Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 84 (2006) 628–634
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Oral contraceptives and neuroactive steroids

Andrea J. Rapkin [a], Giovanni Biggio [b,c], Alessandra Concas [b,c,*]

[a] Department of Obstetrics and Gynecology, David Geffen School of Medicine, UCLA, Los Angeles, CA, United States
[b] Department of Experimental Biology, University of Cagliari, Cagliari, Italy
[c] C.N.R. Institute of Neuroscience, Italy

Available online 18 July 2006

Abstract

A deregulation in the peripheral and brain concentrations of neuroactive steroids has been found in certain pathological conditions characterized by emotional or affective disturbances, including major depression and anxiety disorders. In this article we summarize data pertaining to the modulatory effects of oral contraceptive treatment on neuroactive steroids in women and rats. Given that the neuroactive steroids concentrations are reduced by oral contraceptives, together with the evidence that a subset of women taking oral contraceptives experience negative mood symptoms, we propose the use of this pharmacological treatment as a putative model to study the role of neuroactive steroids in the etiopathology of mood disorders. Moreover, since neuroactive steroids are potent modulators of $GABA_A$ receptor function and plasticity, the treatment with oral contraceptives might also represent a useful experimental model to further investigate the physiological role of these steroids in the modulation of GABAergic transmission.

Keywords: Oral contraceptives; Neuroactive steroids; Mood; $GABA_A$ receptors; Women; Rat brain

1. Introduction

Oral contraceptive pills (OCs) are currently the most widely used method of reversible contraception (Sulak et al., 2002). Since the beginning of wide spread usage in the 1970s there were reports of emotional liability, irritability, depression and magnification of premenstrual symptoms in up to 56% of women (Kurshan and Epperson, 2005; Oinonen and Mazmanian, 2002; Sanders et al., 2001) with almost 30% of women discontinuing OCs due to psychological side effects, often within the first three months (Goldzieher, 1994). However, there is a lack of consensus regarding the psychological effects of contemporary lower dose OC formulations and even more importantly, mechanisms for the mood derangement when it does occur. Here we describe the various OC formulations and briefly review their effects on mood, behavior, and premenstrual syndrome (PMS), highlighting the modulatory effects of OC or the estrogens and progestins comprising the OC on neuroactive steroids in women and rats. We then discuss how the impact of OCs on mood and neuroactive steroids could be a model in which to study the role of these steroids in the modulation of GABAergic transmission.

1.1. Oral contraceptive pill formulations

The combined oral contraceptive pills (OCs) were patented in 1960 with a high concentration of both ethinyl estradiol (EE) and one of various 19-nor testosterone derived progestins. However after the recognition of a dose–response relationship for OC related side effects and adverse cardiovascular events each subsequent generation of OC contained progressively lower doses of estrogen and progestin (Mishell, 1986) (150 μg of ethinyl estradiol to 20–35 μg and 18 mg of norethindrone to 0.5–1 mg, for example). With the removal of the 19 carbon from testosterone, the androgenic properties of these compounds were diminished, however, they were not eliminated completely. The newer "second" and "third" generation progestin compounds and lower progestin doses were intended to minimize androgenic side effects such as acne, hirsutism,

* Corresponding author. Department of Experimental Biology "B. Loddo," University of Cagliari, 09100 Cagliari, Italy. Tel.: +39 070 675 4137; fax: +39 070 675 4166.
E-mail address: aconcas@unica.it (A. Concas).

0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2006.06.008

weight gain, and adverse carbohydrate metabolism and lipid profile; and in concert with reduced estrogen doses, the contemporary OCs have improved cardiovascular safety and less negative and even positive impact on mood and PMS (Oinonen and Mazmanian, 2002).

1.2. Mechanism of action of oral contraceptives

The estrogen/progestin combination contraceptive formulation inhibits the mid-cycle gonadotropin-releasing hormone surge (GnRH) from the hypothalamus, thus preventing ovulation (Lobo and Stanczyk, 1994; Mishell et al., 1977). Additionally, a direct inhibitory effect on the pituitary is evidenced by suppression of pituitary luteinizing hormone (LH) and follicle stimulating hormone (FSH) after GnRH infusion (Mishell et al., 1977). However, neither gonadotropin nor ovarian steroid production is completely suppressed by OCs, and the levels of endogenous estradiol in the peripheral blood have been noted to be similar to or lower than to those found in the early follicular phase of the menstrual cycle (Brenner et al., 1977; Mishell et al., 1972; Rapkin et al., 2006) (Fig. 1).

1.3. Oral contraceptives and mood

Numerous studies have documented negative affect/mood occurring in a subset of women ingesting OCs (Kurshan and Epperson, 2005; Oinonen and Mazmanian, 2002; Sanders et al., 2001). However, there is a lack of agreement regarding the specific psychological effects of OCs; with evidence for mood deterioration as well as mood improvement or stabilization. More recent investigations have assessed mood during exposure to low dose OCs. In aggregate most demonstrate no differences in negative or positive affect between OC users and controls, either or across the cycle or comparing users and non-users, although there is evidence for less day to day variability in mood for OC users (Kurshan and Epperson, 2005; Oinonen and Mazmanian, 2002; Rapkin et al., 2006). A subgroup of women does experience mild to moderate depressive symptoms associated with OC use. Risk factors which predispose this subset of women to negative OC mediated mood change include personal and family history of depression, post partum depression, or oral contraceptive related depression, and possibly PMS (Kahn and Halbreich, 2001; Joffe et al., 2003; Yonkers et al., 2005; Oinonen and Mazmanian, 2002).

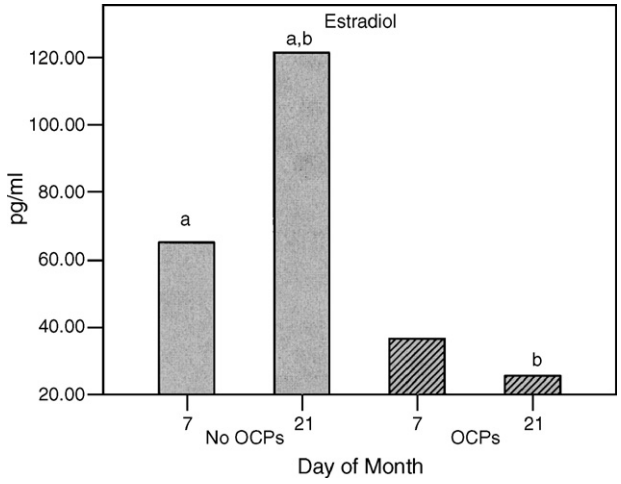

Fig. 1. Estradiol concentrations prior to and during third month of OCP administration. a) Significant increase from baseline day 7 to day 21 ($p<0.001$); b) significant decrease from baseline day 21 and OCP day 21 ($p<0.001$); ■ baseline (prior to OCP); ▨during third month of OCP (reprinted from Rapkin et al., 2006, with permission from Elsevier).

1.4. Effect of OCs on PMS

A large retrospective study found only 16% of women who used OCs experienced mood deterioration, while 12.3% of the women had premenstrual mood improvement (Joffe et al., 2003). The study design included retrospective recall of symptoms as opposed to prospective recording (the current "gold standard"), however it does suggest that newer low dose OCs, generally fail to exacerbate, and may actually relieve PMS symptoms. There is a paucity of randomized controlled trials (RTCs) for PMS utilizing OCs. One early trial using a 35 μg of EE and norethindrone (0.5 mg days 1–7, 1 mg days 8–16 and 0.5 mg days 17–21) containing triphasic OC did not demonstrate improvement in any of the PMS mood symptoms (Graham and Sherwin, 1993).

Recent studies of a new drospirenone containing OC, showed efficacy for symptoms of PMS and premenstrual dysphoric disorder (PMDD), a severe form of PMS (Pearlstein et al., 2005; Sangthawan and Taneepanichskul, 2005; Yonkers et al., 2005). The RTC demonstrating efficacy for PMDD contains 20 μg of EE and 3 mg of drospirenone in an extended pill (24 active/4 placebo as opposed to 21/7) regimen. This newest progestin, drospirenone is a derivative of spirolactone (an analogue of spironolactone) instead of testosterone. This compound has strong progestational properties as well as anti-mineralocorticoid and anti-androgenic properties (Blode et al., 2000). The extended pill regimes more effectively suppress ovulation and there is less increase in GnRH pulse frequency and amplitude and estradiol production compared with a seven-day pill free interval (Spona et al., 1996; van Heusden and Fauser, 2002).

2. Oral contraceptives and neuroactive steroids

The 3α,5α-reduced metabolite of progesterone, allopregnanolone, exerts a rapid, non-genomic, inhibitory effect on excitability of neurons through a direct modulation of the activity of the γ-aminobutyric acid (GABA)-gated chloride channel known as the $GABA_A$ receptor (Biggio and Purdy, 2001). As discussed throughout this issue, the $GABA_A$ receptor is the site of action for benzodiazepines, barbiturates, ethanol, many anticonvulsants, and most importantly endogenous neuroactive steroids, including allopregnanolone, which positively modulate $GABA_A$ receptor function. The interaction between allopregnanolone and the $GABA_A$ receptor appears to underlie the pharmacological actions of this steroid. Thus, allopregnanolone administered systematically or intracerebroventricularly elicits anxiolytic, anticonvulsant and neuroendocrine effects similar to those produced by benzodiazepines and barbiturates (Biggio and Purdy, 2001). Other neuroactive steroids such as pregnenolone sulphate (PS) and

dehydroepiandrosterone sulphate (DHEAS) are in general $GABA_A$ receptor antagonists resulting in reduced GABAergic tone (Dubrovsky, 2005).

Synthesis of these neuroactive steroids occurs in the brain; however, the corpus luteal contribution of progesterone and allopregnanolone to the peripheral plasma augments brain concentrations of neuroactive steroids (Biggio and Purdy, 2001; Ottander et al., 2005). During OC administration, corpus luteal progesterone production is suppressed, and the various progestins and their metabolites may differ from progesterone in their effects on receptor and membrane mediated neuronal activity in the central nervous system (CNS).

Given that $GABA_A$ receptor participates in the regulation of a variety of psychophysiological phenomena, including anxiety, depression, sleep, cognitive functioning, seizures and sexual behavior, changes in these naturally-occurring modulators of $GABA_A$ receptors might also contribute to various neurological and psychiatric disorders. Indeed, altered plasma and cerebrospinal fluid concentrations of allopregnanolone appear to be involved with a number of psychiatric and hormonally related mood and anxiety disorders including major depressive disorder (Pisu and Serra, 2004), pregnancy related depression (Pearson Murphy et al., 2001), panic and anxiety disorders (Dubrovsky, 2005; Pisu and Serra, 2004), premenstrual syndrome (PMS) (Rapkin et al., 1997; Wang et al., 1996), premenstrual dysphoric disorder (PMMD) (Girdler et al., 2001) and stress-related amenorrhea (Genazzani et al., 2002). Moreover, it has been shown that administration of drugs having clinical relevance in the treatment of these pathologies influence the secretion of these steroids (Dubrovsky, 2005; Pisu and Serra, 2004).

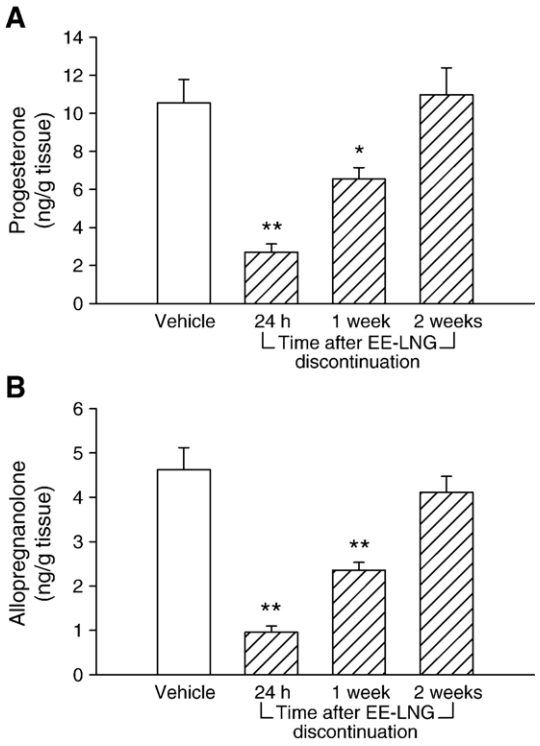

Fig. 2. Effects of long-term treatment with EE and LNG on the concentrations of, progesterone (A), and allopregnanolone (B) in the rat cerebral cortex. Rats were injected daily with the combination of EE (0.030 mg, s.c.) and LNG (0.125 mg, s.c.), or with vehicle (control), for 6 weeks and were then killed 24 h, 1 week or 2 weeks after the last treatment. Data are expressed as nanograms of steroid per gram of cortical protein, and are means±SEM of values obtained from 10 rats per group. *$p<0.01$, **$p<0.001$ vs. control group. (reprinted from Follesa et al., 2002, with permission from Elsevier).

2.1. Animal studies

As stated above, combination treatment with progestins and estrogens is thought to prevent ovulation through inhibition of the hypothalamic–pituitary–ovarian axis, as a result of interference either with the release of gonadotropin-releasing hormone from the hypothalamus or with gonadotropin release from the pituitary (Lobo and Stanczyk, 1994). Each of these effects would result in impaired production of estrogens and progesterone by the gonads. Given that allopregnanolone is a progesterone metabolite, it was predicted that the daily administration of a combination of ethinyl estradiol (EE) and levonorgestrel (LNG) for 4–6 weeks would result in a significant decrease in plasma concentrations of both progesterone (−43%) and allopregnanolone (−40%); an effect not evident in animals in which gonads had been removed (Follesa et al., 2002). The same treatment regimen also reduced by a greater extent the concentrations of progesterone (−74%) and allopregnanolone (−79%) in the cerebral cortex of rats killed 24 h after the last treatment (Fig. 2). This effect was still apparent one week after discontinuation of drug treatment but was no longer statistically significant after two weeks. These results suggest that, in addition to its effects on the hormonal milieu in peripheral steroidogenic tissues (Lobo and Stanczyk, 1994), the combination of EE and LNG induces a persistent and marked decrease in the synthesis and accumulation of progesterone and allopregnanolone in the brain. Moreover, the more pronounced decrease in progesterone and allopregnanolone concentrations elicited by OC treatment in the cerebral cortex than in plasma suggests that in brain, the synthesis/accumulation of neuroactive steroids is not simply a function of their plasma concentrations. Accordingly, OC treatment affects the brain concentrations of progesterone and allopregnanolone in animals in which gonads have been removed (Follesa et al., 2002). It is possible that OC treatment also directly influences the activities of enzymes that contribute to steroidogenesis. Indeed, LNG inhibits in vivo, both the production of progesterone by rat luteal cells (Telleria et al., 1994) and the activity of 5α-reductase in skin (Rabe et al., 2000).

Long-term increases in the cerebrocortical concentrations of neuroactive steroids, either induced by pharmacological treatment with progesterone or allopregnanolone (Smith et al., 1998a; Follesa et al., 2000) or associated with physiological conditions such as pregnancy and estrus cycle (Concas et al., 1998; Fenelon and Herbison, 1996; Lovick et al., 2005; Maguire et al., 2005) affect $GABA_A$ receptor plasticity and behavior. This evidence suggests that impairment of the physiological rhythm of progesterone and allopregnanolone production induced by OCs might also affect $GABA_A$ receptor gene expression. Indeed, the changes in the brain concentrations of these steroids elicited by chronic administration of OC constituents were associated with an increase in the γ_2 subunit immunoreactivity as shown by Western

blot analysis (Fig. 3), as well as in the abundance of γ_2L and γ_2S subunit mRNAs in the cerebral cortex (Follesa et al., 2002). In contrast, the amounts of α_1, α_3, α_4, β_1, β_2 and β_3 subunit mRNAs in the cerebral cortex were not significant affected by OC treatment (Follesa et al., 2002), indicating that the changes in the abundance of the γ_2 subunit mRNAs are specific.

The observation that the OC-induced decrease in the brain concentrations of progesterone and allopregnanolone is in parallel with a selective increase in $GABA_A$ receptor γ_2 subunit gene expression is consistent with the results of previous studies showing that long-lasting fluctuations in the brain concentrations of neuroactive steroids occurring during pregnancy and after delivery affect the expression of this subunit gene (Concas et al., 1998; Fenelon and Herbison, 1996). Thus, the increase in neuroactive steroid concentrations associated with pregnancy is paralleled by a marked decrease in the amount of γ2 subunit mRNA in the cerebral cortex (Concas et al., 1998). This effect of pregnancy on γ2 subunit gene expression is reversed by the marked decrease in the brain concentrations of progesterone and allopregnanolone that precedes parturition, or is prevented by finasteride, a specific inhibitor of 5α-reductase (Concas et al., 1998). A substantial increase in the abundance of γ2 subunit mRNA has also been observed in the posterior paraventricular nucleus in association with low brain concentrations of neuroactive steroids during lactation in rats (Fenelon and Herbison, 1996) and levels of γ2 subunit in the hippocampus fluctuate over the estrus cycle in female rats (Maguire et al., 2005). Furthermore, chronic progesterone treatment down-regulates the expression of γ2L and γ2S subunit mRNAs, as well as those of other $GABA_A$ receptor subunits, in mammalian cerebellar granule cells in culture (Follesa et al., 2000). Thus the observation with OC treatment further suggests that the marked fluctuations in the brain concentrations of progesterone and allopregnanolone seem to be an important determinant in the expression of the $GABA_A$ receptor γ2 subunit.

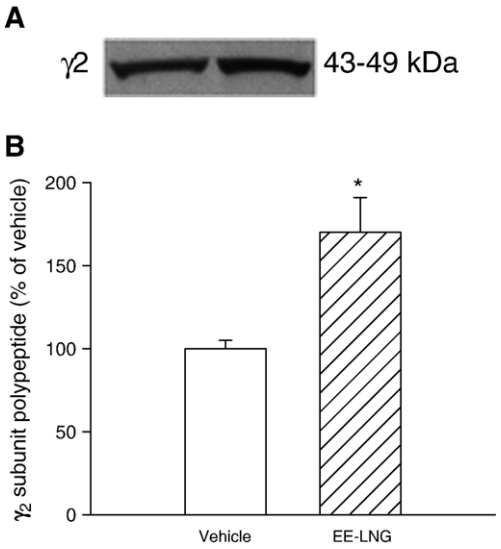

Fig. 3. Effects of long-term treatment with EE–LNG on the abundance of γ2 polypeptide in the rat cerebral cortex. A) Representative immunoblot. The molecular sizes of the immunoreactive band are indicated in kilodaltons. Bands were detected with an ECL detection kit (Amersham, Little Chalfont, UK). The same amounts of proteins were loaded in each well (40 μg). Data were normalized using the corresponding optical intensity of the Ponceau dye stained membrane. B) Bar graph showing the average γ2 polypeptide increase of seven different animals for each experimental group. Values are means ± SEM and are expressed as percentage of control (vehicle-treated) values. *$p<0.05$ vs control (reprinted from Follesa et al., 2002, with permission from Elsevier).

The functional relevance of the changes in γ2 subunit gene expression in the cerebral cortex induced by OC, as well as of those apparent during pregnancy, remains to be determined. The γ2 subunit of the $GABA_A$ receptor forms intersubunit contacts with α and β subunits, contributes to most receptor subtypes, is essential for benzodiazepine action, and is required both for postsynaptic clustering of receptors and for normal inhibitory GABAergic function in cortical neurons (Fritschy and Brunig, 2003; Rudolph and Mohler, 2004). Evidence suggests that GABA, acting at $GABA_A$ receptors, inhibits the secretion of gonadotropin-releasing hormone from the hypothalamus, resulting in an immediate reduction in the release of luteinizing hormone from the pituitary (Leonhardt et al., 1995). Given that the secretion of luteinizing hormone is important for fertility and is suppressed by OCs (Kuhl et al., 1984), an increase in the expression of the γ2 subunit in the hypothalamus and a consequent increase in $GABA_A$ receptor function (Fritschy and Brunig, 2003) might be involved in the prevention by these drugs of the luteinizing hormone surge that is normally apparent at the time of ovulation.

Rats subjected to long-term treatment with OC also exhibited an anxiety-like behavioral profile in the elevated plus-maze test; OC-treated rats thus showed a 50% decrease in the time spent in the open arms of the maze and a 45% decrease in the proportion of entries into the open arms, compared with animals treated with vehicle (Follesa et al., 2002). Given that allopregnanolone exhibits a pronounced anxiolytic action, the decrease in the brain concentration of this progesterone metabolite might account for the anxiety-like behavior apparent in OC-treated rats. This conclusion is consistent with the observation that ovariectomy or administration of finasteride, treatments that reduce both the brain and plasma concentrations of progesterone and allopregnanolone, increase anxiety-like behavior in the elevated plus-maze paradigm (Smith et al., 1998b; Zimmemberg and Farley, 1993).

2.2. Human studies

Human studies evaluating the effect of oral contraceptive pills and neuroactive steroids are limited. Follesa et al. (2002) administered an OC containing LNG and EE to ten healthy women. Prior to OC administration, the expected luteal phase rise in serum concentrations of progesterone precursor pregnenolone, of progesterone, and progesterone metabolite, allopregnanolone was documented, while, during the third month of OC treatment, the increased serum concentrations of these neuroactive steroids were abolished.

The relationship between mood and neuroactive steroids after exposure to another low dose OC was evaluated (Rapkin et al., 2006). Serum neuroactive steroids allopregnanolone, allotetrahydrodeoxycorticosterone (THDOC), dehydroepiandrosterone (DHEA) and neuroactive steroids precursors, progesterone and

Table 1
Serum concentrations of ALLO, THDOC, progesterone (P), pregnenolone (Preg), DHEA, and E2

	Baseline day 7	Baseline day 21	Month 3 OCP day 7	Month 3 OCP day 21
ALLO (ng/ml)	4.15±1.27 [a]	8.94±4.18 [a, b]	4.05±1.13	4.95±2.98 [b]
THDOC (ng/ml)	2.22±0.91 [a, c, d]	3.36±1.34 [a, b]	1.86±0.65 [c]	1.89±0.64 [b, d]
P (ng/ml)	3.22±0.91 [a, c, d]	10.74±6.9 [a, b]	2.28±1.59 [c]	2.51±1.57 [b, d]
Preg (ng/ml)	5.75±2.74 [a, c, d]	8.62±3.31 [a, b]	4.66±2.86 [c]	4.42±3.00 [b, d]
DHEA (ng/ml)	4.48±1.62	4.56±1.93	4.24±2.48	3.74±2.69
E2 (pg/ml)	65.16±45.5 [a, d]	121.9±64.4 [a, b]	36.85±50.1	26.01±22.3 [b, d]

Values are means±SD.
[a] Significant increase from baseline 7 to day 21 in ALLO, THDOC, P, Preg ($p<001$) and E2 ($p=0.024$).
[b] Significant decrease from baseline day 21 to OCP day 21 in ALLO, THDOC, P, Preg, and E2 ($p<0.001$).
[c] Significant decrease from baseline day 7 to OCP day 7 in THDOC ($p=0.023$), P ($p=0.005$) and Preg ($p=0.035$).
[d] Significant decrease from baseline day 7 to OC day 21 in THDOC ($p=0.045$), P ($p=0.015$), Preg ($p=0.016$), and E2 ($p=0.015$). (reprinted from

pregnenolone, were determined on days 7 and 21 both before and during the third month of an OC containing 20 μg of EE and 100 μg of levonorgestrel (LNG) in 31 healthy, new OC users without history of mood disorder or PMS. Mood and anxiety were assessed by PMS daily ratings form, the Beck Depression Inventory, Spielberg State/Trait Anxiety Inventory and Profile of Mood States assessment form. This OC also significantly reduced serum concentrations of neuroactive steroids allopregnanolone and THDOC, neuroactive steroid precursors progesterone and pregnenolone as well as estradiol but had no effect on DHEA (Table 1 and Fig. 4). Prior to the administration of the OC, progesterone, pregnenolone, allopregnanolone, and THDOC increased significantly from day 7 to day 21 (Table 1, Fig. 4). DHEA did not differ between the two sampling intervals (Table 1).

Again, during OC administration, the normal luteal phase rise in steroids was obliterated, resultant in allopregnanolone concentrations was in the low follicular phase range, and there were no significant differences between the day 7 and day 21 serum concentrations for any of the measured steroids (Table 1). The suppression of neuroactive steroids by the OC did not elicit negative mood alterations as determined by the Becks, Speilberger, Profile of Mood States or the PMS daily rating form.

The OC mediated diminution in neuroactive steroids was not associated in this human study with mood deterioration, perhaps because the neuroactive steroids concentrations were still in the follicular phase range and were not profoundly suppressed. The diminished allopregnanolone concentrations in rodents and humans manifesting anxiety-like and depressive behavior (Follesa et al., 2002; Pisu and Serra, 2004) may be associated with more profound perturbations of neuroactive steroids. The majority of women taking OC fail to develop negative mood, but as noted, the neuroactive steroids were not radically suppressed. There appear to be threshold effects of neuroactive steroids on mood, with concentrations below which adverse emotional symptoms may occur (Andréen et al., 2005). Alternatively, the hormonally induced fluctuations in $GABA_A$ receptor subunit configuration and resultant decreased sensitivity to the anxiolytic effects of GABAergic agonists (Concas et al., 1998; Smith et al., 1998a) may be more important for mood regulation than the absolute level of neuroactive steroids. Further neuroactive steroids/OC studies in women with risk factors for depression have yet to be completed.

A third investigation of OCs and neuroactive steroids entailed a 30 μg/EE and 3 mg of drospirenone OC administered to 10 women and compared to 12 control women (Paoletti et al.,

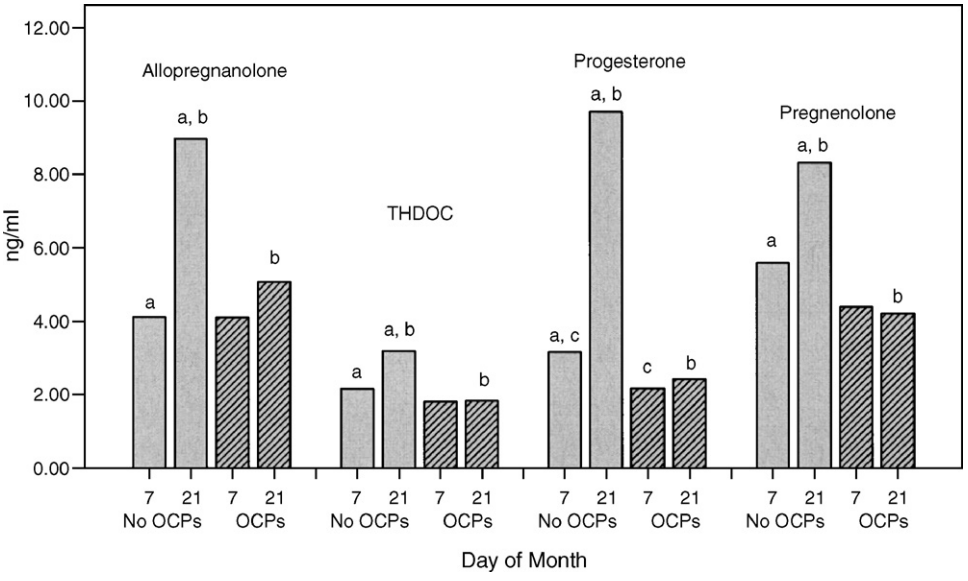

Fig. 4. Allopregnanolone, THDOC, progesterone, and pregnenolone prior to and during third month of OCP administration. a) Significant increase from baseline day 7 to day 21 ($p<0.0001$); b) significant decrease from baseline day 21 and OCP day 21 ($p<0.0001$); c) significant decrease from baseline day 7 to OCP day 7 ($p=0.005$); ■ baseline (prior to OCP); ▨during third month of OCP (reprinted from Rapkin et al., 2006, with permission from Elsevier).

2004). Psychological symptoms were evaluated by the Italian Psychometric Self-Report Clinical Scale SCL-90. The baseline SCL-90 and DHEA sulphate did not vary across the menstrual cycle. During the 3rd month of OC treatment, the values of progesterone, allopregnanolone and THDOC were significantly lower than those measured during the previous luteal phase of the baseline month in the OC and the control women. However, the neurosteroid concentrations after OC intake were not different from those measured during the follicular phase before treatment in either group. DHEAS concentrations were significantly lower in the OC group after treatment compared with the baseline follicular and luteal phases or with the DHEAS values in controls at any time period. The total SCL-90 global score was significantly reduced during the OC exposure compared with the baseline month or the control subjects scores. The symptoms explaining the significant decrease in the SCL-90 scale included anxiety, and paranoid ideation. The authors concluded that the relative lowering of the GABA antagonist DHEAS may play a role in mood improvement in this group of women undergoing treatment with EE/DRSP. The drospirenone pill was not however compared with a norgestrel containing OC, and it remains unclear whether the effect on DHEAS is specific (Carlstrom et al., 2002).

3. Conclusions and directions for future research

A potential role for neuroactive steroids on mood regulation has been suggested. The concentration of neuroactive steroids in plasma or cerebrospinal fluid is decreased in individuals with depression and antidepressant medications, such as selective serotonin reuptake inhibitors and even the amino acid precursor of serotonin, tryptophan, increases these neuroactive steroid modulators of the $GABA_A$ receptor (Pisu and Serra, 2004; Rasgon et al., 2001). Moreover, allopregnanolone elicits an antidepressant-like effect in mice subjected to the forced swim test (Khisti et al., 2000). Given that the brain and plasma concentrations of neuroactive steroids are reduced by OC treatment, together with the evidence that a subset of women taking these compounds experience negative mood symptoms, OC treatment might be a useful pharmacological model in which to evaluate the role of neuroactive steroids in the etiopathology of mood disorders. The data presented here clearly demonstrate that the OC mediated reduction in neurosteroids during the luteal phase of the menstrual cycle is not associated with negative psychological symptomatology in healthy women. Further studies are necessary to determine the putative correlation between circulating neuroactive steroids levels and negative symptoms in women taking OCs with a history of mood disorders or PMS.

Moreover, the different effects of OCs on mood might also be attributed to different progestin compounds or possibly, their oestrogen ratios. Therefore an extensive study should be undertaken in order to compare the effects of different progestins on neuroactive steroids levels both in women and animals. For instance, the progestin ethynodial diacetate, a compound structurally derived from norethindrone present in one OC formulation, was noted in one study to undergo metabolism to allopregnanolone (Simic et al., 1998) and the acute administration of 5α- reduced metabolites of levonorgestrel induced anxiolytic effects in rats (Picazo et al., 1998).

The evidence that the persistent decrease in the plasma and brain concentrations of progesterone and allopregnanolone induced by chronic OC treatment of female rats is associated with a plastic adaptation of $GABA_A$ receptor gene expression in brain, suggests that this pharmacological treatment also represents a good experimental model in which to further investigate the physiological role of these steroids in the modulation of GABAergic transmission.

Future investigations should also establish which component, i.e. estrogens or progestins or both, is responsible for the effects of OC on neurosteroid concentrations, $GABA_A$ receptor subunit gene expression and animal behavior. In fact, both estrogens and progestins independently might decrease neuroactive steroids production by suppressing gonadotropins release from the pituitary (Lobo and Stanczyk, 1994; Kuhl et al., 1984) and affect $GABA_A$ receptor plasticity. Given the importance of $GABA_A$ receptor-mediated neurotransmission in the modulation of brain function, the changes in $GABA_A$ receptors induced by OC treatment might be relevant to some of the side effects exhibited by women taking these compounds.

Acknowledgements

This research was supported by grant CE00042735 (Project Center of Excellence for the Neurobiology of Dependence, D.M. 21 January 2001), FIRB grant RBNE0195AX, and PRIN grants 2005059123 from the Ministry of Instruction, Universities, and Research, Italy; by the Sardinian Health Ministry; and, in part, by GIO.I.A. Foundation (Pisa).

References

Andréen L, Sundström-Poromaa I, Bixo M, Andersson A, Nyberg S, Bäckström T. Relationship between allopregnanolone and negative mood in postmenopausal women taking sequential hormone replacement therapy with vaginal progesterone. Psychoneuroendocrinology 2005;30:212–24.

Biggio G, Purdy RH, editors. Neurosteroids and brain function (International review of Neurobiology), vol. 46. New York: Academic Press; 2001.

Blode H, Wuttke W, Loock W, Roll G, Heithecker R. A 1-year pharmacokinetic investigation of a novel oral contraceptive containing drospirenone in healthy female volunteers. Eur J Contracept Reprod Health Care 2000;5:256–64.

Brenner PF, Mishell Jr DR, Stanczyk FZ, Goebelsmann U. Serum levels of d-norgestrel, luteinizing hormone, follicle-stimulating hormone, estradiol, and progesterone in women during and following ingestion of combination oral contraceptives containing dl-norgestrel. Am J Obstet Gynecol 1977;129:133–40.

Carlstrom K, Karlsson R, Von Schoultz B. Diurnal rhythm and effects of oral contraceptives on serum dehydroepiandrosterone sulfate (DHEAS) are related to alterations in serum albumin rather than to changes in adrenocortical steroid secretion. Scand J Clin Lab Invest 2002;62:361–8.

Concas A, Mostallino MC, Porcu P, Follesa P, Barbaccia ML, Trabucchi M, et al. Role of brain allopregnanolone in the plasticity of γ-aminobutyric acid type A receptor in rat brain during pregnancy and after delivery. Proc Natl Acad Sci U S A 1998;95:13284–9.

Dubrovsky BO. Steroids, neuroactive steroids and neurosteroids in psychopathology. Prog Neuro-psychopharmacol Biol Psychiatry 2005;29:169–92.

Fenelon VS, Herbison AE. Plasticity in $GABA_A$ receptor subunit mRNA expression by hypothalamic magnocellular neurons in the adult rat. J Neurosci 1996;16:4872–80.

Follesa P, Serra M, Cagetti E, Pisu MG, Porta S, Floris S, et al. Allopregnanolone synthesis in cerebellar granule cells: roles in regulation of $GABA_A$ receptor expression and function during progesterone treatment and withdrawal. Mol Pharmacol 2000;57:1262–70.

Follesa P, Porcu P, Sogliano C, Cinus M, Biggio F, Mancuso L, et al. Changes in $GABA_A$ receptor γ_2 subunit gene expression induced by long-term administration of oral contraceptives in rats. Neuropharmacology 2002;42:325–36.

Fritschy JM, Brunig I. Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. Pharmacol Ther 2003;98:299–323.

Genazzani AD, Luisi M, Malavasi B, Strucchi C, Luisi S, Casarosa E, et al. Pulsatile secretory characteristics of allopregnanolone, a neuroactive steroid, during the menstrual cycle and in amenorrheic subjects. Eur J Endocrinol 2002;146:347–56.

Girdler SS, Straneva PA, Light KC, Pedersen CA, Morrow AL. Allopregnanolone levels and reactivity to mental stress in premenstrual dysphoric disorder. Biol Psychiatry 2001;49:788–97.

Goldzieher JW. Are low-dose oral contraceptives safer and better? Am J Obstet Gynecol 1994;171t:587–90.

Graham CA, Sherwin BB. The relationship between mood and sexuality in women using an oral contraceptive as treatment for premenstrual syndrome. Psychoneuroendocrinology 1993;18:273–81.

Joffe H, Cohen LS, Harlow BL. Impact of oral contraceptive pill use on premenstrual mood: predictors of improvement and deterioration. Am J Obstet Gynecol 2003;189:1523–30.

Kahn LS, Halbreich U. Oral contraceptives and mood. Expert Opin Pharmacother 2001;2:1367–82.

Khisti RT, Chopde CT, Jain SP. Antidepressant-like effect of the neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one in mice forced swim test. Pharmacol Biochem Behav 2000;67:137–43.

Kuhl H, Weber W, Mehlis W, Sandow J, Taubert HD. Time- and dose-dependent alterations of basal and LH-RH-stimulated LH-release during treatment with various hormonal contraceptives. Contraception 1984;30:467–82.

Kurshan N, Epperson CN. Oral contraceptives and mood in women with and without premenstrual dysphoria: a theoretical model. Arch Womens Ment Health 2005;9:1–14.

Leonhardt S, Seong JY, Kim K, Thorun Y, Wuttke W, Jarry H. Activation of central $GABA_A$- but not of $GABA_B$-receptors rapidly reduces pituitary LH release and GnRH gene expression in the preoptic/anterior hypothalamic area of ovariectomized rats. Neuroendocrinology 1995;61:655–62.

Lobo RA, Stanczyk FZ. New knowledge in the physiology of hormonal contraceptives. Am J Obstet Gynecol 1994;170:1499–507.

Lovick TA, Griffiths JL, Dunn SM, Martin IL. Changes in GABA(A) receptor subunit expression in the midbrain during the oestrous cycle in Wistar rats. Neuroscience 2005;131:397–405.

Maguire JL, Stell BM, Rafizadeh M, Mody I. Ovarian cycle-linked changes in GABA(A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety. Nat Neurosci 2005;8:797–804.

Mishell Jr DR. Oral steroid contraceptives. In: Mishell Jr DR, Davajan Val, editors. Infertility, contraception and reproductive endocrinology. 2nd ed. California: Medical Economic Company Inc; 1986. p. 593–622.

Mishell Jr DR, Thorneycroft IH, Nakamura RM, Nagata Y, Stone SC. Serum estradiol in women ingesting combination oral contraceptive steroids. Am J Obstet Gynecol 1972;114:923–8.

Mishell Jr DR, Kletzky OA, Brenner PF, Roy S, Nicoloff J. The effect of contraceptive steroids on hypothalamic–pituitary function. Am J Obstet Gynecol 1977;128:60–74.

Oinonen KA, Mazmanian D. To what extent do oral contraceptives influence mood and affect? J Affect Disord 2002;70:229–40.

Ottander U, Poromaa IS, Bjurulf E, Skytt A, Backstrom T, Olofsson JI. Allopregnanolone and pregnanolone are produced by the human corpus luteum. Mol Cell Endocrinol 2005;239:37–44.

Paoletti AM, Lello S, Fratta S, Orrù M, Ranuzzi F, Sogliano C, et al. Psychological effect of the oral contraceptive formulation containing 3 mg of drospirenone plus 30 μg of ethinyl estradiol. Fertil Steril 2004;81:645–51.

Pearlstein TB, Bachmann GA, Zacur HA, Yonkers KA. Treatment of premenstrual dysphoric disorder with a new drospirenone-containing oral contraceptive formulation. Contraception 2005;72:414–21.

Pearson Murphy BE, Steinberg SI, Hu FY, Allison CM. Neuroactive ring A-reduced metabolites of progesterone in human plasma during pregnancy: elevated levels of 5 alpha-dihydroprogesterone in depressed patients during the latter half of pregnancy. J Clin Endocrinol Metab 2001;86:5981–7.

Picazo O, Fernandez-Guasti A, Lemus AE, Garcia GA. A-ring reduced derivatives of two synthetic progestins induce anxiolytic effects in ovariectomized rats. Brain Res 1998;796:45–52.

Pisu MG, Serra M. Neurosteroids and neuroactive drugs in mental disorders. Life Sci 2004;74:3181–97.

Rabe T, Kowald A, Ortmann J, Rehberger-Schneider S. Inhibition of skin 5α-reductase by oral contraceptive progestins in vitro. Gynecol Endocrinol 2000;14:223–30.

Rapkin AJ, Morgan M, Goldman L, Brann DW, Simone D, Mahesh VB. Progesterone metabolite allopregnanolone in women with premenstrual syndrome. Obstet Gynecol 1997;90:709–14.

Rapkin A, Morgan M, Sogliano C, Biggio G, Concas A. Decreased neuroactive steroids induced by combined oral contraceptive pills are not associated with mood changes. Fertil Steril 2006;85:1371–8.

Rasgon N, Serra M, Biggio G, Pisu MG, Fairbanks L, Tanavoli S, et al. Neuroactive steroid–serotonergic interaction: responses to an intravenous L-tryptophan challenge in women with premenstrual syndrome. Eur J Endocrinol 2001;145:25–33.

Rudolph U, Mohler H. Analysis of GABAA receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. Annu Rev Pharmacol Toxicol 2004;44:475–98.

Sanders SA, Graham CA, Bass JL, Bancroft J. A prospective study of the effects of oral contraceptives on sexuality and well-being and their relationship to discontinuation. Contraception 2001;64:51–8.

Sangthawan M, Taneepanichskul S. A comparative study of monophasic oral contraceptives containing either drospirenone 3 mg or levonorgestrel 150 μg on premenstrual symptoms. Contraception 2005;71:1–7.

Simic B, Kniewald J, Kniewald Z. Influence of ethynodiol diacetate on the formation of A-Homo-3-Oxa-5α-Pregnane-4,20-dione in female rats. Endocr Regul 1998;32:125–31.

Smith SS, Gong QH, Hsu F-C, Markowitz RS, ffrench-Mullen JMH, Li X. $GABA_A$ receptor α_4 subunit suppression prevents withdrawal properties of an endogenous steroid. Nature 1998a;392:926–30.

Smith SS, Gong QH, Li X, Moran MH, Bitran D, Frye CA, et al. Withdrawal from 3α-OH-5α-pregnan-20one using a pseudopregnancy model alters the kinetics of hippocampal $GABA_A$-gated current and increases the $GABA_A$ receptor α_4 subunit in association with increased anxiety. J Neurosci 1998b;18:5275–84.

Spona J, Elstein M, Feichtinger W, Sullivan H, Lüdicke F, Müller U, et al. Shorter pill-free interval in combined oral contraceptives decreases follicular development. Contraception 1996;54:71–7.

Sulak PJ, Kuehl TJ, Ortiz M, Shull BL. Acceptance of altering the standard 21-day/7-day oral contraceptive regimen to delay menses and reduce hormone withdrawal symptoms. Am J Obstet Gynecol 2002;186:1142–9.

Telleria CM, Carrizo DG, Deis RP. Levonorgestrel inhibits luteinizing hormone-stimulated progesterone production in rat luteal cells. J Steroid Biochem Mol Biol 1994;50:161–6.

van Heusden AM, Fauser BCJM. Residual ovarian activity during oral steroid contraception. Hum Reprod Updat 2002;8:345–58.

Yonkers KA, Brown C, Pearlstein TB, Foegh M, Sampson-Landers C, Rapkin A. Efficacy of a new low-dose oral contraceptive with drospirenone in premenstrual dysphoric disorder. Obstet Gynecol 2005;106:492–501.

Wang M, Seippel L, Purdy RH, Backstrom T. Relationship between symptom severity and steroid variation in women with premenstrual syndrome: study on serum pregnenolone, pregnenolone sulfate, 5 alpha-pregnane-3,20-dione and 3 alpha-hydroxy-5 alpha-pregnan-20-one. J Clin Endocrinol Metab 1996;81:1076–82.

Zimmemberg B, Farley MJ. Sex differences in anxiety behavior in rats: role of gonadal hormones. Physiol Behav 1993;54:1119–24.