Molecular and Cellular Endocrinology 290 (2008) 8–13

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Caveolin proteins and estrogen signaling in the brain

Jessie I. Luoma, Marissa I. Boulware, Paul G. Mermelstein*

Department of Neuroscience, University of Minnesota, 6-145 Jackson Hall, 321 Church Street S.E., Minneapolis, MN 55455, United States

ARTICLE INFO

Article history:
Received 3 April 2008
Accepted 5 April 2008

Keywords:
Estradiol
Estrogen receptor
mGluR
Lordosis
Membrane receptor
Rapid actions
Caveolae
Flotillin

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 traffic and cluster estrogen receptors to caveolae. Interactions between the estrogen receptors ERα and ERβ 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.

1. 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 ERα and ERβ acts to affect gene expression and protein synthesis (McKenna et al., 1999; McInerney et al., 1998). 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 (Odor, 1955; Gorski and Yanase, 1981; Young et al., 1941; Levine and Mullins, 1964; Feder and Whalen, 1965; Booth, 1977). 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 (McEwen and Alves, 1999; McEwen, 2002; Becker et al., 1982; Hampson and Kimura, 1988; Hampson, 1990; Riley et al., 1999; Brinton, 2001). The majority of these reported membrane-initiated actions of estradiol in the nervous system appear dependent on a subpopulation of ERα and/or ERβ that are localized to the membrane surface (McCarthy, 2004; Vasudevan and Pfaff, 2007a; Boulware and Mermelstein, 2005, but see Prossnitz et al., 2007; Ronnekleiv et al., 2007; Toran-Allerand, 2004). To this end, several 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.

1.1. 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 (Glenney and Soppet, 1992; Rothberg et al., 1992). Caveolae when associated with the plasma membrane exhibit invaginations described as omega- or cave-like structures that cluster functionally related membrane-associated proteins (Parton et al., 2006). Caveolae were first recognized in epithelial cells by electron micrograph techniques over 50 years ago (Yamada, 1955). There are three known caveolin proteins, caveolin 1 (CAV1, with splice variants α and β), caveolin 2 (CAV2), and caveolin 3 (CAV3) (Tang et al., 1996; Scherer et al., 1996; Glenney, 1992). CAV1 and CAV2 have overlapping expression patterns in a variety of cell types including, neurons and glia (Galbiati et al., 1998; Ikezu et al., 1998a),

* Corresponding author: Tel.: +1 612 624 8977; fax: +1 612 626 5009.
E-mail address: pmerm@umn.edu (P.G. Mermelstein).

0303-7207/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mce.2008.04.005

endothelial (Lisanti et al., 1994a), and epithelial cells (Vogel et al., 1998). Disruption of CAV2 expression does not affect caveolae formation *in vivo* (Razani et al., 2002a), inasmuch it is hypothesized that CAV2 only forms caveolae as hetero-oligomers with CAV1, and not in isolation (Scherer et al., 1997). 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 (Galbiati et al., 2001; Razani et al., 2002b). Notably, it was originally believed that expression of CAV3 was restricted to skeletal and smooth muscle cells (Tang et al., 1996; Song et al., 1996; Way and Parton, 1995; Chang et al., 1994). We now know this not to be the case, as expression of CAV3 is more widespread, including its presence in nervous tissue (Ikezu et al., 1998a).

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 (Lisanti et al., 1994b; Simons and Toomre, 2000). 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 (Anderson, 1993, 1998; Stefanova et al., 1991; Patel et al., 2008).

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 (Kong et al., 2007), M1 muscarinic receptor (Shmuel et al., 2007), angiotensin II type 1 receptor (Wyse et al., 2003), and glucagon-like peptide 1 receptor (Syme et al., 2006). Notably, caveolins also play a role in receptor endocytosis (Shmuel et al., 2007; Lajoie and Nabi, 2007; Pelkmans and Helenius, 2002; Hommelgaard et al., 2005; Becher and McIlhinney, 2005; de Weerd and Leeb-Lundberg, 1997) 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 in breast cancer (Sotgia et al., 2006; Williams et al., 2004; Chen et al., 2004), vascular abnormalities (Li et al., 2005; Drab et al., 2001; Razani et al., 2001), pulmonary malfunction (Razani et al., 2002a), and muscle disease (Galbiati et al., 2001; Song et al., 1996).

1.2. Caveolin proteins and estrogen receptors

A functional link between caveolin proteins and membrane estrogen receptors was first reported in non-neuronal cells approximately 10 years ago. Initial studies identified ERα-dependent nitric oxide (NO) production required ERα to be associated with caveolae that contained endothelial nitric oxide synthase (eNOS) (Kim et al., 1999; Chambliss et al., 2000). Concurrent with these studies, CAV1 was shown to potentiate classical ERα-mediated gene expression (Schlegel et al., 1999), a process dependent upon the direct interaction between ERα and CAV1 (Schlegel et al., 1999). 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 ERα interaction with CAV1 (Schlegel et al., 1999; Acconcia et al., 2005), and estrogens modulate expression of caveolins (Zschocke et al., 2002), providing additional levels of regulation. Of note, ERβ can also associate with caveolae, and they too are functionally coupled to enzymatic signaling machinery via this process. For example, ERβ within eNOS containing caveolae affords estradiol/ERβ regulation of NO production (Chambliss and Shaul, 2002).

A necessary step in estrogen receptor localization to caveolae is the palmitoylation of the receptor. Although research has focused primarily on palmitoylation of ERα and its variants (Acconcia et al., 2005, 2004; Li et al., 2003; Pedram et al., 2002), the same mechanism for caveolae association has been described for ERβ (Acconcia et al., 2005; Pedram et al., 2007). Specifically, palmitoylation of human ERα 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 ERα. In addition, the physical interaction between ERα and CAV1 is abolished, and membrane estrogen effects are eliminated (Acconcia et al., 2005; Pedram et al., 2007). It is through regulation of palmitoylation that estradiol appears to affect the interaction between ERα and CAV1. In particular, stimulation of HELA cells with estradiol reduces ERα binding to CAV1 with a corresponding reduction in membrane ERα (Acconcia et al., 2005; Pedram et al., 2007). It is significant to note that it is the palmitoylation of a single amino acid that regulates the trafficking of ERα, as this residue is well conserved across species. Similarly, a single cysteine residue in ERβ (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 (Mitchell et al., 2006), this determination may not be rapidly forthcoming.

A second amino acid within the ligand-binding domain of ERα is also necessary for interaction of ERα with CAV1. Mutation of mouse serine 522 to an alanine reduces ERα binding to CAV1 by ~60% in CHO cells and reduces membrane-localization of ERα by a similar percentage (Razandi et al., 2003). The S522A mutation also acts as a dominant-negative in relation to membrane estrogen receptor signaling (Boulware et al., 2007). Currently, the mechanism by which serine 522 facilitates ERα binding to CAV1 and caveolin-dependent trafficking of the receptor to the membrane remains unknown. Mutation of residues within the palmitoylation motif (ERα: F449A, IL456-7AA; ERβ: Y416A, IL423-4AA), have also been shown to interfere with palmitoylation and membrane localization of mouse ERα and ERβ.

The physiological relevance of caveolin proteins and estrogen receptors is best described in relationship to breast cancer. Both ERα 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 (Anderson and Clarke, 2004), which may be a result of elevated levels of estrogen receptors in breast cancer tissue (Shoker et al., 1999) as these proliferative effects of estrogen can be reduced by treatment with anti-estrogens (Song et al., 2002). 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 (Razandi et al., 2004). Mutations of CAV1 are found in ~35% of ERα-positive human breast cancer samples (Li et al., 2006), and CAV1 RNA and protein levels are reduced in many cases of human primary breast carcinomas (Bouras et al., 2004). Mechanistically,