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The antidepressant-like effects of the 3β-hydroxysteroid dehydrogenase inhibitor trilostane in mice is related to changes in neuroactive steroid and monoamine levels

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ABSTRACT

In the present study, we analyzed the effects of a systemic treatment with the competitive 3β-hydroxysteroid dehydrogenase (3β-HSD) inhibitor trilostane on: (i) neurosteroid and monoamine levels in the brain, and (ii) the antidepressant activity of steroids and antidepressants in the forced swimming test (FST). 3β-HSD converts pregnenolone (PREG) into progesterone (PROG) or dehydroepiandrosterone (DHEA) into androstenedione. These neuroactive steroids are known to regulate neurotransmitters effects in the brain, particularly glutamate, γ-aminobutyric acid (GABA) and serotonin (5-HT), with consequences on mood and depression. We previously reported that trilostane showed antidepressant-like properties in the FST and concomitantly regulated plasma adrenocorticotropin (ACTH) and corticosterone levels, markers of the stress-induced hypothalamus–pituitary–adrenal (HPA) axis activation. We here observed that adrenalectomy/castration blocked the trilostane effect, outlining the importance of peripheral steroid levels. Trilostane (25 mg/kg) decreased hippocampus PROG contents and paradoxically increased circulating PROG levels. It also increased PREG levels in the hippocampus and frontal cortex. In the FST, a co-treatment with trilostane facilitated DHEAS (5–20 mg/kg) antidepressant activity, but showed only an additive, not facilitative, effect with PREGS (10–40 mg/kg), PROG (10–40 mg/kg) or allopregnanolone (ALLO, 1–8 mg/kg). Trilostane (25 mg/kg) treatment significantly increased 5-HT and (-)-norepinephrine (NE) turnovers in the hippocampus, an effect likely related to its antidepressant action. In co-administration studies, trilostane further decreased immobility following fluoxetine (30–60 mg/kg), sertraline (20–40 mg/kg) and imipramine (20–40 mg/kg), but not desipramine (20–40 mg/kg), treatments. A significant additive effect was observed for the selective 5-HT reuptake inhibitors (SSRI) at their highest dose. This study confirmed that a systemic administration of trilostane directly affected peripheral and brain levels in neuroactive steroids and monoamine turnover, resulting in antidepressant activity. The drug could be proposed as a co-treatment with SSRI.

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Abbreviations: ALLO, 5α-pregnan-3α-ol,20-one, allopregnanolone; 3α-HSOR, 3α-hydroxysteroid oxydoreductase; 3α/3β-HSD, 3α/3β-hydroxysteroid dehydrogenase; 5β-DHP, 5β-dihydroprogesterone; 5-HIAA, 5-hydroxyindole-3-acetic acid; 5-HT, serotonin; ACTH, adrenocorticotropin; AdX/CX, adrenalectomized/castrated; BDNF, brain-derived neurotrophic factor; CSF, cerebrospinal fluid; DA, dopamine; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate ester; DOC, deoxycorticosterone; DOPAC, 3,4-dihydroxyphenylacetic acid; Epi, (-)-epinephrine; EPIALLO, 3β-hydroxy-5α-pregnan-ol-20-one, epiallopregnanolone; HVA, homovanillic acid; i.p., intraperitoneally; MHPG, 4-hydroxy-3-methoxyphenylglycol (MHPG); NE, (-)-norepinephrine; PREG, pregnenolone; PREGS, pregnenolone sulfate ester; PROG, progesterone; s.c., subcutaneously; SSRI, selective serotonin reuptake inhibitor; THDOC, 3α,5α-tetrahydrodeoxycorticosterone.

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1. Introduction

Neuroactive steroids are steroid hormones that exert rapid non-genomic effects on nervous cells. A pool of neuroactive steroid is synthesized *de novo* by neurons and glial cells and contributes to the higher concentrations found for several steroids in the brain (Corpéchot et al., 1981). These so-called '*neurosteroids*' (Baulieu, 1981) include pregnenolone (PREG), dehydroepiandrosterone (DHEA), progesterone (PROG) and its tetrahydro-reduced metabolites, such as 5α-pregnan-3α-ol,20-one (allopregnanolone, ALLO). The role of neuroactive steroids is to regulate the inhibitory/excitatory balance in the brain and their impact in several psychiatric conditions is well known. For instance, some neuroactive steroids have been reported to possess anti-stress, anxiolytic, antidepressant, antipsychotic, anticonvulsant, ataxic, and/or anesthetic properties in animals (Crawley et al., 1986; Landgren et al., 1987; Belelli et al., 1990; Bitran et al., 1993; Korneyev and Costa, 1996; Urani et al., 2001). Their role in depressive states has gained major attention (see the recent reviews by Van Broekhoven and Verkes, 2003; Eser et al., 2006; Girdler and Klatzkin, 2007; Reddy, 2010). In patients suffering from major depression, plasma and cerebrospinal fluid (CSF) levels in tetrahydro-reduced PROG metabolites, including ALLO and pregnanolone, were found to be significantly lower than those measured in control subjects (Uzunova et al., 1998). In contrast, 3α,5α-tetrahydrodeoxycorticosterone (THDOC) plasma levels were found to be significantly increased (Rupprecht, 2003; Ströhle et al., 1999). Plasma concentrations of both steroids returned to physiological baseline levels following a successful treatment with selective serotonin reuptake inhibitors (SSRI), after a period of 30 days (Romeo et al., 1998; Ströhle et al., 2000). Griffin and Mellon (1999) have reported that the SSRI fluoxetine increases the affinity for its substrates of 3α-hydroxysteroid dehydrogenase (3α-HSD), the enzyme that catalyzes the reduction of the dihydroxy-reduced metabolites of PROG and deoxycorticosterone (DOC). The levels of pregnanolone and ALLO were found to be lower in the CSF and plasma of depressed patients as compared within healthy volunteers. Moreover, successful antidepressant treatment resulted in increases up to normal levels and proportional to the mood improvement (Romeo et al., 1998; Uzunova et al., 1998; Ströhle et al., 1999, 2000). In animal models, injection of fluoxetine or paroxetine to male rats also resulted in a rapid increase in the brain content of ALLO and a concomitant decrease in 5α-DHP, without any change in PREG, PROG or DHEA (Uzunov et al., 1996). Moreover, the role of neuroactive steroids on mood is particularly important in females, due to the natural fluctuations in estrogens and PROG levels, with particular impacts during menstrual cycle or gestation in predisposed individual. Indeed, women suffer twice more from major depression than men (Weissman and Olfson, 1995). For instance, women suffering from premenstrual dysphoric disorders showed a blunted response to ALLO, responsible for a diminished functional activity of $GABA_A$ receptors (Girdler and Klatzkin, 2007). Moreover, estrogens participate in the modulation of depression associated with the endocrinal changes along the life of women (Stahl, 1998; Robinson, 2001). In depressive women, therapeutic treatments with estrogens decrease depressive symptoms and improve the effects of antidepressants including imipramine, sertraline, and fluoxetine (Amsterdam et al., 1999; Halbreich and Kahn, 2001; Oppenheim, 1983; Robinson, 2001; Schneider et al., 1997). In rodents submitted to the FST, estrogen co-treatment facilitated that action of antidepressants like venlafaxine, fluoxetine or desipramine, by shortening their onset of action (Estrada-Camarena et al., 2004, 2008). Chronic treatment with estrogens affected the metabolism of antidepressants by interacting with monoamine oxidase (Holschneider et al., 1998; Ma et al., 1995) or cytochrome P450 (Wang and Strobel, 1997). Moreover, both estrogens and antidepressants alter the levels of pregnane steroids by affecting enzymes involved in the synthesis of ALLO (Griffin and Mellon, 1999), in relation with the relief of depressive symptoms during the premenstrual syndrome and the perimenopausal period (Eser et al., 2006; Marx et al., 2006; Uzunova et al., 2006). It appears therefore that several studies illustrate how PROG and estrogen levels are responsible for the sensitivity of women to mood disorders in direct relation to changes in steroid levels.

DHEA and its sulfate ester DHEAS may also play a role in depressive symptoms in humans, since the disease altered their serum and urinary levels (Tollefson et al., 1990; Thomas et al., 1994). In open-label or double-blind randomized placebo-controlled clinical trials, oral administration of DHEAS decreased depressive symptoms in patients with major forms of depression (Wolkowitz et al., 1995, 1997, 1999a; Bloch et al., 1999). Moreover, in rodents, DHEA counteracted glucocorticoid actions by inhibiting glucocorticoid enzyme activity, a plausible mechanism for its antidepressant effect (Svec and Lopez, 1989; Browne et al., 1992; Wolkowitz et al., 1995, 1997, 1999b). DHEAS improves performance of rodents in the forced swimming test (FST), a procedure that relies on behavioral despair response in rodents and is routinely used to measure the antidepressant-like activity of drugs (Reddy et al., 1998; Urani et al., 2001).

The serotonergic system has also long been implicated in the neurobiology of mood disorders because, amongst other observations, most antidepressant treatments resulted in an enhanced serotonin (5-HT) neurotransmission through various mechanisms of action (Blier & de Montigny, 1994; Owens, 1996). Interestingly, numerous studies indicate that steroids modulate gene expression and functional activity of different components of the 5-HT system (Bethea et al., 2000). Steroids, particularly, modulate the electrical activity of dorsal raphe nucleus 5-HT neurons. A 7-day administration of 5β-pregnane-3,20-dione (5β-DHP), ALLO or DHEA increased the firing activity of 5-HT neurons in female rats (Robichaud and Debonnel, 2004). Testosterone and 17β-estradiol, in both male and female rats, also increased the 5-HT neuronal firing activity (Robichaud and Debonnel, 2005). Together, these data strongly suggest a reciprocal modulation between the 5-HT system and neuroactive steroids, mainly involving the dihydroxy-reduced metabolites of PROG and/or DHEA.

Trilostane is an effective inhibitor of the conversion of Δ^5-3β-hydroxysteroids into the corresponding Δ^4-3-ketosteroids. It acts as a competitive inhibitor of 3β-hydroxysteroid dehydrogenase (3β-HSD) activity with a Ki of 230 nM (Potts et al., 1978). The concentrations of PREG and PROG are largely and significantly increased by trilostane (Young et al., 1994). In adrenalectomized/castrated male rats, trilostane significantly increased the brain concentration of PREG, while it significantly decreased the brain concentration of PROG, as expected from a substrate-to-product relationship. This observation confirmed that PROG is a neurosteroid as defined by Baulieu (Jung-Testas et al., 1989; Corpéchot et al., 1993; Young et al., 1994).

A treatment with trilostane is therefore expected to directly modulate the brain and circulating levels in PROG, its reduced metabolites, and PREG and its metabolites. Consequently, a change in the activity of $GABA_A$ and 5-HT systems must be expected, that could lead to modulations of the antidepressant response in pathological conditions. We have indeed reported that trilostane showed antidepressant-like effect in mice submitted to the forced swimming test (FST) and anxiolytic effect in the elevated plus-maze and black-and-white exploration box (Espallergues et al., 2009). Trilostane also reduced the increase in plasma corticosterone and adrenocorticotropin (ACTH) levels provoked by a 15-min duration forced swimming stress in mice, showing direct effect on HPA axis