

## NEUROACTIVE STEROIDS: OLD PLAYERS IN A NEW GAME

R. C. MELCANGI<sup>a\*</sup> AND G. C. PANZICA<sup>b</sup>

<sup>a</sup>Department of Endocrinology and Center of Excellence on Neurodegenerative Diseases, University of Milan, Via Balzaretti 9, 20133 Milano, Italy

<sup>b</sup>Neuroscience Research Institute, Laboratory of Neuroendocrinology, Dept. Anatomy, Pharmacology and Forensic Medicine, University of Torino, C.SO M. D'Azeglio 52, 10126 Torino, Italy

**Abstract**—It is now clear that the study of the effects exerted by steroids on the nervous system may be considered as one of the most interesting and promising topics for biomedical research. Indeed, new effects, mechanisms of action and targets are becoming more and more evident suggesting that steroids are not only important key regulators of nervous system function but they may also represent a new therapeutic tool to combat certain diseases of the nervous system. The present review summarizes recent observations on this topic indicating that while the concept of the nervous system as a target for steroid hormones has been appreciated for decades, a promising new era for the study of these molecules and their actions in the nervous system has been initiated in the last few years. © 2005 Published by Elsevier Ltd on behalf of IBRO.

**Key words:** neurosteroids, brain, spinal cord, peripheral nerves, steroid receptors, non-classical steroid receptors.

The notion that the nervous system is an important target of gonadal products can be traced to observations of F. J. Gall in 1818 and by J. Vimont in 1835, indicating that unilateral castration causes atrophy of the contralateral hemisphere of the cerebellum and Arnold Adolph Berthold's experiments on testicular transplant in 1849 (Medvei, 1982). In the early 20th century the products of the gonads and adrenal glands were identified and purified as steroid hormones, such as 17 $\beta$ -estradiol, progesterone, testosterone, corticosterone, and aldosterone. These were finally purified between 1929 and 1954, and subsequently demonstrated to affect a wide array of neurophysiological parameters, controlling sexual differentiation of the brain, reproduction, behavior, memory, etc. (McEwen, 1981, 1994; Fink et al., 1991). The mechanisms by which steroids exert their effects on the nervous system were construed as a classical endocrine mechanism involving steroid production by endocrine glands such as the adrenals and gonads, secretion into the bloodstream, crossing of

the blood–brain barrier and then regulating the CNS in various ways. Moreover, a further advance in understanding the role of steroid hormones in the differentiation of rodent brain was the finding that androgens produced by testis and the adrenal may act in the CNS through their local conversion (the so-called “peripheral conversion”) into more active molecules such as estrogens: this idea was identified as “the aromatization hypothesis” (Naftolin and MacLusky, 1984). Finally, the unexpected discovery by Baulieu and coworkers in 1981 (Corpechot et al., 1981; Baulieu et al., 1999) of the synthesis of steroids directly in the CNS (i.e. the formation of the so-called *neurosteroids*), has added also paracrine and/or autocrine mechanisms to the list of ways steroids can regulate brain function in addition to the previously described endocrine mechanism.

### The CNS as a target for steroids

Steroid hormone receptors in the brain were discovered in the 1960s with the use of autoradiography (Stumpf et al., 1975), initial observations suggested that their distribution was mainly restricted to the hypothalamic region. These receptors (now named classical steroid receptors) are localized in the cytoplasm and, when activated by binding to the hormone, translocate into the nucleus where they exert a regulatory action on the genome (Yamamoto, 1985). Examples of these are progesterone (Blaustein, 2003), estrogen (Shupnik, 2002), androgen (Cato and Peterziel, 1998), glucocorticoid and mineralocorticoid receptors (McEwan et al., 1997). The activation of these receptors may explain the medium- and long-term effects of steroid hormones (such as the regulation of the secretion of hypophyseal hormones, or the sexual differentiation of brain circuits). However, additional studies indicate that steroids might also induce short-term effects (i.e. effects that take place in seconds or minutes), thus suggesting the existence of other receptors (i.e. the so-called non-classical steroid receptors) located within the membrane and thus able to act as mediators of short-term actions. Examples of these are GABA type A and B (GABA-A receptor, GABA-B receptor), serotonin type 3 (5-HT<sub>3</sub>), *N*-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate receptor, and an atypical intracellular receptor like the sigma 1 (Melcangi et al., 2005; see in this issue Belelli et al., Frye et al., Henderson LP et al.). In addition, very recent observations demonstrate the existence of a small pool of classical estrogen receptors (ER $\alpha$  and ER $\beta$ ) at the plasma membrane that can rapidly affect cellular physiology through the activation of second messenger pathways (Chaban et al., 2004; Razandi et al., 2004; see in this issue Mhyre and Dorsa). As a final point, recent papers from Pfaff and colleagues (Kow and

\*Corresponding author. Tel: +39-02-50318238; fax: +39-02-50318204. E-mail address: roberto.melcangi@unimi.it (R. C. Melcangi).

**Abbreviations:** DOC, deoxycorticosterone; EDC, endocrine disrupting chemical; ER, estrogen receptor; PBR, *Peripheral Benzodiazepine Receptor*; SERM, selective estrogen receptor modulator; StAR, Steroidogenic acute Regulatory protein; THDOC, allotetrahydrodeoxycorticosterone; WHI, Women's Health Initiative; WHIMS, Women's Health Initiative Memory Study.

Pfaff, 2004; Vasudevan et al., 2005) suggest that, one effect of estrogen through membrane receptors may be to potentiate their genomic action.

Other important players are molecules that can interfere with or enhance the activity of intracellular steroid receptors (i.e. co-repressors, co-activators) (see in this issue Meijer et al.), including also some neurotransmitters (e.g. dopamine) that can activate steroid-receptors in a “ligand-independent” manner by influencing the dynamic equilibrium between neuronal phosphatases and kinases (see in this issue Mani).

Interestingly, recent observations have indicated that not only cortical brain areas but also spinal cord and peripheral nervous system express classical and non-classical steroid receptors, as well as co-activators, suggesting that these structures are also likely targets of steroids (Melcangi et al., 2005).

### Steroidogenesis in the nervous system

The various steps of the *in situ* synthesis of steroids in the brain have not yet been fully elucidated, however, the recent discovery of a large distribution of the StaR (Steroidogenic acute Regulatory) protein within the brain (see in this issue Lavaque et al.), together with the presence of several steroid-forming enzymes (Melcangi et al., 2004), further suggests that this synthesis can directly start from cholesterol and that steroidogenesis is a generalized process within the CNS. In accordance with this view, some studies on the distribution of androgen receptors (see in this issue DonCarlos et al.) demonstrate that the most prominent forebrain target for androgen action with respect to the number of androgen receptors is the cerebral cortex, rather than the well-characterized hypothalamic and limbic brain regions that are known to control reproductive functions. These observations imply that higher cortical functions such as memory, learning and motion behavior, may be directly influenced by steroid hormones and/or neurosteroids (see in this issue Grobin et al.).

The activity of the enzymes involved in steroidogenesis may be influenced in several ways. For example, the enzyme aromatase is regulated by long-term (hours or days) steroid-induced modifications of transcription, as well as rapid (within minutes) non-genomic mechanisms such as variations in concentration of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ , or ATP. These two modes of control provide variations in the local availability of estrogens and match well with the genomic and non-genomic action of these steroids on neural circuits and related behaviors (see in this issue J. Balthazard et al.).

Estradiol is one of the end products of the brain steroidogenesis, as well as of the transformation of circulating androgens. For example, estrogens are synthesized “de novo” in the adult hippocampal neurons and the local release of estradiol is modulated by glutamatergic transmission (see in this issue: Prange-Kiel and Rune; Mukai et al.). Even more interesting is the localization of ER alpha at the level of synaptic membranes, suggesting therefore a rapid non-genomic synaptic action of estradiol (see in this issue Takata et al.), as it was previously hypothesized on the basis of the synaptic location of the enzyme aromatase

(Naftolin et al., 1996). Finally, synthesis of steroids is not restricted to the brain but is also present in spinal cord and peripheral nerves. Indeed, expression of StAR and steroidogenic enzymes, such as cytochrome P450scc (i.e. the enzyme converting cholesterol to pregnenolone) and 3 beta-hydroxysteroid dehydrogenase, which convert pregnenolone into progesterone, or enzymes further converting steroids, such as 5alpha-reductase (i.e. the enzyme converting progesterone and testosterone into dihydroprogesterone and dihydrotestosterone respectively) has been demonstrated (Melcangi et al., 2005).

### The concept of neuroactive steroids

All of these observations indicate that the nervous system is a target for two different pools of steroids, one coming from the peripheral glands (i.e. steroid hormones) and the second one originating directly in the nervous system (i.e. neurosteroids). However, because in many circumstances it is difficult to discriminate whether the steroid effect is due to *in situ* synthesis, to the peripheral hormones, or to an enzymatic activation of steroids in metabolites which are more active and in some cases utilize a different mechanism of action, some investigators in this field use now the term *neuroactive steroids* (Paul and Purdy, 1992).

In addition, a family of compounds that are biologically active and often mimic endogenous steroid hormones, binding to steroid hormones' receptors (mainly to the ERs), thereby altering hormone-modulated responses, has been recently revealed. They belong to the class of the so-called endocrine disrupting chemicals (EDCs) and are of either synthetic (i.e. bisphenol), or biological derivation (i.e. phytoestrogens). Their actions have been largely studied on non-nervous structures and with a toxicological approach (Witorsch, 2002). However, in recent years it became clear that there are other possible mechanisms of action of EDCs leading to biological effects. In particular, the timing of exposure to EDCs is a critical factor, such that the effects of a particular EDC will vary over the lifecycle of the animal as well as across species and phyla. Often, embryonic exposure to estrogenic EDCs will have lifelong effects due to action of the estrogenic compounds on sexual differentiation of brain structures and behaviors. Some compounds target neuroendocrine systems, thereby affecting reproductive endocrine systems as well as other endocrine systems. Therefore, exposure to the estrogenic chemicals during embryonic development has consequences beyond impaired function of the reproductive axis. This makes it very challenging to evaluate the short- and long-term effects of EDCs.

The disturbance of hormonal systems by EDCs with estrogenic action, particularly during the sensitive periods of organogenesis and sexual differentiation of the brain, can alter the functionality of the reproductive organs and the neurochemistry and organization of cortical circuits, and thus, the behavioral responses of the individuals exposed to these substances (Panzica et al., 2005b). Several recent studies have investigated subtle modifications of the animal behaviors (e.g. reproductive, aggressive) induced by EDCs that are probably related to alterations of

specific neural pathways (Lephart et al., 2005; Panzica et al., 2005a). These compounds are therefore a third player within the nervous system and the evolutionary implications of having them in the normal food supply for certain human populations (i.e. phytoestrogen derivatives from soy) (Naftolin and Stanbury, 2002), as well as for wild and farm animals have not yet been discussed.

### Gonadal hormones, brain and behavior development

Sex steroid hormones play a fundamental role in brain development. These hormones may induce permanent changes in the architecture of nervous circuits, including changes in cell number, density of axonal connections, dendritic architecture and neurotransmitter phenotype, as a consequence they also profoundly influence the establishment of related behaviors (see in this issue Kudwa et al.). The mechanisms to reach these effects are different and have been largely studied in a variety of experimental model systems based on vertebrate species (for reviews, see Panzica et al., 1995; Breedlove et al., 1999). One of the main mechanisms of estrogen action is through the regulation of caspase dependent, apoptotic-like programmed cell death. In addition, by acting on genes such as those for BDNF or semaphorin, estrogen may also modulate sexually dimorphic pathways within the brain (Simerly, 2002). The use of a gene “knock-out” mouse provides a direct identification of at least one molecule required in this process (Bax) and suggests a molecular mechanism for the development of neural sex differences: testosterone may regulate the activity of Bax, or of proteins that interact with Bax, to engender sex differences in neuron number (see in this issue Forger). Many neural circuits have been investigated to explain the role of sexual steroids in differentiating and/or controlling circuits (Panzica et al., 1995; Cooke et al., 1998). In this special issue some of them are described, as the neural song system of songbirds (see Fusani and Gahr), the vasopressin and vasotocin systems of vertebrates (see De Vries and Panzica), the NO-producing system (see Panzica et al.), and the amygdaloid complex (see Cooke).

Effects of gonadal steroids on hippocampus-mediated behaviors may result at least in part from hormone-based actions, which is discussed in this issue with respect to the role of testosterone in maintaining normal hippocampal structure (see MacLusky et al.). A similar role is played by estrogens and androgens in the synaptic remodelling of the hypothalamus (see in this issue Parducz et al.). Also astroglial elements may play a role in these mechanisms by regulating glutamatergic neurotransmission (see in this issue Mong and Blutstein). Finally, the development of transgenic animals with modified sex chromosomes (i.e., with altered expression of Sry gene, the gene determining in mammals the development of masculine sex) provides a powerful tool to investigate the determination of sexually dimorphic circuits (Arnold et al., 2003) as well as that of gonadal steroid dependent behaviors (see in this issue Kudwa et al.).

### Neuroactive steroids as protective agents

In the last few years, the finding that neuroactive steroids may be considered as neuroprotective agents has attracted the attention of several investigators. Indeed, it is now clear that neuroactive steroids, like progesterone and its derivatives (i.e. dihydroprogesterone and allopregnanolone), dehydroepiandrosterone, and estrogens exert a variety of neuroprotective effects, which suggests that they may be candidates as new therapeutic tools to counteract neurodegenerative events (Garcia-Ovejero et al., 2005; Schumacher et al., 2003; Veiga et al., 2004). For instance, in an experimental model of Niemann-Pick type C (NP-C) disease (a fatal, autosomal recessive childhood neurodegenerative disease), the cerebral levels of allopregnanolone are decreased and the neonatal administration of this steroid results in a delay of the onset of neurological symptoms, and a doubling the lifespan of these animals (Griffin et al., 2004). Moreover, other steroids have been recently evaluated in terms of their potential therapeutic activity for neurodegeneration. For instance, it has been demonstrated that treatment with 7beta-hydroxy epiandrosterone has a neuroprotective effect in animal models displaying Alzheimer's disease lesions (Dudas et al., 2004). On the other hand, some observations also suggest that neuroactive steroids may induce damage. For instance, it has been recently demonstrated that testosterone treatment amplifies excitotoxic damage of oligodendrocytes in culture (Caruso et al., 2004). In opposition, estradiol (which, as mentioned above, is formed from testosterone by the action of the enzyme aromatase) protects oligodendrocytes from cytotoxicity *in vitro* (Takao et al., 2004).

An interesting alternative to treatment with neuroactive steroids might be synthetic receptor modulators, like for instance selective estrogen receptor modulators (SERMs). Examples of these are tamoxifen, raloxifene, lasofoxifene, bazedoxifene etc. Very recent observations have indicated that SERMs are also neuroprotective agents in experimental animal models of central neurodegeneration (Ciriza et al., 2004; O'Neill et al., 2004).

Moreover, a further alternative therapeutic strategy might be the use of pharmacological agents that increase the synthesis of endogenous neuroactive steroids within the nervous system. With this perspective, ligands of *Peripheral Benzodiazepine Receptor* (PBR) may represent an interesting option. PBR is mainly present in the mitochondrial outer membrane, where it promotes, in cooperation with the StaR protein, the translocation of cholesterol to the inner mitochondrial membrane. The mitochondrial translocation of cholesterol is a limiting step in steroidogenesis, since it allows the transformation of cholesterol into pregnenolone. Reports from different laboratories indicate that PBR ligands stimulate steroid synthesis in adrenal, placental, testicular, ovarian and glial cells (see in this issue Papadopoulos et al.). Indeed, very recent observations have shown that treatment with PBR ligands, like for instance Ro5-4864, exerts neuroprotective effects both in central (Veiga et al., 2005) and peripheral nervous system (Leonelli et al., 2005).

The therapeutic potential of neuroactive steroids as neurogenic molecules has been also recently proposed. For instance, pregnenolone sulfate, which has been demonstrated to be able to counteract age-dependent cognitive impairments, is able to stimulate *in vivo* neurogenesis in the dentate gyrus of adult and aged rats (Mayo et al., 2005). In agreement, allopregnanolone has been demonstrated to promote proliferation of neuroprogenitor cells derived from the rat hippocampus and of human neural stem cells derived from the cerebral cortex (Wang et al., 2005). Furthermore, it has been suggested recently that dehydroepiandrosterone is also involved in the maintenance and division of human neural stem cells (Suzuki et al., 2004).

The neuroprotective effects of estradiol are presented in this special issue in some animal and cellular models. Thus, estrogen treatment in ovariectomized female rats has neuroprotective effects on status epilepticus-induced hippocampal damage and prevents the loss of inhibition in the dentate gyrus (see in this issue Veliskova). In an animal model of prenatal exposure to estradiol, an increase of inactive glycogen synthase kinase-3 $\beta$  (i.e. a condition protective for Alzheimer disease; Bhat et al., 2004) is evident in the postnatal hippocampus (see in this issue Manthey and Behl). In both young and aged female rats, treatment with physiological concentrations of estradiol decreases experimentally induced ischemic injury by almost 50%, compared with oil-treated controls (see Wise in this issue). In an animal model for Parkinson's disease, estradiol modulates the glial neuroinflammatory reaction in the protection of mesencephalic dopaminergic neurons, showing, in particular, that astrocyte and microglia response to methyl-phenyl-tetrahydropyridine (MPTP) injury. Moreover, the expression of pro-inflammatory mediators, may vary according to the estrogenic status with direct consequences for dopaminergic neuron survival, recovery and repair (see in this issue Morale et al.). Finally, studies in young adult non-human primate females suggest that some aspects of cognition fluctuate with the menstrual cycle, but that ovariectomy and estrogen replacement have only modest effects on cognitive function. In contrast, data in aged, naturally or surgically menopausal monkeys indicate that estrogen modulates a broad range of cognitive domains (see in this issue Lacreuse). Indeed, estrogen treatment increases spine numbers in the prefrontal cortex of ovariectomized female rhesus monkeys (Tang et al., 2004).

In late pregnancy, oxytocin neuron responses to systemic interleukin-1 $\beta$  are suppressed by allopregnanolone and endogenous opioids. Allopregnanolone may act independently, via GABA-A receptors on oxytocin neurones, and by inducing opioid expression (see in this issue Russell and Brunton).

Extensive evidence indicates that glucocorticoid hormones influence cognitive performance, mainly activating glucocorticoid-sensitive pathways that enhance the consolidation of long-term memory (Lupien et al., 2005). Glucocorticoid effects on memory consolidation involve noradrenergic activation of the basolateral amygdala. They

may interact with the noradrenergic system both at a postsynaptic level, increasing the efficacy of the beta-adrenoceptor-cAMP/PKA system, as well as presynaptically in brainstem noradrenergic cell groups that project to the basolateral amygdala. In contrast, memory retrieval and working memory performance are impaired with high circulating levels of glucocorticoids (see in this issue Roozendaal et al.).

In addition to cortisol, a significant amount of the mineralocorticoid deoxycorticosterone (DOC) is also released during stress. DOC undergoes biotransformation to allotetrahydrodeoxycorticosterone (THDOC), a neuroactive steroid with anxiolytic and anticonvulsant properties. THDOC is a potent positive allosteric modulator of the GABA-A receptor. Although the role of THDOC within the brain is undefined, recent studies indicate that stress induces THDOC to levels that can activate GABA-A receptors. These results might have significant implications for human stress-sensitive conditions such as epilepsy, panic disorder, post-traumatic stress disorder, and major depression (see in this issue Reddy).

Interestingly, neuroactive steroids may be considered as neuroprotective agents not only in the CNS, but also in spinal cord and peripheral nervous system. For instance, recent observations have demonstrated the efficacy of treatment with dehydroepiandrosterone (Fiore et al., 2004) or progesterone (Gonzalez et al., 2004) after spinal cord injury.

Recently, a direct link between neuropathic pain and neuroactive steroid formation in spinal and supra-spinal networks has been proposed. In fact, rat sciatic nerve ligation induces an increase of neuroactive steroids, such as pregnenolone and allopregnanolone, in the spinal cord dorsal horn (Patte-Mensah et al., 2004). In addition, an acute blockade of the endogenous synthesis of estrogen in the quail spinal cord dorsal horn, induces a reduction of behavioral responsiveness to thermal painful stimulus (Evrard and Balthazart, 2004). Lastly, progesterone and/or its derivatives, dehydroepiandrosterone and androgens have been recently identified as neuroprotective agents for acquired (after trauma, during aging etc.) or inherited peripheral neuropathy, such as Charcot-Marie-Tooth disease (Melcangi et al., 2005; Sereda et al., 2003).

Altogether these new findings suggest that neuroactive steroids may represent a promising strategy to counteract neurodegenerative events both in central and peripheral nervous system.

### Clinical studies

The transition from animal or cellular models to human therapies is fraught with difficulty, and in this arena, the clinical and pre-clinical results have some apparent inconsistencies. For example, a large clinical trial on the effects of hormonal therapy in women (Women's Health Initiative, WHI) has been performed in the USA. However, the results of the WHI suggest that some hormone therapies may increase the risk of several diseases, including stroke (Rossouw et al., 2002). The results of WHI may be due to the doses, specific hormones used, age at which hor-

mones were administered relative to the perimenopausal transition, or the sub-population of women who were included in this study. Thus, it is important to determine whether other hormone regimens may afford protection against stroke and neurodegenerative diseases, as well as to design therapies that favor beneficial effects of therapy and minimize the risks of treatment (Wise et al., 2005).

Several clinical trials are discussed in this special issue. In particular, it has been demonstrated that androgen deprivation, caused by prostatic surgery, induces memory loss in men, and that estrogen may restore the function, whereas androgens may have an important role for the long-term consolidation of verbal information (see in this issue, Janowsky). In women, estrogen helps to maintain aspects of cognitive functioning in premenopausal women who undergo a surgical menopause when it is administered immediately following the surgery (see in this issue, Sherwin). Neuroactive steroids are important endogenous modulators of depression and anxiety-related behavior and might have therapeutic potential for the treatment of depression and anxiety disorders. Novel therapeutic strategies might either be based on synthetic derivatives of endogenous 3 alpha-reduced neuroactive steroids or on the modulation of neurosteroidogenic enzymes, e.g. by ligands of the PBR (see in this issue, Eser et al.).

Basic science studies, observational studies, and randomized clinical studies suggest that women in the earlier stages of the menopausal transition might gain cognitive benefits from estrogen therapy. To address this important issue, observational trials of hormone therapy and dementia risk, randomized clinical trials of hormone therapy and cognitive function, and basic science studies were performed. These lines of research provide suggestive, but not definitive, evidence that early initiation of hormone therapy may provide cognitive benefits, particularly to verbal memory and other hippocampus-mediated functions (see in this issue, Maki).

Estrogen has the potential to influence brain processes implicated in Alzheimer's disease pathogenesis. Therefore, estrogen-containing hormone therapy after menopause might be expected to influence the risk of Alzheimer's disease. However clinical evidence from the Women's Health Initiative Memory Study (WHIMS) demonstrates that oral conjugated equine estrogens, with or without a progestin, increased the incidence of dementia for postmenopausal women age 65 years or older (Rapp et al., 2003). The WHIMS findings may not generalize to estrogen use by relatively young postmenopausal women, a class of women who were ineligible for the WHIMS trial, or across formulations and treatment regimens. Although there is no clinical trial evidence from randomized clinical trials that hormone therapy at any age protects against Alzheimer's disease, it remains to be determined whether the age at which hormone exposure occurs or the timing of hormone therapy initiation in relation to the menopause (the "critical window" hypothesis) modifies treatment outcomes on dementia risk (see in this issue, Henderson VW).

## CONCLUSION

The observations summarized in this paper, along with the papers collected in this special issue of Neuroscience (derived from the 3rd edition of the International Meeting on Steroids and Nervous System that was held in Torino, February 2005), which provide additional detailed information about the topics discussed, strengthen the conclusion that this topic represents a critically important area of neuroscience and a very promising arena for translational biomedical research. Indeed, even though it has been known for some time that the nervous system is a target for steroid hormones, the observations obtained in the last few years have revealed many potential applications of these molecules and related modulators as a new therapeutic strategy to combat aging and neurodegenerative events.

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Information about this series of conferences is available at a dedicated website, <http://www.dafml.unito.it/anatomy/panzica/neurosteroids/index.html>, where the extended abstracts of all the editions of the meeting are available for downloading.

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