exhibit a similar pattern, falling mostly within the first hour after the

delivery. The decrease in PIC is shifted to the interval within the first hour and first day after delivery (M. Hill, et al., 2001; Klak, et al., 2003). The PIC/PI ratios significantly decrease within the first hour and the first day after delivery in all PI (M. Hill, et al., 2001; Klak, et al., 2003). These results indicate an intensive sulfation of GABAergic substances in the maternal compartment during pregnancy but attenuating sulfation activity shortly after labor. The sulfation of GABAergic steroids (transforming them to antagonistic substances) might represent a mechanism counterbalancing their placental overproduction. The ratios of 3α/3β-PI decrease around parturition (M. Hill, et al., 2001; Klak, et al., 2003), which may indicate that the placental and possibly also the liver reductive conversion of the 3-oxo- and 3β-hydroxy-5α/β-PI to the 3α-isomers may be of importance for pregnancy sustaining.

4.6 Effects of 5α/β-reduced pregnanes in pregnant women and fetuses

4.6.1 The role of progestogens and their 5α/β-reduced metabolites in pregnancy sustaining and induction of labor

Pregnant women and fetuses have exceedingly elevated levels of steroids positively modulating NMDA-r (including the 5α-PIC) (M. Hill, et al., 2007; M. Hill, Parizek, Cibula, et al., 2010; M. Hill, Parizek, Kancheva, et al., 2010; Malayev, Gibbs, & Farb, 2002; Weaver, et al., 2000). On the other hand, the 5β-PIC exert an antagonistic effect on NMDA-r (Malayev, et al., 2002; Park-Chung, et al., 1997; Weaver, et al., 2000) and promote their desensitization (Kussius, Kaur, & Popescu, 2009). Like the levels of other PIC, the levels of conjugated 3α,5β-THP are also extremely elevated in pregnant women and (in contrast to slightly increasing, stagnating or even decreasing levels of GABAergic PI) pronouncedly rise in the late pregnancy (Gilbert Evans, Ross, Sellers, Purdy, & Romach, 2005; M. Hill, et al., 2007; M. Hill, Parizek, Cibula, et al., 2010; M. Hill, Parizek, Kancheva, et al., 2010; Luisi, et al., 2000; Parizek, et al., 2005; Pearson Murphy, et al., 2001). These findings allow a speculation whether the conjugated 3α,5β-THP might serve as an endogenous antinociceptive agent around parturition (Hering, et al., 1996; Kallela, Haasio, & Korttila, 1994).

On the other hand, rising steroid sulfation that catabolizes both 3α,5α-THP and 5β-reduced steroids, produces high amounts of PIC. PIC induce neuroexcitatory effect via $GABA_A$-r and may shift the biological activity towards induction of labor (Park-Chung, et al., 1999). Majewska and Vaupel (Majewska & Vaupel, 1991) reported that 3α,5α-THP interact with $GABA_A$-r to modulate uterine contractility: 3α,5α-THP inhibits while PregS increases contractions. Further, 3α,5α-THP rapidly antagonizes the stimulatory effect of PregS, but progesterone inhibits the contractions after a delay, suggesting that the known pregnancy sustaining effect of progesterone on the uterus is at least partly mediated via the metabolite 3α,5α-THP, which potentiates the neuroinhibitory function of $GABA_A$-r (Majewska & Vaupel, 1991). On the other hand, Lofgren et al. (Lofgren, Holst, & Backstrom, 1992) reported contradictory data. Concerning the 3α-hydroxysteroid oxidoreductase-mediated turnover of 5α-DHP and 5β-DHP to their metabolites 3α,5α-THP and 3α,5β-THP, respectively, which reflects the ratios between these GABAergic 3α-PI and their inactive precursors, Gilbert Evans et al. (Gilbert Evans, et al., 2005) reported that the turnover of 5α-DHP to 3α,5α-THP rise during pregnancy and drops at the late prenatal visit. At 6 weeks postpartum, all steroids are significantly reduced compared with late prenatal values. Although, we have found no significant change of the ratio 3α/3β-PI during pregnancy (Parizek, et al., 2005), our more recent study shows contradictory results to the data of Gilbert Evans et al. and demonstrates a moderate but significant shift from the 3α-PI to the-3-oxo-isomers (M. Hill, Parizek, Cibula, et al., 2010).

When testing the capacity to inhibit the *in vitro* motility of rat uterus, progestins with their ring A reduced in the 5β-position are significantly more potent than Δ^4-3-oxo and 5α-reduced progestins (Kubli-Garfias, Medrano-Conde, Beyer, & Bondani, 1979; Perusquia & Jasso-Kamel, 2001). The 5α/β-PM elicit an immediate relaxing effect that is dose-dependent. With the exception of two 5α/β-PM (5α-DHP and 3β,5α-THP), the remaining ones used in the present study are more potent than progesterone. It is important that when the tissues are washed, the contractile activity is recovered. This rapid and reversible relaxing effect is not blocked by antiprogestin RU 486, which suggests its independence of receptor-mediated genomic action (Perusquia & Jasso-Kamel, 2001).

Being already mentioned, the abundance of progesterone, 3α-hydroxy-5α/β-pregnane-steroids and estradiol levels in pregnancy is high. Some of them like 3α,5α-THDOC, 3α,5α-THP and progesterone induce opening of voltage-dependent K^+ channels and relaxes myometrium while estradiol is their antagonist (Knock, Tribe, Hassoni, & Aaronson, 2001; Perusquia & Jasso-Kamel, 2001; Yoshihara, et al., 2005). Therefore the ratios progesterone/estradiol and 3α-PI/estradiol may be of importance for sustaining the uterine quiescence. Whereas 3α,5α-THP stagnates from the 36th week of gestation (M. Hill, et al., 2007), estradiol still shows an increasing trend (Buster, et al., 1979; Parizek, et al., 2005; Turnbull, et al., 1974), which might induce uterine contractions resulting in parturition onset.

Whereas the turnovers of 5α-DHP/progesterone and 5β-DHP/progesterone in the 3rd trimester show that the metabolism of progesterone to 5α-DHP inconspicuously culminates in the 35th week, the conversion of progesterone to 5β-DHP significantly declines from the 31st week of gestation (M. Hill, et al., 2007). This is in accordance with results of other authors as well as with our current data (Gilbert Evans, et al., 2005; M. Hill, Parizek, Cibula, et al., 2010; M. Hill, Parizek, Kancheva, et al., 2010; Sheehan, 2006; Sheehan, et al., 2005). Besides the modulation of ionotropic receptors, the 5β-reduced metabolites of progesterone may act chronically in pregnancy as uterine relaxants through a mechanism mediated by pregnane X-type receptors. Moreover, acute *in vitro* treatment with 5β-DHP causes rapid uterine relaxation that is independent of pregnane X-type receptors (Mitchell, et al., 2005; Putnam, Brann, Kolbeck, & Mahesh, 1991). The aforementioned data demonstrate that the progesterone metabolite 5β-DHP is a potent tocolytic (Mitchell, et al., 2005). In the placenta and myometrium, the relative expression of AKR1D1 decreases in association with labor by about two-fold and 10-fold, respectively (Sheehan, et al., 2005). Therefore, it is likely that the decrease in AKR1D1 activity during the third trimester is associated with a reduced ability to sustain the pregnancy (Gilbert Evans, et al., 2005; M. Hill, et al., 2007; M. Hill, Parizek, Cibula, et al., 2010; M. Hill, Parizek, Kancheva, et al., 2010; Sheehan, et al., 2005). AKR1D1 activity participates in the formation of almost 40% of pregnancy- sustaining PI.

4.7 Effects of 5α/β-reduced pregnanes on pain perception, induction of tolerance, receptor plasticity

4.7.1 The effects of 5α/β-reduced pregnanes in the fetal CNS

3α,5α-THP may interact with $GABA_A$-r to inhibit fetal CNS activity from mid-gestation. This inhibition may contribute to maintaining the sleep-like behavior and low incidence of arousal-type activity typical of fetal life (Crossley, et al., 2003). Mellor et al. reviewed the role of endogenous neuro-inhibitors that contribute to fetal sleep states, and thus mediate the suppression of fetal awareness. The authors show that there are several suppressors *in utero*, which inhibits neural activity in the fetus to a far greater degree than is seen postnatally in

the infant. The authors suggest that the uterus plays a key role in keeping the fetus continuously asleep. Despite the presence of intact nociceptive pathways from around mid-gestation, the critical aspect of cortical awareness in the process of pain perception is missing. The mechanism providing the permanent sleeping status in the fetus combines neuroinhibitory actions of a powerful EEG suppressor and sleep inducing agent (adenosine), two GABAergic steroids anesthetics (3α,5α-THP, 3α,5β-THP) and a potent sleep-inducing hormone (prostaglandin D2), acting together with a putative peptide inhibitor and other factors produced by the placenta (Mellor, Diesch, Gunn, & Bennet, 2005). Concerning the role of GABAergic steroids in suppressing the nociceptive pathways in the fetus, our current data shows 2-3 times lower 3α,5α-THP levels in the fetal circulation than in the maternal one, while 3α,5β-THP levels in UV exceed those in MV 1-2.5 times (M. Hill, et al., 2011). The total amount of GABAergic PI is only slightly higher in the fetal compartment than in the maternal, mainly due to the contribution of unconjugated 3α,5β-THP. These results indicate that the peripheral GABAergic steroids exert a comparable effect on the maternal and fetal CNS. Even when considering the 1.5-3 fold excess of progesterone in the fetal circulation when compared to the maternal blood, a possibility of progesterone transport into the brain, and its conversion to the GABAergic steroids herein, the resulting contribution of GABAergic steroids originating from peripheral sources do not pronouncedly differ between mother and fetus. Therefore the importance of GABAergic steroids for maintenance of permanent fetal sleeping is open to discussion.

4.7.2 The effects of 5α/β-reduced pregnanes in the maternal CNS

Increases in the brain levels of 5α/β-PM during pregnancy are causally related to changes in the expression of specific $GABA_A$-r subunits and the function of extrasynaptic $GABA_A$-r in the cerebral cortex and hippocampus (Concas, Follesa, Barbaccia, Purdy, & Biggio, 1999; Mostallino, Sanna, Concas, Biggio, & Follesa, 2009). Turkmen et al. demonstrated that 3α,5α-THP treatment induce a partial tolerance against acute 3α,5α-THP effects in the Morris water maze (Turkmen, Lofgren, Birzniece, Backstrom, & Johansson, 2006). Alterations in δ$GABA_A$-r expression during pregnancy result in region-specific increases in neuronal excitability in brain that are restored by the high levels of 3α,5α-THP under normal conditions. On the contrary, under pathological conditions may result in neurological and psychiatric disorders associated with pregnancy and postpartum period (Maguire, Ferando, Simonsen, & Mody, 2009). Besides the GABAergic effects in the CNS and periphery, 5β-PM also exert peripheral analgesic effects via blockade of testosterone-type calcium channels controlling pain perception (Todorovic, et al., 2004). These data as well as those mentioned previously, allow a speculation, whether these steroids might operate as endogenous analgesics around parturition.

4.7.3 Neuroprotective and excitotoxic effects of 5α/β-reduced pregnanes

The synthesis of neurosteroids from cholesterol in late gestation persists into neonatal life but SRD5A expression is greater in the fetus compared to the neonate. Fetuses exposed to stress during labor produce higher progesterone, which may protect them against the sequelae of hypoxia (Antonipillai & Murphy, 1977; Shaxted, Heyes, Walker, & Maynard, 1982). It is likely that the increasing fetal progesterone levels in stressful situations are associated with increased activity of the FZ producing extreme amounts of PregS. Physiologic concentrations of progesterone metabolite 3α,5α-THP provide protection

against both necrotic and apoptotic injury induced by NMDA excitotoxicity via positive modulation of $GABA_A$-r (Yawno, Hirst, Castillo-Melendez, & Walker, 2009). This modulation limits excitatory neurotransmission (Crossley, et al., 2003; Lockhart, et al., 2002). Growth restriction is a potent stimulus for neurosteroid synthesis in the fetal brain in late pregnancy. The low concentrations of 3α,5α-THP in the growth-restricted postnatal brain suggest a delay in the capacity of the adrenal gland or brain to synthesize pregnane steroids or their precursors and may render the postnatal brain vulnerable to hypoxia-induced injury (Westcott, Hirst, Ciurej, Walker, & Wlodek, 2008). At birth, the 3α,5α-THP concentrations in the brain fall markedly, probably due to the loss of placental precursors; however, stressors, including hypoxia and endotoxin-induced inflammation, lift up 3α,5α-THP concentrations in the newborn brain. (Hirst, Yawno, Nguyen, & Walker, 2006). Abrupt changes in neonatal levels of 3α,5α-THP could be related to the susceptibility to neurodevelopmental disorders (Darbra & Pallares, 2010).

GABAergic PI may reduce the excitotoxicity induced by N-methyl-D-aspartate (Lockhart, et al., 2002). In pregnant women and fetuses, this effect might be of importance when considering exceedingly elevated levels of steroids, which positively modulate N-methyl-D-aspartate receptors (NMDA-r). The positive NMDA-r modulators (like the sulfated Δ^5 steroids and sulfates of 5α-PI) may induce excitotoxic effect (Guarneri, et al., 1998; M. Hill, et al., 2007; M. Hill, Parizek, Cibula, et al., 2010; M. Hill, Parizek, Kancheva, et al., 2010; Malayev, et al., 2002; Weaver, et al., 2000). Moreover, the sulfated 5β-PI, 3α,5β-THP, the levels of which pronouncedly rise in the late pregnancy (M. Hill, et al., 2007; M. Hill, Parizek, Kancheva, et al., 2010), have also antagonistic effect on NMDA-r (Malayev, et al., 2002; Park-Chung, et al., 1997; Weaver, et al., 2000) and promote their desensitization (Kussius, et al., 2009).

5. Summary

Although the effects of bioactive reduced progesterone metabolites in human and laboratory animals were extensively studied, their physiological importance remains commonly uncertain due to the lack of metabolomic data. Therefore, we focused on the intersection between steroid metabolomics and neurophysiology so as to give a comprehensive insight into the physiological and pathophysiological relevance of the aforementioned compounds.

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7. References

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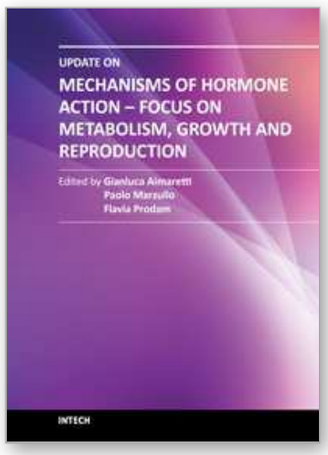

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The purpose of the present volume is to focus on more recent aspects of the complex regulation of hormonal action, in particular in 3 different hot fields: metabolism, growth and reproduction. Modern approaches to the physiology and pathology of endocrine glands are based on cellular and molecular investigation of genes, peptide, hormones, protein cascade at different levels. In all of the chapters in the book all, or at least some, of these aspects are described in order to increase the endocrine knowledge.

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