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REVIEW ARTICLE

Actin Cytoskeleton Remodelling by Sex Steroids in Neurones

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Cell morphology and its interaction with the extracellular environment are integrated processes involving a number of intracellular controllers orchestrating cytoskeletal proteins and their interaction with the cell membrane and anchorage proteins. Sex steroids are effective regulators of cell morphology and tissue organisation, and recent evidence indicates that this is obtained through the regulation of the actin cytoskeleton. Intriguingly, many of these regulatory actions related to cell morphology are achieved through the rapid, nonclassical signalling of sex steroid receptors to kinase cascades, independently from nuclear alteration of gene expression or protein synthesis. The identification of the mechanistic basis for these rapid actions on cell cytoskeleton has special relevance for the characterisation of the effects of sex steroids under physiological conditions, such as for the development of neurone/neurone interconnections and dendritic spine density. This is considered to be critical for gender-specific differences in brain function and dysfunction. Recent advancements in the characterisation of the molecular basis of the extranuclear signalling of sex steroids help to clarify the role of oestrogen and progesterone in the brain, and may turn out to be of relevance for clinical purposes. This review highlights the regulatory effects of oestrogens and progesterone on actin cytoskeleton and neurone morphology, as well as recent progresses in the characterisation of these mechanisms, providing insights and working hypotheses on possible clinical applications for the modulation of these pathways in the central nervous system.

Key words: sex steroids, moesin, focal adhesion kinase, WASP, dendritic spine formation.

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Sex steroid hormones are fundamental regulators of cell growth, proliferation and migration. Oestrogens direct the development of tissues and organs (1) and orchestrate cell growth and proliferation during adult life (2). Cells respond to the environment through the expression of transmembrane receptors that sense extracellular stimuli and activate an elaborate network of intracellular signalling molecules. Given the large number of different signalling molecules and the complex relationships between them, a major challenge is to understand how specific signals generate unique responses. The architecture of signalling networks plays an important role in achieving signalling specificity (3).

Cell morphology and its interaction with the extracellular environment requires a number of regulators orchestrating the different cytoskeletal components and their interactions with the cell membrane and anchorage proteins (4). Recent findings indicate that the extranuclear signalling of oestrogen is a fundamental regulator of cell morphology in diverse cellular lines, including neurones (5,6), and that many of these actions are played via rapid signalling to the actin cytoskeleton achieved via actin recruitment (7–11).

The present review describes and discusses the current understanding of the regulatory actions of sex steroids on the cytoskeleton of neurones, with the aim of highlighting how these hormones influence the turnover of dendritic spines.

Sex steroids and the brain

The brain is an important target of sex steroids that play multiple regulatory roles. These hormones are powerful mediators of dynamic brain differentiation into a male or female phenotype. This phenomenon has been originally described in studies on neuronal cell migration in the developing pre-optic area/anterior hypothalamus (12–15). The effects of sex hormones turn into differences in neuronal cell number, glial complexity, neurochemical expression

and synaptic connectivity (16–18) and are related to oestrogens in the brain. Oestrogen acts as a neurotrophin regulating cell death, neurogenesis, neurotransmitter plasticity (19), neuronal migration and synaptogenesis (20), and modulates brain ontogeny by influencing neuronal cell movement (21,22). Our knowledge on how oestrogen interferes with mammalian brain functions and development has recently broadened. In the adult brain, oestrogen is not only involved in the neuroendocrine feedback regulation at the hypothalamic and pituitary level, but also in the control of motor and cognitive functions. After the demonstration that the oestrogen-synthesising enzyme aromatase and both oestrogen receptors (ER) α and β are expressed in many brain areas, it was realised that oestrogen modulates neuronal differentiation, notably by influencing cell migration, survival and death, as well as the synaptic plasticity of neurones. These effects were initially seen in the classical target area for oestrogen (i.e. the hypothalamus), although later studies revealed actions in other regions.

Oestrogens do not act solely on the early stages of neuronal development, but also on mature neurones for the maintenance and reorganisation of dendritic structures. Dendrites are sites where neurones receive, process and integrate inputs from their presynaptic partners. Both the shape of dendritic trees and the density of their spines undergo significant changes during the development and life of a neurone. Dendritic spines are formed out of actin microfilaments. These structures are instrumental for the development of neuronal circuits under the regulation of several extracellular stimuli (23). Oestrogen is involved in the formation of dendritic spines during development and in their plasticity at mature synapses, controlling actin filaments (15,19).

One of the most intriguing actions that these hormones perform is the control of brain plasticity, particularly through neuronal/glial remodelling, which is critical for memory, learning and cognition (16,17,24,25). At the basis of brain plasticity is the ability of neurones and glial cells to remodel their mutual connections, which requires major changes in cell morphology (26,27). These morphological modifications depend on the generation of dynamic structural changes of the actin cytoskeleton, as well as on the development of protrusive membrane structures, such as lamellipodia and filopodia, that are involved in the generation of cell–cell interconnections (4).

Clinical studies also suggest that lack of oestrogens, such as in women after menopause, may be related to the progression of brain degenerative diseases, such as Alzheimer's disease or Parkinson's disease (17,28–30), and the hypothesis that oestrogen administration to postmenopausal women might decrease the progression of these conditions is enduring (31).

Sex steroids and synaptic plasticity

The most remarkable property of synapses is not that they convey information from one neurone to another but that they can readily alter the efficiency with which they do this. This property, known as synaptic plasticity, enables us to store and use vast amounts of information in the form of learnt behaviours and conscious memories (32).

The discovery of oestradiol-dependent spine generation and the formation of excitatory synapses on neurones in the adult hippocampus (33,34) has shed light on the role of oestrogen in structural plasticity. This indicated that, beyond controlling dendritic spine morphology and function, oestrogen also regulated the *de novo* formation of these structures. This has led to the identification of oestrogen as an important factor affecting the excitability of hippocampal network (35). Additional evidence for an important role of oestradiol in the hippocampus comes from behavioural experiments. Intra-hippocampal oestradiol administration improves spatial memory (36). Interestingly, GABAergic interneurones in the hippocampus express ERs, and possibly mediate oestradiol actions on the local circuits (37). However, although the actions of oestradiol on glutamatergic system have been extensively described (35), its role in regulating the GABAergic system is less understood. To date, few studies have addressed the impact of oestradiol on GABAergic transmission in the adult hippocampus, and even less is known about the role of oestradiol during development.

Oestradiol and $GABA_A$ receptor function

Oestradiol alters hippocampal function via classical nuclear and extranuclear actions (35,38). For example, in the CA1 region, oestradiol induces neurone depolarisation and spontaneous firing (39). Moreover, oestradiol increases the amplitude of the dendritic excitatory postsynaptic potential in various regions of the hippocampus (40) and is important for long-term potentiation (40), as well as long-term depression (41). Such enhancement of neuronal excitability and facilitation of synaptic plasticity could partly be a result of the suppression of GABAergic drive in these cells through a direct modulation of $GABA_A$ receptors. These receptors possess binding sites for neurosteroids (42,43) and are readily modulated by many of them, either positively or negatively (44). For this reason, a direct effect of oestradiol on $GABA_A$ receptors has been tested in several studies, which show that oestrogen is a potent modulator of GABAergic transmission in the hippocampus. At the current stage of research, it is assumed that, during development, when dendritic growth, spinogenesis and synaptogenesis are particularly intense in the rodent brains (45), oestradiol would increase the impact of excitatory GABAergic transmission to hippocampal neurones. In the adult brain instead, oestradiol appears to suppress GABAergic inhibitory transmission, thus favouring increased excitability in CA1 and facilitating the formation of new dendritic spines and local circuit connectivity. The effects of oestradiol on different elements of the GABAergic system are complex and the molecular basis underlying these effects remains to be elucidated. Altogether, it appears that oestradiol should no longer be recognised solely as a steroid hormone but also as an important signalling molecule in the hippocampus.

Sex steroids and the actin cytoskeleton

Three different types of filaments form the cytoskeleton: actin, microtubules and intermediate filaments. These cytoskeletal complexes interact together as a dynamic network contributing to cell

functions, such as structural integrity, shape, division and cell motility (46,47).

Sex steroid hormones are fundamental modulators of cell morphology and motility in different cell types (7–11,48–50). Through binding their receptors, sex hormones modulate rapid intracellular cascades such as G proteins, tyrosine kinases, c-Src, small GTPases and protein kinase pathways, leading to the activation of actin remodelling and cell movement. In this context, we recently described how physiological amounts of oestradiol and progesterone lead to a rapid remodelling of the actin cytoskeleton with a loss of stress fibres (7–10,48,50,51). This cytoskeletal rearrangement, associated with the development of specialised cell membrane structures, such as ruffles and pseudopodia, is obtained through the activation of the actin-regulatory protein, moesin (7,8,48,50,51). A number of studies indicate the fundamental contribution of the ezrin–radixin–moesin (ERM) family of proteins to the cytoskeletal processes responsible for many vital cellular functions such as cell motility (8,52).

Moesin and actin remodelling

Moesin, a member of the ERM family, is an actin-binding protein that plays an important role in cell motility by linking the actin cytoskeleton to a variety of membrane-anchoring proteins (53,54). Under quiescent conditions, moesin exists in an auto-inhibited conformation, and its phosphorylation on Thr^{558} within the C-terminal actin binding domain by the Rho-associated kinase (ROCK) results in a conformational change and in the association with the scaffold protein, ERM-binding protein 50 (EBP50), on the NH_2-terminal end of moesin and with F-actin on the COOH-terminal end of moesin to mediate the linkage of microfilaments to membranes in cell surface microvilli (48,55). Sex steroids activate moesin in neurones through a rapid, extranuclear signalling cascade originated by the interaction of ERα/progesterone receptor A. with the G protein Gα13. This process leads to the recruitment of RhoA and Rho associated kinase, ROCK-2, as well as moesin activation. This pathway leads to the formation of membrane ruffles and pseudopodia, which interact with the extracellular matrix and with nearby cells (7,8,48,50,51). The control of cell morphology may be relevant for the actions of oestrogen in the central nervous system, where morphological changes in neurone/neurone interconnections and dendritic spine density ensue that are related to cyclical changes in oestrogen levels (7,56,57).

Focal adhesion kinase (FAK) and focal adhesion complexes

Once actin is remodelled, focal adhesions (FAs) are formed (58). FAs are composed of a group of structural proteins and signalling molecules, including the tyrosine kinases c-Src and FAK, integrin proteins, actin-binding proteins such as vinculin, and adaptor proteins such as paxillin (59). FAK is the key enzyme regulating the formation of FAs. Under the stimulation of multiple factors, FAK is activated via the phosphorylation at Tyr^{397} and begins to partner with cell-membrane integrins with the assistance of other proteins such as p130CAS, paxillin and vinculin, resulting in FA formation (60).

Sex steroids modulate the activity and expression of FAK (9,11,61,62). Oestrogen-induced phosphorylation of FAK (10) turns into a dual action on cells: an initial increase in cell spreading and focal contact formation, followed by a later gradual disruption of these structures (63,64).

Oestrogen activates FAK via phosphorylation on Tyr^{397}, triggered through a c-Src/phosphoinositide 3-kinase (PI3K)-dependent cascade. In addition, oestrogen controls FAK by regulating Cdc42 and its effector N-WASP (10). N-WASP is a scaffold protein that links upstream signals to the activation of the Arp2/3 complex, leading to a burst of actin polymerisation (65–67). Sex steroid regulation of FAK is emerging as a relevant process in neurones, where these hormones increase the tyrosine phosphorylation of FAK, increasing the development of FAs and dendritic spines.

WAVE1 and formation of dendritic spines

A large amount of literature supports a key role of the Rho family of GTPases (Rho, Rac1 and cdc42) in regulating polymerisation and the turnover of the actin cytoskeleton (68) through the activation of different downstream effectors such as c-Src kinase, PI3K, phosphatidylinositol 3-phosphatase and the WASP family of proteins. Signalling originating from small GTPases to the WASP family members WASP, N-WASP and WAVE promotes the formation and branching of actin filaments in different settings (69–71). WAVE1 is well established as a key player for actin branching and spine formation in neurones (72) (Table 1). In addition, WAVE1 is important for axon elongation (73). From a mechanistic standpoint, Kim *et al.* (74) recently reported that, during spine formation, cyclin-dependent kinase-5 phosphorylates the proline-rich region of WAVE1. These phosphorylation sites are not conserved in WAVE2 and WAVE3 (74). A loss of WAVE1 function *in vivo* or in cultured neurones results in a decrease in mature dendritic spines (74) and WAVE1 knockout mice exhibit deficits in learning and memory (75). N-WASP has also been implicated with processes related to spine formation in neurones (70,76,77).

Regulatory actions of sex steroids via moesin, FAK and WAVE1

Oestrogen regulates brain function through modifications of axons and dendrites, influencing brain plasticity and, possibly as a result of this, cognitive and behavioural functions. The classical mode of oestrogen action is through the activation of ERα and ERβ (78). However, new signalling mechanisms based on interaction of oestrogen receptors with scaffolds and signalling intermediates have been recently identified. Such actions are broadly identified as extranuclear effects of ERs to indicate the fact that they mostly occur at the cell membrane or within the cytoplasm. Within this wide set of nonconventional signalling actions of ERs is the interaction with G proteins at the cell membrane, which appears to be key for the neuronal regulatory actions of oestrogens.

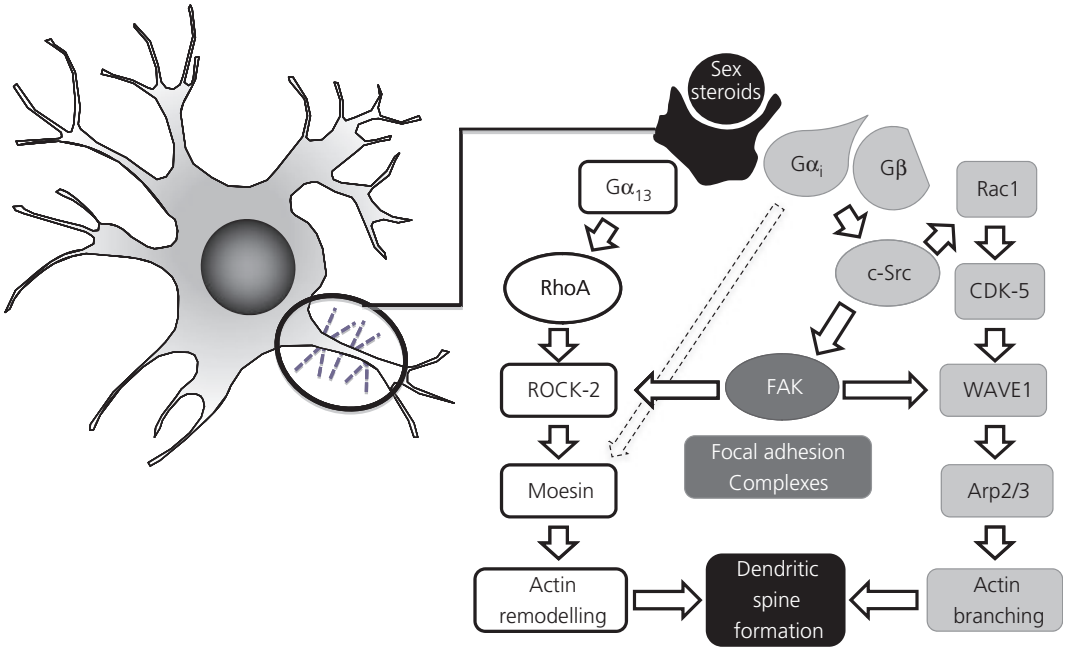

Fig. 1. Moesin, focal adhesion kinase (FAK) and WAVE1 signalling. Binding of sex steroids to the specific membrane receptors induces the phosphorylation of the moesin, FAK and WAVE1 cascade. The steroids receptors use the actin controllers moesin, FAK and WAVE1 to induce a rapid cytoskeletal remodelling supporting the formation of dendritic spines. In the presence of sex steroids, such as oestrogen or progesterone, the steroids receptors (oestrogen receptors or progesterone receptors) recruit a $G\alpha_i$-$G\beta$/c-Src/Rac1/cyclin-dependent kinase-5/WAVE1 cascade. This turns into WAVE1 phosphorylation and translocation to the sites where the actin cytoskeleton and the cell membrane are actively remodelled, as well as in the parallel membrane localisation of the Arp2/3 protein complex, which is responsible for the extension and branching of actin filaments and thus for spine dendrite formation. The parallel recruitment of the ERM (ezrin–radixin–moesin) actin-binding protein, moesin, via a $G\alpha_{13}$/RhoA/Rho-associated kinase-2 pathway contributes to the remodelling of the cytoskeleton and is required for oestrogen-induced dendritic spine formation. Moesin activation is finely modulated by Gαi protein-dependent intracellular pathways. FAK acts as a central pivot, regulating the moesin and WAVE-1 signalling pathway. CDK, cyclin-dependent kinase; FAK, focal adhesion kinase; ROCK, Rho-associated kinase.

Interaction of ERα with $G\alpha_i$ and $G\beta\gamma$ has been recently reported in endothelial, breast cancer and neuronal cells (7,10,79). During binding of ERα with $G\beta_1$, the interaction of $G\alpha_{i1}$ and $G\beta_1$ is decreased. Consistent with our previous results obtained in endothelial and breast cancer cells (8,50,51), ERα also interacts with $G\alpha_{13}$ in the presence of 17β-oestradiol in neurones. Through the activation of $G\alpha_{13}$ at the cell membrane, ERα has a privileged access to the pathways that signal to the actin cytoskeleton. Indeed, $G\alpha_{13}$ is responsible for the activation of the RhoA/ROCK-2/moesin cascade pathway (80), and we have demonstrated that this connection is also functional in neuronal cells, providing the first evidence of a regulatory effect of oestrogens on this important signalling pathway and providing the indication that the activation of this pathway is implicated in oestrogen-induced dendritic spine turnover (7). In addition, our preliminary results demonstrate that FAK also regulates ROCK-2/moesin and WAVE1 in cortical neuronal cells, via $G\alpha_{13}$ and $G\alpha_i/G_{\beta 1}$, respectively, contributing to dendritic spine remodelling (AM Sanchez and T Simoncini, unpublished). Thus, oestrogen-dependent actin remodelling is made possible in neurones by extranuclear signalling to actin controllers. This first identified set of actions is relevant for the modulation of actin concentration at the cell membrane at sites where dendritic spines are formed (7).

In addition, when oestradiol hits ERα, a rapid signalling cascade to cyclin-dependent kinase-5 is also recruited in neurones (7). This leads to the localisation of WAVE1 at the cell membrane, where it promotes the branching of actin filaments, creating the structural platform necessary to enact the mechanical force for neurite extension and growth-cone translocation.

Thus, a number of actions of oestrogens in neurones emerge to be exerted via rapid extranuclear signalling pathways (81–85), which qualify oestrogens as flexible modulators of neuronal function. Whether such rapid modifications of the structure of neuronal membrane and of the number of dendritic spines can be related to improved or preserved cognition or memory is difficult to say. However, it is well-established that brain function undergoes rapid modifications with changing oestrogen concentrations, as shown by endocrine manipulations in animals or by the clinical alterations (e.g. vasomotor symptoms, headache) observed at times of rapid oestrogen withdrawal, such as after delivery, with surgical menopause, or, to some extent, during the pill-free intervals in oral contraceptive users. These observations support the idea that sex steroids might be effective fast regulators of the central nervous system (7). Similarly, the increased prevalence of cognitive impairment, as well as some degenerative disorders, observed under conditions of chronic oestrogen withdrawal might possibly be the

result of a less efficient control of actin and membrane remodelling in neurones. Thus, the identification of the signalling cascades through which oestrogen controls neuronal cell morphology may be useful for achieving a better understanding and the possible development of new therapeutic strategies with the aim of treating conditions such Alzheimer's disease or Parkinson's disease.

Conclusions

Neuronal membrane morphology and the turnover of dendritic spines are complex and integrated processes that are orchestrated by multiple factors. These processes are key for the control of single cells, as much as for brain organisation. A deeper understanding of the underlying events that allow neurones to modify their cytoskeleton and membrane and to establish interconnections may be essential to gain insight into several degenerative brain disorders. Recently, new signalling mechanisms of sex steroids have been identified that are of relevance in this area. Such rapid signalling actions do not require modulation of gene expression; thus, they can trigger fast effects, such as rapid neuronal membrane remodelling or dendritic spine turnover. These extranuclear signalling avenues may represent a natural way of using sex steroids as flexible modulators of neuronal function, allowing dynamic changes in response to surrounding stimuli. Further investigations in this area will lead to better understanding of the mechanistic basis through which sex steroids dynamically control brain physiology and could help with the engineering of newer and more selective pharmacological tools for endocrine therapies against important neurological diseases.

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