Neuroactive steroids and the nervous system: further observations on an incomplete tricky puzzle.

NEUROACTIVE STEROIDS AND THE NERVOUS SYSTEM: FURTHER OBSERVATIONS ON AN INCOMPLETE TRICKY PUZZLE.

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Introduction

In this special issue, we have collected reviews based on the invited lectures of the VIIth International Meeting on Steroids and Nervous System (Torino, Italy, February 2013). They cover the majority of the actual and future hot topics in this field of the neuroendocrinology.

The relationships among steroid hormones and the nervous system have been the topic of many studies, that started from very old experiments illustrating the effects of castration on both brain structure and behaviour [see Gall, 1818, Vimont 1835 and Berthold 1849, cited by (1)]. For a long time the brain and the peripheral nervous system were indicated as targets of the action of the steroid hormones produced by gonads or adrenals. This view was confirmed by the discovery of a wide distribution of steroid hormone receptors within the nervous tissue of all vertebrates (2, 3) and by the finding that steroid hormone receptors were nuclear receptors able to regulate gene expression (4). Thus, this research field was focused for a long time in a paradigm that can be summarized as follows: peripheral glands may regulate the development, differentiation and activity of neural circuits through the interactions of their hormones with the appropriate nuclear receptors expressed by nerve cells. According to this view, the chief action of steroid hormones should be the long-term regulation of neural processes that, in the case of neural development and differentiation, may result in permanent organizational effects.

However, this view of the action of the steroid hormones on the nervous tissue was incomplete and in contrast with earlier studies illustrating rapid changes in biochemical reactions in the cytoplasm (5) and with several studies completed in the late 70s and in the 80s showing that steroids (or some of their metabolites) can bind specifically to purified synaptic membranes (6), rapidly modify electrophysiological properties (7) or modulate the action of some receptors (8). More or less at the same time it was discovered that androgens produced by the testis and the adrenal may be converted within the brain into more active molecules such as oestrogens (the aromatization hypothesis) (9). This last discovery opened the door to the idea that the steroid-dependent neural functions are conditioned not only by the actions of the endocrine glands, but that the nervous system can modify and/or interfere hormones' actions. This hypothesis was strongly reinforced by the unexpected discovery by Baulieu and coworkers in 1981 of the synthesis of steroids (called neurosteroids) directly in the CNS (10).

Thus, during the 90s the view of how steroids can interact with the nervous system turned to a complex system in which endocrine glands and nerve cells cooperate through classical endocrine, but also with paracrine and/or autocrine mechanisms to regulate brain functions (11). This regulation is not only a long-term adaptation, but in many cases involves short-term alterations of

nerve cells activity, thus the search for so called non-classical steroid receptors and mechanisms of action received a new input from these discoveries (12).

Therefore, in the last fifteen years the study of the interactions between steroids, now named neuroactive steroids (13), and the nervous system has received great attention from the scientific community (e.g., the elucidation of various signalling pathways involving classical and non-classical steroid receptors, their role in sexual differentiation of the brain, and in the control of reproduction and behaviours) (14). These molecules may also represent interesting tools for therapeutic strategies against neurodegenerative and psychiatric disorders, that in many cases show sex differences in their incidence, symptomatology, neurodegenerative outcome, and, interestingly, in the levels of neuroactive steroids (15-17)

Genomic, non-genomic, and epigenetic effects of neuroactive steroids: mechanisms of action

The genomic action of steroid hormones is performed through nuclear receptors (i.e. oestrogen, androgen, progesterone or glucocorticoid receptors) that, in the absence of hormone, are linked with several chaperone molecules. After binding the appropriate hormone, these receptors undergo a conformational change that causes dissociation of these molecules and allows receptors to dimerize and to bind directly to specific steroid response elements (SRE) and SRE-like sequences in the promoter regions of target genes (18). However, it has been discovered that the mechanism involves also the presence of nuclear receptor coregulators that enhance (coactivators) or repress (corepressors) the transcriptional activity of steroid receptors (19). Hundreds of coregulators have been identified and the knowledge of their functions in behaviour, physiology and disease is growing rapidly (20). In this issue, Tetel et al. (21) discuss the p160 family of coactivators (including SRC-1, SRC-2 and SRC-3) in the regulation of steroid action in brain and behaviour. The differential regulation and expression of these coactivators may provide a mechanism by which single cells in specific brain regions can differentially respond to steroids in response to changes in external stimuli.

As reported previously, it has been long established that hormones exert long-term influences on the developing brain, which direct the response in adulthood (organizational effects), but the cellular mechanisms have not been satisfactorily elucidated. However, the recent renewed interest in epigenetic mechanisms, in particular those that are temporary and responsive to changes in the environment, drug exposure or experience (context- dependent epigenetics) has stimulated several studies on their effects on brain circuits and related behaviours (22, 23). In this issue, epigenetic mechanisms have been discussed in relation to brain sexual differentiation (24), sexual differences in mental health risk (25), and hippocampal synaptic plasticity and cognitive functions (26). At the

hippocampal level sex hormones are also regulating other phenomena, such as adult neurogenesis (27). Gonadal and stress hormones may modulate multiple aspects of neurogenesis (cell proliferation and cell survival) in both male and female rodents. The function of adult neurogenesis in the hippocampus is linked to spatial memory and depression and provides early evidence of the functional links between hormonal modulation of neurogenesis to regulate cognition and stress.

Non-classical mechanisms of action are discussed in two reviews. The classical actions of progesterone (PROG) in the nervous system are the regulation of gonadotrophin-releasing hormone release and elicitation of feminine reproductive behaviours. In addition, PROG may modulate, through non-classical signalling molecules and effector systems, diverse neural processes such as cognitive functions and emotion, neurogenesis, neuroinflammation, neuroprotection and neuronal cell death (28).

Oestrogens trigger several pathways at the plasma membrane that exert beneficial actions against neurodegenerative diseases. The study of Marin et al. (29) demonstrates that oestrogen mechanisms of neuroprotection may occur not only by its interaction with neuronal protein targets through non-genomic and genomic mechanisms, but also through its participation in membrane architecture stabilization via "lipostatic" mechanisms.

Recently, an orphan G-protein coupled receptor was identified as an oestrogen- sensitive receptor in cancer cells. This receptor, now termed G-protein estrogen receptor 1 (GPER1) has been the subject of many investigations including its distribution within the brain (30). Srivastava and Evans (31) highlight some of the more recent advances in the understanding of the distribution and subcellular localisation of this receptor in the brain, as well as some of the evidence for the potential role that this receptor may play in the brain and some of the controversies surrounding the pharmacology of this receptor.

Endocrine disrupting chemicals (EDC) are useful tools to understand mechanisms of action of steroid hormones. In addition they represent a major environmental concern for the animal and human health. During the last years we have observed an increasing interest in the field of EDCs, mainly related to the potentially adverse effects on the sexual differentiation of brain and behaviour (14). In this special issue Bourguignon et al. (32) reviewed the literature on the effects of EDC on the timing of puberty in women, concluding that human evidence of altered female pubertal timing after exposure to endocrine disrupting chemicals (EDCs) is equivocal. Among limiting factors, most studies evaluate exposure to single EDCs at the time of puberty and can hardly assess the impact of lifelong exposure to mixtures of EDCs. Rodent and ovine studies indicate a possible role of fetal and neonatal exposure to EDCs, along the concept of early origin of health and disease. Such effects possibly involve neuroendocrine mechanisms (33) since the hypothalamus is a site

where homeostasis of reproduction as well as control of energy balance are programmed and regulated. The authors show that neonatal exposure to the potent synthetic oestrogen diethylstilbestrol (DES) is followed by early or delayed puberty depending on the dose, with consistent changes in developmental increases of GnRH pulse frequency. Moreover, DES results in reduced leptin stimulation of GnRH secretion in vitro, an effect that is additive with prenatal food restriction. Thus, using puberty as an endpoint of EDC effects, it appears necessary to consider pre- and perinatal exposure to low doses and to pay attention to the other conditions of prenatal life such as energy availability, keeping in mind the possibility that puberty could be not only advanced but also delayed through neuroendocrine mechanisms.

Oestrogen-regulated synaptic connectivity

Aromatase, the enzyme that converts testosterone to oestradiol in the brain, is also expressed in presynaptic terminals and modulated within minutes by calcium-dependent phosphorylation. In 2006 Balthazart and Ball (34) suggested the hypothesis that brain oestrogens display many, if not all, functional characteristics of neuromodulators or even neurotransmitters. In this special issue we have several contributions about the role played by oestrogens in the regulation of synaptic connectivity.

Oestrogen may act directly on actin networks through direct activation of small GTPases by oestrogen receptors on the synaptic membrane, in addition, the hormone stimulates TrkB receptors for Brain-Derived Neurotrophic Factor (BDNF), a neurotrophin shown to stimulate RhoA, cofilin, and LTP. Collectively, these results point to the conclusion that E2 can rapidly act on neighbouring membrane receptors to influence actin signalling, synaptic physiology, and plasticity (35).

Oestradiol membrane signalling involves the transactivation of metabotropic glutamate receptors (mGluR) that transduce steroid information through PKC signalling cascades producing rapid activation of lordosis regulating circuits. Micevych and Sinchak (36) review recent findings about oestradiol membrane signalling, including oestrogen receptor alpha, GPER-1 and other receptors, inducing dendritic spine formation in the arcuate nucleus that is critical for oestradiol induction of sexual receptivity and activation of lordosis circuits. New methods for tracking single receptor molecule mobility in neuronal membranes (37) represent the next frontiers to better understand steroid-membrane relationships.

Neuroactive steroids and psychiatric disorders: clinical and preclinical studies

A role for neuroactive steroids in modulating mood and exerting an important component in psychiatric disorders has been proposed (38, 39). In this issue, Biagini et al. (40) discuss data obtained in an animal model of epilepsy indicating that the enzyme cytochrome P450 cholesterol side-chain cleavage (P450scc) is associated with a delayed appearance of spontaneous recurrent seizures induced by pilocarpine. Moreover, by inhibiting the formation of neuroactive steroids, such as allopregnanolone, it is possible to terminate the latent period in pilocarpine-treated rats. Indeed, the inhibitor finasteride induces seizures in the chronic period of epileptic rats, suggesting that 5α-reduced neuroactive steroids are continuously produced to counteract seizures. It is interesting to note that in humans, exacerbation of epilepsy has been also described in patients occasionally exposed to finasteride.

Changes in neuroactive steroid biosynthesis, by finasteride, have been related to another psychiatric disorder, Tourette syndrome (41, 42). This is a neurodevelopmental disorder characterized by recurring motor and phonic tics, which is thought to reflect dysregulations in the signaling of dopamine and other neurotransmitters, leading to excitation/inhibition imbalances in cortico-striato-thalamocortical circuits. As described in this issue (43), clinical and preclinical studies indicate that the block of the enzyme 5α-reductase exerts marked anti-DAergic and tic-suppressing properties in Tourette syndrome. This suggests that the impairment of DA and other neurotransmitter signalling depends on the imbalances in steroid homeostasis and this may ultimately result in the facilitation of tics and other behavioural abnormalities in Tourette syndrome.

Infants with infantile spasms, a devastating epileptic syndrome of infancy with characteristic spastic seizures, chaotic irregular waves on interictal electroencephalograms (EEG; hypsarhythmia) and mental deterioration, have decreased concentrations of adrenocorticotrophic hormone (ACTH) and cortisol in cerebrospinal fluid strongly suggesting the involvement of hypothalamic dysfunction (44). In animal models, prenatal exposure to corticosteroids has long-term postnatal somatic and neurodevelopmental consequences, including hypothalamic function (45). In this issue Jacobas at al. (46) discuss a model of human infantile spasms developed by using repeated prenatal exposure to betamethasone and postnatal trigger of developmentally relevant spasms with N-methyl-D-aspartic acid (NMDA). The spasms triggered in prenatally primed rats are more severe compared to controls and respond to ACTH, a treatment of choice for infantile spasms in humans. Transcriptomic analysis of the arcuate nucleus after prenatal priming with betamethasone but before trigger of spasms indicates that prenatal betamethasone exposure down-regulates, in a sexually differentiated way, genes encoding several important proteins participating in glutamatergic and GABAergic transmission. Changes in transcript expression and their coordination may contribute to a molecular

substrate of permanent neurodevelopmental changes (including infantile spasms) found after prenatal exposure to corticosteroids.

Neuroprotection

It is now clear that neuroactive steroids exert important protection against neurodegeneration. Among the different neuroactive steroids considered so far, PROG and oestrogens seem to represent interesting candidates for therapeutic strategies in the case of several neurodegenerative diseases like for instance Alzheimer, Parkinson, multiple sclerosis, or other diseases (47, 48). In particular, PROG, as demonstrated in several experimental models of neurodegeneration, exerts an important role on neuroinflammation (49). As reported in this issue (50), PROG restored the expression of neuronal markers, decreased the activity of nitric oxide synthase and enhanced complex I respiratory activity and Mn-superoxide dismutase type 2 in an experimental model of motoneurone degeneration, such as the Wobbler mouse (51). Long-term treatment with PROG increased muscle strength, biceps weight and survival. Indeed, as demonstrated in other experimental models of multiple sclerosis (52), PROG blocked proinflammatory mediators, reactive microglial cells and attenuated clinical signs of EAE in experimental autoimmune encephalomyelitis induced in mice (50).

This neuroactive steroid has been tested with success also in another pathological event, the stroke (53, 54), but the majority of pre-clinical studies have focused on using young, healthy adult animals. In terms of cerebral stroke, males and postmenopausal females, represent the groups at highest risk of cerebral stroke and these categories can be modelled using either aged or ovariectomized female animals. In this special issue, Wong et al. (55) discuss the importance of conducting experimental studies in aged animals compared to young, healthy animals. The studies that have been conducted to date examining the neuroprotective potential of PROG in aged animals provide further support to a therapeutic option based on PROG following ischaemic stroke.

Oestrogen also is effective on stroke (56). Interestingly, several evidences indicate that oestrogen acts in concert with growth factors to initiate neuroprotection (57). Oestrogen and insulin like growth factor (IGF)-1 act cooperatively to influence cell survival and combined steroid hormone/growth factor interaction has been well documented in the context of neurones, astrocytes and microglia.

The importance of glial cells in the synthesis of neuroactive steroids is not limited to the mammalian brain, also in other vertebrates, specifically in birds, glial cells are playing important roles. Oestrogens, for example, have profound effects on avian neuroanatomy and neurophysiology throughout life, and importantly, are synthesized at high levels within neurones of the avian brain

(58-62). However, as in mammals, disruption of the neuropil by multiple forms of perturbation may induce aromatase expression in astrocytes and radial glia (63). In this special issue Saldanha et al. (64), discuss the mechanisms underlying the induction of aromatase expression in glial cells in the songbird brain, the emerging interactions between the neuroendocrine and neuroimmune systems with respect to brain injury, the influence of glial aromatization on neuroplasticity, and the sex differences in the induction of glial aromatase in the zebra finch.

Rapid regulation of behaviour

The regulation of many steroid-dependent behaviours (such as reproductive behaviour), has been considered, for a long time, a typical physiological activity dependent on the classic genomic action of gonadal hormones linked to cytoplasmic or nuclear receptors. Nucleus-initiated events control a wide array of physiological and behavioural responses, covering long periods (several hours, days or the entire life in the case of organizational effects). However, many data have been accumulated showing that steroids can rapidly regulate these behaviours (65).

In this special issue we collected reviews covering some important examples of the rapid action of steroids to regulate behaviours. The Japanese quail is an interesting model to study the regulation of male sexual behaviour. Cornil et al. (66) discuss data indicating that 17β-oestradiol, or its membrane impermeable analogues, acutely enhances measures of male sexual motivation but does not affect copulatory behaviour. These effects depend on the activation of membrane-initiated events and local oestrogen production. The regulation of brain oestrogen synthesis through post-translational modifications of the enzyme aromatase, occurring in vivo following variations in the social and environment context, provide a mechanism of acute regulation of local oestrogen availability. These distinct modes of oestrogenic action (membrane- vs. nucleus-initiated) acting in different time frames (short- vs. long-term) interact to control different components (motivation vs. performance) of the same behavioural response and improve reproductive fitness.

Remage-Healey et al. (67) review several recent findings from studies of songbirds showing how the identified neural circuits that govern auditory processing and sensorimotor integration are modulated by the local and acute production of oestrogens. Studies using in vivo microdialysis demonstrate that oestrogens fluctuate in the auditory cortex when songbirds are hearing song and interacting with conspecifics. In addition, oestrogens rapidly boost the auditory-evoked activity of neurones in the same auditory cortical region, enhancing auditory processing. Local pharmacological blockade of oestrogen signalling in this region impairs auditory neuronal responsiveness as well as behavioural song preferences. It is likely that the receptor for this rapid

action of oestradiol is in neuronal membranes, and that traditional nuclear oestrogen receptor agonists do not mimic these rapid actions.

In mammals, the rapid effects of oestradiol and ER agonists have been tested on both social and non-social learning paradigms (68). Social learning refers to a paradigm in which an animal acquires information and modifies its behaviour based on observation of another animal, commonly studied using the social transmission of food preferences paradigm. Oestradiol rapidly improves social learning on this task but no ER agonist has definitive, comparable improving effects. Conversely, ERα and GPER1 play a larger role than ERβ in the rapid improving effect of oestrogens on non-social learning, including social and object recognition. In addition, oestrogens also rapidly improve memory consolidation in a variety of learning paradigms: object recognition, object placement, inhibitory avoidance, and the Morris water maze, indicating that oestradiol affects the consolidation of multiple types of memory, probably through an action at the level of the dorsal hippocampus where selective activation of all the three ERs show rapid improving effects on spatial learning comparable to oestradiol. Future research should further elucidate the roles of ERs in the enhancing effects of oestradiol on learning and memory, and determine where in the brain oestradiol acts to affect social and non-social learning.

PROG have actions in the midbrain ventral tegmental area (VTA) to mediate motivated behaviours, such as those involved in reproductive processes, among female rodents (69). In the VTA, formation and actions of one metabolite, such as 5α-pregnan-3α-ol-20-one (3α,5α-THP; 3α,5α-THP), are necessary and sufficient to facilitate sexual responding (measured by lordosis) of female rodents. Although 3α,5α-THP can be produced following metabolism of ovarian PROG, it is also a neurosteroid produced de novo in brain regions, such as the VTA. There can be dynamic changes in 3α,5α-THP production associated with behavioural experience, such as mating. Pregnane Xenobiotic Receptor (PXR) may be a novel factor involved in 3α,5α-THP metabolism in the VTA (as well as a direct target of 3α,5α-THP) and manipulating PXR in this region reduces 3α,5α-THP synthesis and alters lordosis as well as affective and social behaviours. In addition, recent studies have focused on the role of membrane PROG receptors (mPRs). Two of the common forms of these receptors (mPRα and mPRβ) are present in female rats. Expression of mPRα was observed in peripheral tissues and brain areas, including hypothalamus and midbrain. Expression of mPRβ was only observed in brain tissues and was abundant in the midbrain and hypothalamus. Studies to date suggest that mPRβ may be an important target of progestogens in the VTA for lordosis. Together, these studies demonstrate that PXR is involved in production of 3α,5α-THP in the midbrain VTA. Moreover, mPRs may be a target for PROG and its derivatives' actions in the VTA for lordosis.

Together, these and other emergent studies provide support for rapid, brain-derived steroid signalling in regulating sensorimotor integration, learning and perception.

Age-related steroid hormone changes

As with many other functions of the body, the neuroendocrine system also shows important alterations during aging, with a reduction in the production of peripheral hormones such as gonadal and adrenal hormones (70, 71).

Rhesus macaques (Macaca mulatta) are large, long-lived diurnal primates, and represent a pragmatic animal model in which to examine the mechanisms by which these steroidal changes contribute to perturbed sleep-wake cycles and cognitive decline in the elderly (72). In these animals the circulating levels of dehydroepiandrosterone (DHEA) sulphate, as well as oestradiol and testosterone, decline markedly in old monkeys. Furthermore, genes associated with the conversion of DHEA to oestradiol and testosterone (e.g., 3βHSD, 17βHSD, and aromatase) are highly expressed in brain areas associated with cognition and behaviour, including the hippocampus, prefrontal cortex, and amygdala. Taken together, these findings suggest that administration of supplementary DHEA in the elderly may have therapeutic potential for cognitive and behavioural disorders, but with fewer negative side effects outside of the central nervous system. This was tested through a novel steroid supplementation paradigm involving oral administration of DHEA and testosterone at physiologically relevant times of the day to mimic the circadian hormone patterns observed in young adults. This steroid supplementation paradigm could reverse age-associated disorders, including perturbed sleep-wake cycles and cognitive decline as well as impaired immune responses.

Animal and in vitro studies have shown that sex steroids can have protective effects on the ageing brain (73, 74). In humans the data are sparse and in some way contrasting. In this issue, Hogervorst (75) report the results of a literature review using the following keywords: testosterone, (o)estradiol/(o)estrogens, menopaus(*) AND memory or cognitive. In all studies participants were usually over 65 years of age, and were not using sex hormone treatment. In general, sex steroid changes associated with natural menopause in women have very few global effects on cognitive change and oestrogen treatment studies seem to show only time limited positive effects in postmenopausal women; only for a couple of months, regardless of age. For older women (60+ and/or those who are at risk for dementia through lifestyle, morbidity and genetic risk factors) available data may suggest that oestrogens should not be prescribed for longer than 6 months to maintain cognition or prevent dementia as initial positive effects may reverse after that time. A number of large well controlled treatment studies and several observational studies have shown that

high oestrogen levels in older women were related to worse cognitive function. The role of oestrogens in older men in cognitive decline and dementia risk is not clear, but data suggest that the same may apply. Several observational studies on the other hand found negative associations between high testosterone levels and worse cognitive function in older women. Age, health status, duration of treatment and gender may thus modify the effects of longer term elevated sex steroid levels on brain function.

Altogether, reviews collected in this special issue provide new insights on the mechanisms of action (genomic, non-genomic and epigenetic) and on the roles played by neuroactive steroids as key physiological regulators of central and peripheral nervous functions (regulation of synaptic connectivity, neurogenesis, rapid regulation of behavior, aging). In addition, these molecules are involved in phenomena such as mental diseases or neuroprotection and therefore they might also represent an interesting therapeutic strategy for neurodegenerative events.

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