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NEUROACTIVE STEROIDS: OLD PLAYERS IN A NEW GAME

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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 17β-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-HT3), *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 (ERalpha and ERbeta) 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

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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.

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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 Ca^{++} and 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. Balthazart 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