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Caveolin Proteins and Estrogen Signaling in the Brain

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Abstract

Best described outside the nervous system, caveolins are structural proteins that form caveolae, functional microdomains at the plasma membrane that cluster related signaling molecules. Caveolin associated proteins include G protein coupled receptors and G proteins, receptor tyrosine kinases, as well as protein kinases, ion channels and various other signaling enzymes. Not surprisingly, a wide array of biological disorders are thought to be rooted in caveolin dysfunction. In addition, caveolins also traffic and cluster estrogen receptors to caveolae. Interactions between the estrogen receptors ER α and ER β with caveolins appear critical in many non-neuronal cell types, e.g. disruption of normal function may underlie many forms of breast cancer. Recent findings suggest caveolins may also play an essential role in membrane estrogen receptor function in the nervous system. Not only are they expressed in neurons and glia, but different caveolin isoforms also appear necessary to generate distinct functional signaling complexes. With membrane estrogen receptors responsible for the efficient activation of a multitude of intracellular signaling pathways, which in turn influence a wide variety of nervous system functions, caveolin proteins are poised to act as the central coordinators of these processes.

Keywords

estradiol; estrogen receptor; mGluR; lordosis; membrane receptor; rapid actions; caveolae; flotillin

Introduction

The role of estradiol on brain function and its consequent influence upon behavior has been studied for over 60 years. Once thought to be the sole mechanism of estrogen action, estradiol binding to the intracellular estrogen receptors ER α and ER β acts to affect gene expression and protein synthesis [1,2]. This classical mechanism of estrogen action, i.e. stimulation of steroid regulated transcription factors, plays a crucial role in brain functions involving sexual development, sexual maturation, and the expression of sexual receptivity [3–8]. However, in addition to its actions on intracellular estrogen receptors, estradiol can also affect a variety of cellular processes through stimulation of surface membrane receptors. Not only have these rapid acting effects of estrogens been shown to play a role in sex behavior, but also in brain and spinal cord regions involved with, but not limited to, learning and memory, motor function, nociception and drug addiction [9–15]. The majority of these reported membrane-initiated actions of estradiol in the nervous system appear dependent on a subpopulation of ER α and/or ER β that are localized to the membrane surface [16–18], but see [19–21]. To this end, several

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biological questions remain unanswered: (1) how are intracellular estrogen receptors trafficked to the membrane, and (2) once in the membrane, how are estrogen receptors targeted to the appropriate signaling complexes for the precise activation of specific intracellular signaling cascades? This review will focus on the putative role caveolin proteins may play in mediating these two processes.

Caveolins: Important for the Trafficking and Clustering of Membrane-Associated Signaling Proteins

Caveolin proteins are the fundamental components of caveolae, which form distinct structural and functional microdomains in many cell types [22,23]. Caveolae when associated with the plasma membrane exhibit invaginations described as omega- or cave-like structures that cluster functionally related membrane-associated proteins [24]. Caveolae were first recognized in epithelial cells by electron micrograph techniques over 50 years ago [25]. There are three known caveolin proteins, caveolin 1 (CAV1, with splice variants α and β), caveolin 2 (CAV2), and caveolin 3 (CAV3) [26–28]. CAV1 and CAV2 have overlapping expression patterns in a variety of cell types including, neurons and glia [29,30], endothelial [31], and epithelial cells [32]. Disruption of CAV2 expression does not affect caveolae formation *in vivo* [33], inasmuch it is hypothesized that CAV2 only forms caveolae as hetero-oligomers with CAV1, and not in isolation [34]. In comparison, knockout of either CAV1 or CAV3 results in a loss of caveolae formations in the specific cell type for which they are expressed [35,36]. Notably, it was originally believed that expression of CAV3 was restricted to skeletal and smooth muscle cells [26,37–39]. We now know this not to be the case, as expression of CAV3 is more widespread, including its presence in nervous tissue [30].

The cavernous structure of caveolae supports a functional domain where various proteins can cluster and associate for efficient activation of discrete signaling pathways. As such, caveolae are often described as signaling regulators that serve to orchestrate the interaction of receptors and signaling molecules, modulating transmembrane signaling in a rapid and specific manner [40,41]. This is thought to occur via direct protein-protein interactions between caveolins and signaling components found at the plasma membrane. In various cell types, caveolin proteins have been shown to be associated with G protein-coupled receptors, G protein subunits, tyrosine kinase receptors, various intracellular kinases, voltage-gated ion channels, ion pumps, and various second messenger molecules [42–45].

In addition to its role of clustering related signaling molecules, caveolin proteins also play a role in the trafficking of various receptors to the membrane. Surface receptors in which trafficking to the membrane has been reported to be dependent on caveolin function include, but are not limited to, the D1 dopamine receptor [46], M1 muscarinic receptor [47], angiotensin II type 1 receptor [48], and glucagon-like peptide 1 receptor [49]. Notably, caveolins also play a role in receptor endocytosis [47,50–54] providing an additional regulatory mechanism to modulate cell signaling. Caveolin-dependent endocytosis is a mechanism involving internalization of membrane components within caveolae resulting in the diminution of function. Caveolin-dependent sequestration of receptors can be thought of as a means to negatively modulate signaling via the storage of signaling complexes within the cell.

With their importance for the trafficking and clustering of various signaling transduction molecules, it stands to reason caveolins play critical roles in many cellular processes. Indeed, alteration/disruption of caveolin expression has been implicated, breast cancer [55–57], vascular abnormalities [58–60], pulmonary malfunction [33], and muscle disease [35,37].

Caveolin Proteins and Estrogen Receptors

A functional link between caveolin proteins and membrane estrogen receptors was first reported in non-neuronal cells approximately ten years ago. Initial studies identified ER α -dependent nitric oxide (NO) production required ER α to be associated with caveolae that contained endothelial nitric oxide synthase (eNOS) [61,62]. Concurrent with these studies, CAV1 was shown to potentiate classical ER α -mediated gene expression [63], a process dependent upon the direct interaction between ER α and CAV1 [63]. Thus, the signaling of estradiol via classical and novel actions appears to be intertwined, with activation of each influenced by the other. Furthermore, estradiol appears to directly influence ER α interaction with CAV1 [63,64], and estrogens modulate expression of caveolins [65], providing additional levels of regulation. Of note, ER β can also associate with caveolae, and they too are functionally coupled to enzymatic signaling machinery via this process. For example, ER β within eNOS containing caveolae affords estradiol/ER β regulation of NO production [66].

A necessary step in estrogen receptor localization to caveolae is the palmitoylation of the receptor. Although research has focused primarily on palmitoylation of ER α and its variants [64,67–69], the same mechanism for caveolae association has been described for ER β [64, 70]. Specifically, palmitoylation of human ER α at cysteine 447 (mouse 451) is essential for receptor interaction with CAV1 and its subsequent localization to the plasma membrane. In CHO cells, mutation of cysteine 447 to an alanine results in a loss of membrane ER α . In addition, the physical interaction between ER α and CAV1 is abolished, and membrane estrogen effects are eliminated [64,70]. It is through regulation of palmitoylation that estradiol appears to affect the interaction between ER α and CAV1. In particular, stimulation of HELA cells with estradiol reduces ER α binding to CAV1 with a corresponding reduction in membrane ER α [64,70]. It is significant to note that it is the palmitoylation of a single amino acid that regulates the trafficking of ER α , as this residue is well conserved across species. Similarly, a single cysteine residue in ER β (mouse 418) appears to be the critical amino acid for palmitoylation and trafficking to the membrane. The question to which enzyme(s) is/are responsible for palmitoylation of the estrogen receptors remains unanswered. As 23 separate palmitoyl acyl transferases are known to exist and appear to be ubiquitously expressed across various tissues [71], this determination may not be rapidly forthcoming.

A second amino acid within the ligand-binding domain of ER α is also necessary for interaction of ER α with CAV1. Mutation of mouse serine 522 to an alanine reduces ER α binding to CAV1 by ~60% in CHO cells and reduces membrane-localization of ER α by a similar percentage [72]. The S522A mutation also acts as a dominant-negative in relation to membrane estrogen receptor signaling [73]. Currently, the mechanism by which serine 522 facilitates ER α binding to CAV1 and caveolin-dependent trafficking of the receptor to the membrane remains unknown. Mutation of residues within the palmitoylation motif (ER α : F449A, IL456-7AA; ER β : Y416A, IL423-4AA), have also been shown to interfere with palmitoylation and membrane localization of mouse ER α and ER β .

The physiological relevance of caveolin proteins and estrogen receptors is best described in relationship to breast cancer. Both ER α and caveolin proteins have been implicated in breast cancer etiology, as alterations in the expression or function of either is prevalent in many forms of the disease. In addition, proliferation of estrogen receptor-positive breast cancer cells has been shown to be sensitive and facilitated by estrogen [74], which may be a result of elevated levels of estrogen receptors in breast cancer tissue [75] as these proliferative effects of estrogen can be reduced by treatment with anti-estrogens [76]. Of note is the finding that cell cycle progression in breast cancer cells has been shown to be mediated through mechanisms activated by membrane-localized estrogen receptors [77]. Mutations of CAV1 are found in ~35% of ER α -positive human breast cancer samples [78], and CAV1 RNA and protein levels are

reduced in many cases of human primary breast carcinomas [79]. Mechanistically, it has been theorized that these alterations in CAV1 function or expression contribute to the increased sensitivity of mammary tissue to estrogen by leading to an increase of ER α expression [78, 80]. Moreover, overexpression of CAV1 in mammary tissue protects against the tumorigenic phenotype that mammary tissue lacking CAV1 shows [81]. Collectively, these data indicate a possible role for both proteins in the biology of a subset of breast cancers that may result from a loss of caveolin-mediated modulation of estrogen receptor availability in breast cancer tissue. This provides additional support for a mechanism of membrane estrogen signaling that depends on interactions between caveolins and estrogen receptors.

In terms of caveolin proteins and nervous system function, until a decade ago, it was believed caveolin expression was limited to glial cells [82]. Thus, when first hypothesizing that caveolae-like structures may too be responsible for the localization of membrane estrogen receptors in neurons, a review article by Dominique Toran-Allerand discussed structures comprised of the caveolin-related, flotillin proteins [21]. Indeed, caveolar-like microdomains (CLMs) were termed as the neuronal counterpart to non-neuronal caveolae. More recently, however, various reports have demonstrated the expression of all three caveolin isoforms in neurons [29,30,83–86]. Data described below is consistent with reports from non-neuronal tissue and the initial descriptions in Dominique Toran-Allerand's review that caveolin proteins play an essential role in brain membrane estrogen receptor function.

Our experiments in hippocampal neurons delineated two distinct signaling pathways by which membrane estrogen receptors regulate cell function. The first pathway found estradiol to activate ER α , leading to stimulation of the metabotropic glutamate receptor mGluR1a. This in turn led to activation of Gq, PLC, IP3 and MAPK signaling, and eventually, phosphorylation of the transcription factor CREB. The second pathway was initiated by estradiol activation of either ER α or ER β . Under these conditions, stimulation of mGluR2/3 led to Gi/o signaling, with a subsequent decrease in L-type calcium channel currents due to inhibition of PKA. As such, L-type calcium channel-dependent CREB phosphorylation was attenuated [87]

In a follow-up report, we found CAV1 and CAV3 to be responsible for the segregation and functional compartmentalization of these two distinct signaling pathways. ER α activation of mGluR1a signaling was dependent on CAV1. Correspondingly, ER α /ER β activation of mGluR2/3 was dependent on CAV3 [73]. Presumably, CAV1 is responsible for the localization of ER α with mGluR1a and its downstream signaling partners. Conversely, CAV3 traffics and/or clusters either ER α or ER β with mGluR2/3 and its second messengers (Figure 1). These data are both consistent with and expand upon previous work. Previous studies have not only indicated CAV1 to localize with ER α , but also mGluR1a [88]. Similarly, L-type calcium channels cluster in CAV3-generated caveolae [89,90]. As such, we find several individual signaling molecules linked to caveolin proteins to which they were previously ascribed. In addition, distinct caveolae generated by different caveolin isoforms, responsible for the functional isolation of separate signaling pathways in the same cell illustrates the complexity by which neurons transmit information from the membrane surface.

The hypothesis that CAV1 and CAV3 play important roles in membrane estrogen receptor signaling expands to paradigms outside the hippocampus. mGluRs appear required for membrane estrogen receptor signaling in several other systems including neurons of the arcuate nucleus [91], striatal neurons [92], dorsal root ganglia [93] and hypothalamic astrocytes [94]. Thus far, it has been determined that caveolin proteins functionally link estrogen receptors with mGluRs in striatal neurons [92]; other regions await verification. Through influencing these additional regions, rapid estrogen receptor signaling has been suggested to modulate multiple cellular processes, such as motor control and drug addiction [12,95–97], sexual receptivity [91], the control of the estrous cycle [98], and nociception [14,99]. Furthermore, while we and

others hypothesize GPCRs to act as intermediaries between estrogen receptors and G proteins, others have postulated that estrogen receptors directly activate the G proteins [100]. This alternative hypothesis does not negate the possibility that caveolin proteins are essential for membrane estrogen receptor function. In fact, with caveolin proteins clustering specific G proteins, as well as GPCRs, their influence on membrane estrogen receptor signaling within the nervous system may be far reaching. Consistent with this idea, there are many parallels between caveolin and estrogen receptor function in brain. A wealth of information regarding caveolins and their influence on brain function has recently emerged. Topics include, but are not limited to, caveolin regulation of synaptic strength, motor control, and Alzheimer's disease [101–106]. These studies demonstrate that caveolins play critical roles in both neuronal and glial processes. Interestingly, each of these processes has also been demonstrated to be under the regulation of estrogens [9,107–110].

It is intriguing to consider that divergent functions in neurons are often localized to discrete functional regions [111,112]. The notion of subcellular compartmentalization of various signaling and effector proteins has been best demonstrated in neurons in regard to transsynaptic signaling. For example, PDZ domain-containing scaffolding proteins have been studied in great detail, elucidating their significance in the localization and clustering of proper neurotransmitter receptors, downstream second messengers, and cytoskeletal proteins [111]. Thus, the separation of Gq-coupled mGluR1a and Gi/o-coupled group II mGluRs, imparted by their localization within distinct caveolae, makes sense in terms of glutamatergic synaptic function. The spatial segregation of various estrogen receptor-dependent signaling pathways is necessitated by the isolation of separate glutamatergic receptors, but can it also be significant in terms of steroid-regulated processes? We now know that estrogens can be synthesized and released in various brain regions [113–116]. Based upon these data, it has been suggested that estrogens may play physiological roles similar to neurotransmitters [117]. Thus, whereas ovarian estradiol reaching the hippocampus would most likely activate both mGluR signaling pathways simultaneously, locally synthesized estradiol could potentially activate one pathway over another. Increased temporal and spatial control over estradiol-sensitive signaling by the synthesis and release of the steroid in brain is an exciting possibility that deserves future study.

Conclusions

Caveolae play an important role in the organization of signal transduction by shuttling signaling proteins and molecules to and from the membrane surface. At the membrane surface they may function by partitioning signaling components into particular combinations, increasing specificity without increasing complexity. Additionally, caveolae can rapidly modulate signaling through an endocytotic mechanism by which the caveolae internalize receptors or other signaling components so that they are non-functional. These endocytosed structures can remain near the membrane surface so that they can be made available for signaling by reintroduction to the membrane surface facilitated by the caveolae [46,118,119].

Membrane estrogen receptors can affect a variety of cellular processes both in and outside the nervous system. Caveolin proteins have been demonstrated to play critical roles in many of these systems, whereby they both traffic estrogen receptors as well as cluster the additional signaling machinery at the membrane surface into functional caveolae. While current research in brain has been limited to examination of estradiol regulation of CREB signaling, undoubtedly many other cellular processes dependent on membrane estrogen receptors will too require the proper functioning of caveolins.

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Figure 1.

Caveolin proteins are responsible for the isolation of functionally distinct signaling pathways in neurons. Schematic of caveolin 1 and caveolin 3 comprised caveolae in hippocampal neurons. Caveolin 1 is required for ER α activation of mGluR1a signaling, leading to MAPK-dependent CREB phosphorylation. In comparison, caveolin 3 mediates ER α or ER β activation of mGluR2/3 signaling, leading to a decrease in L-type calcium channel-dependent CREB phosphorylation.