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Estrogen receptors in lipid raft signalling complexes for neuroprotection

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1. ABSTRACT

Estrogens exert a plethora of actions conducted to brain preservation and functioning. Some of these actions are initiated in lipid rafts, which are particular microstructures of the plasma membrane. Preservation of lipid raft structure in neurons is essential for signal transduction against different injuries, such as Alzheimer's disease (AD). These membrane structures appear to be disrupted as this neuropathology evolves, and that may largely contribute to dysfunction of raft resident proteins involved in intracellular signalling. This review includes a survey of some protein interactions that are involved in the structural maintenance and signal transduction mechanisms for neuronal survival against AD. Particularly relevant are the rapid mechanisms developed by estrogen to prevent neuronal death, through membrane estrogen receptors (mER) interactions with a voltage-dependent anion channel (VDAC) and other protein markers within neuronal lipid rafts. These interactions may have important consequences in estrogen mechanisms to achieve neuroprotection against amyloid beta (Abetą-induced toxicity).

2. ESTROGEN ACTIONS TO PRESERVE THE BRAIN

2.1. Diverse roles of estrogens in the brain

Estrogens are versatile molecules that, acting through their binding to specific estrogen receptors (ERs) or other molecular targets, play important roles in the regulation of growth, differentiation and functioning of a wide variety of tissues, including not only the reproductive organs, but also the vascular endothelium, the cardiovascular system, the urogenital tract, intestinal muscle, and even the regulation of lipid and carbohydrate metabolism (1-5). In particular in the nervous system, estrogens develop crucial bioactivities to modulate homeostasis, synaptic plasticity and neurotrophic and neuroprotective mechanisms that modulate memory, cognitive and mood processes (6). Much of the work on the effects of estrogen on neurotrophism and neuroprotection have been conducted in cellular and animals models, and corroborated by different epidemiological data reporting a direct correlation of low values of estrogen after the menopause and acceleration of cognitive decline (7-8).

Although controversially, estrogen replacement therapy for women in postmenopausal periods has proven beneficial in protecting against cognitive deterioration in neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), schizophrenia, depression and stroke (5, 9-17). Furthermore, estrogens have been proposed to decrease the risk, and to delay the onset, of AD (18-19). However, some large clinical trials have reported that hormone replacement therapies do not improve cognition, and even raised the risk of dementia (20-21), thus contradicting, both, preliminary clinical trials and experimental results on cellular and animal models. Some investigators have claimed that these controversies may be due to the time at which the therapy is applied (22), as estrogens efficacy in neuronal defence may depend on the cell capacity to elaborate beneficial responses. Consequently, initiation of estrogen treatment at the onset of menopause may provide protection of cognitive functions as well as cardiovascular benefit, whereas hormone administration following a considerable delay in menopause may not have significant results on cognition (23-24). These facts are related to the dysfunction in the molecular availability required for establishing estrogen survival responses that may decline in the absence of the hormone as a consequence of aging. Despite the conflicting data from human studies, experimental *in vitro* investigations strongly support that estrogen treatment increases the viability, survival and differentiation of neuronal types from different brain regions (including amygdala, hippocampus, cortical areas, substantia nigra or hypothalamus) that may respond against a wide variety of toxicities, from oxidative stress to amyloid-beta (Abeta) toxicity, serum deprivation and excitotoxicity (13, 25-26). Therefore, a deep understanding of estrogen mechanisms of action is necessary to solve these apparent conflicts, in the aim of obtaining optimal results for hormone therapies.

2.2. Neuronal classical and alternative mechanisms of estrogen.

For more than 40 years, the classic genomic theory of estrogen action underpins that the hormone binds to specific estrogen receptors (ERs), which are nuclear transcription factors that modify target gene expression (27). These effects require a significant delay (hours) to observe a cellular response, and have been traditionally named genomic or classical mechanisms of action. In contrast, alternative non-genomic mechanisms are evidenced by their rapid onset of action (within seconds to a few minutes) which involves the activation of different signal transduction pathways (28-30). Thus, steroids rapidly modulate intracellular levels of second messengers such as cAMP, cGMP and calcium, which lead to the activation of a variety of kinases involved in different pathways, including mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK), phosphatidylinositol-3 kinase/Akt (PI3-K/Akt), protein kinase A and C (PKA, PKC), glycogen synthase kinase-3beta (GSK-3beta), tyrosine kinase (Src) family, and calcium-calmodulin-dependent protein kinase II (4, 31-33). In addition to regulation of kinase activation, estrogens can exert rapid regulation of a variety of calcium and potassium channels in neurons from different brain areas involved in learning and memory (17, 31, 34).

Over the last decade, much of the interest has been focus in understanding the mechanisms of estrogen in "non-reproductive" effects in the brain, specifically in the modulation of plasticity, cognition and protection following induction of different injuries related to inflammation, ischemia, AD and PD neuropathologies, which have been the topic of numerous and interesting reviews (4, 13, 26, 35-37). A crucial aspect of these beneficial actions in the central nervous system is the involvement of rapid alternative mechanisms of estrogen action, promoting hormone interaction with different targets present at the plasma membrane.

Substantial experimental work has been carried out to decipher the membrane molecular markers in various brain regions which may participate in cognitive and survival processes. These actions may be versatile and complex, since estrogens are known to bind to a variety of membrane proteins in hippocampal, septal, cerebellar, neostriatal and cortical neurons, including ion channels, neurotransmitter receptors, membrane estrogen receptors (mER) and unidentified ligand receptors (17, 26, 31, 38-40). The cumulative evidence argues for the existence of membrane-associated ERs which, through its binding to estrogens for a short period of time, may importantly contribute to brain preservation (38). The identity of ERs at the neuronal membrane has been a matter of intense investigation due to the technical difficulties to characterize these dynamic molecules. Some evidences, based upon the high similarities in immunoreactivity to antibodies raised against classical ERs, support that at least some membrane estrogen targets have a similar structure to canonical ERalpha and ERbeta (41, 42).

Membrane ERs have been shown to have a pivotal role in neuroprotection in response to different damages including Abeta exposure, glutamate toxicity, serum deprivation and oxidative stress (26, 33, 38, 43-47). In addition, other data have reported the presence of estrogen-binding receptors unrelated to classical ERs, which appear to play a physiological role against neurodegeneration. Thus, Toran-Allerand's group has postulated the existence of an ER-X at the neuronal membrane of cortical neurons that preferentially binds to the enantiomer 17-alpha estradiol (48), and is regulated during development and after ischemia (49). More recently, it was also discovered a transmembrane G-protein-coupled receptor called GPR30, which is distributed in various regions in the brain, and has a potential role in rapid estrogen actions (4, 17, 50). Furthermore, other mERs exhibiting distinct electrophoretic properties have also been reported in different experimental models (51-54), although their physiological relevance in cell preservation has not still been elucidated. Overall, these findings support the view that ER neuroprotective actions triggered at the plasma membrane represent a widespread phenomenon in a complex scenario.

One of the controversial aspects of membrane estrogen receptors have been the manner these molecules without hydrophobic, membrane-spanning regions, may be inserted into the plasma membrane, enabling extracellular estradiol to interact. This phenomenon has been in part elucidated by the recent findings of the anchoring of ERs to

lipid rafts (55, 56). Lipid rafts are membrane compartments of the plasma membrane showing a particular lipid composition, where numerous signalling proteins are recruited (57). Indeed, these domains are keys to neuronal development, functioning and degeneration (58, 59). Interestingly, increasing evidences indicate that the rearrangement of ERs in lipid rafts may be important in the modulation of signalling for neuronal defence, as further discussed below.

3. LIPID RAFTS: KEYS TO SIGNALLING PLATFORMS IN NEURONS

3.1. Lipid interactions with raft integral proteins

Intracellular signal transduction is initiated by a plethora of protein interactions, including receptors, kinases and channels that are crucial for the correct neuronal communication and functions, and whose modifications determine cognitive and neurological impairments. An important concept is that cognitive decline that occurs with normal aging and is exacerbated in neuropathologies, is mainly related to functional changes in signal transduction cascades and cellular communication that modify neuronal responses, rather than to morphological modifications which are not evident during aging (60). Although a set of cell surface proteins are found in liquid disordered regions of the plasma membrane, a large fraction of signalling proteins are located in liquid-ordered domains, or lipid rafts, which are the preferential locations for these proteins, due to the particular physico-chemical properties of these microdomains. In this regard, Lisanti and coworkers (61) were the first to put forth the "caveolae/raft signalling hypothesis", that is, the compartimentalization of proteins involved in transduction signals to provide a mechanism for the regulation and interaction between different intracellular pathways (61). These macromolecular complexes may be considered specialized signalling platforms, or "signalosomes" (61). In particular in neurons, signalling molecules preferentially located in lipid rafts include transmembrane proteins and lipid-modified proteins, as well as intracellular signalling intermediates, such as trimeric and small GTPases, Src tyrosine kinases (STKs) family, lipid second messengers and a variety of cytosolic signal transducers (58, 62), known to participate in neuronal growth, differentiation, preservation and survival.

Furthermore, proteins in these microdomains also interact with resident lipids, suggesting that specific lipids may also take part in the processes developed by signalling molecules. In fact, lipid rafts are presently considered dynamic microenvironments where proteins and lipids can move and interact with different kinetics, changing their size and composition in response to a variety of intra- or extra-cellular stimuli that may ultimately favour specific protein interactions and signalling cascades (57). Therefore, the intrinsic composition and distribution of lipid hallmarks of rafts that modulate membrane fluidity, such as cholesterol, gangliosides and polyunsaturated fatty acids (PUFA), may affect movement of proteins and presumably alter their function and signal transduction. Thus, many raft intrinsic proteins preferentially contain lipid-modified structures that may contribute to their stabilization and correct functioning. In this sense, one of the earlier discoveries was the localization in these domains of glycosylphosphatidylinositol (GPI)-anchored proteins that generally have saturated acyl chains and are preferentially anchored in the outer leaflet of the cell membrane (63). Although the GPI anchor does not completely cross the plasma membrane, it is crucial to initiate signalling events, probably through its association with other transmembrane proteins involved in intracellular signalling (64, 65). Among the GPI-anchored proteins involved in neuropathology, one of the better characterized is the cellular prion protein (PrP^c) implicated in the pathogenesis of prion disease (66). Prion disease is an amyloid disease characterized by the formation within neurons and other brain cells of protein plaques leading to cell death, which involves the conformational modification of normal PrP^c in a pathogenic scrapie form, PrP^{Sc} (67). PrP^c is constitutively expressed in neurons as a GPI-anchored protein localized in lipid rafts, and depletion in cholesterol but not sphingolipids, affects its distribution in these microdomains (68). Interestingly, there is an increasing body of evidence that lipid raft environment plays a direct role in PrP^c conversion into PrP^{Sc} (67). The downstream signalling of PrP^c is dependent on its localization to rafts, which induces the activation of some STKs, possibly Lyn, Src, Lck or Fyn (69, 70). Thus, PrP^c may be part of a multimolecular signalling complex which may be important in neuronal function (71).

Additional lipid modifications of signalling proteins inserted in rafts take place by binding to alternative saturated-chain lipids, such as palmitoylation and myristoylation. These modifications are found, among others, in STKs, scaffolding proteins, steroid receptors and GPI-anchored proteins that may contribute to their stabilization and correct functioning in these microstructures (72-76). In addition, hallmark proteins of lipid rafts such as caveolin and flotillin undergo palmitoylation (77) and, in the case of caveolin, this requirement allows the coupling of Cav-1 to c-Src tyrosine kinase (73). These evidences suggest that lipid-modified nature of proteins integrated in lipid rafts may serve not only to target them to these domains but also to modulate the protein interactions occurring within rafts.

3.2. Estructural proteins of neuronal lipid rafts

Together with GPI-anchored proteins, scaffolding proteins are the most abundant integral molecules of lipid rafts. They represent a particular group that have the intrinsic capacity to form lipid shells around themselves including, apart from caveolins and flotillins, the proteolipid MAL, stomatin, and some transmembrane proteins, such as presenilin-1 (78-82). These structural proteins not only provide stabilizing scaffolds for lipid raft maintenance, but also participate in vesicular trafficking and signal transduction that modulate the final cellular response. Numerous demonstrations have concluded that the members of caveolin family (caveolins 1, 2 and 3) serve to compartmentalize specific signalling molecules within lipid rafts, or caveolae, with the prospect of rapidly and selectively modulating cell signalling events, thereby

proposing a "caveolae signalling hypothesis" (61). In this regard, caveolins are known to regulate a variety of key signalling elements, including G-proteins, STKs and some components of PI3K and MAPK pathways (83, 84). Although the structure of caveolae and caveolin isoforms have not been fully elucidated in the nervous system, evidences suggest that neuronal lipid rafts may serve as docking points for numerous cell surface receptors which are recruited to this microdomain when bound to their specific ligands, activating numerous intracellular processes related to neuronal functioning. Among these receptors known to interact with caveolins are membrane estrogen receptors (ERs), which are regulated by interactions with these proteins (85). Recent evidences suggest that, at least caveolin-1 (Cav-1), may play a crucial role in membrane ER function in the brain, which in turn determines many nervous system activities related to its preservation and maintenance (54, 86). Also, Cav-1 has been claimed to be involved in Abeta processing, suggesting that Abeta generation depends on the interactions of Cav-1 with the amyloid precursor protein (APP) (87). Furthermore, Cav-1 expression is increased in senescent cells and AD brains, suggesting an involvement of this resident protein of lipid rafts in brain degeneration (88, 89).

Flotillins belong to the so-called SPFH (stomatin/prohibiting/Flotillin/ HflK/C) protein family forming specialized rafts that, similar to caveolae, provide stable platforms for multiprotein complexes assembly (80). Flotillins not only are important for coordinated recruitment of the machinery for regulation of cytoskeletal remodeling but they have also been linked to the pathogenesis of Alzheimer's disease. Indeed, flotillins appear to be up-regulated in the cortex of patients with AD, where they accumulate at sites of amyloid beta peptide (Abeta) production and secretion (80, 90). However, further studies are required to fully elucidate the role of flotillin-1 in the progression of AD pathology.

3.3. Neurotrophic signalling and neurotransmission in lipid rafts.

A large body of evidence have demonstrated that lipid rafts are also platforms for neurotrophic signalling under the control of neurotrophins and glial-derived neurotrophic factor (GDNF)-family ligands which are essential for synaptic transmission, axon guidance and cell adhesion (58). Src kinase activity is one of the main signalling proteins required to elicit GDNF-bioactivity related to neurite outgrowth and neuronal survival (91). Numerous receptor tyrosine kinases are located in lipid rafts, including TrkA, insulin receptor (IR), EGFR (epidermal growth factor receptor) and PDGFR (platelet-derived growth factor receptor) (83, 92-94). Accordingly, we have found the enrichment of IGF-1R (insulin growth factor-1 receptor) in lipid rafts from human cortex and hippocampus, in a complex with ERalpha and caveolin-1, suggesting that this receptor may also take part of multimolecular complexes in lipid rafts (as discussed below).

Emerging evidence also indicates that such rafts are important for neuronal synaptic transmission, and different neurotransmitter receptors and ion channels, e.g. the voltage-gated K^+ channel Kv2.1., nicotinic acetylcholine receptor (nAChR) and $GABA_bR$ receptor are biochemically located in lipid rafts (58, 95-97). Localization of ion channels to these microstructures appears to vary depending upon the specific channel, a fact that modifies channel properties (95). In this order of ideas, our recent work in neuronal cell lines, and human and mouse brain cortex and hippocampus have demonstrated that the pro-apoptotic plasma membrane voltage-dependent anion channel (VDAC) is located in lipid rafts in physical contact with Cav-1 and ERalpha (55, 56), a fact that might be relevant in AD neuropathology, as discussed in the next sections.

Thus, lipid rafts not only represent structurally components of neuronal membranes, but also integrate protein signalling platforms which are crucial for the development of neuronal physiological activities related to neuroprotection.

4. MEMBRANE ESTROGEN RECEPTORS WITHIN MACROMOLECULAR PLATFORMS INVOLVED IN NEUROPROTECTION

4.1. Molecular components of macrocomplexes interacting with mERs in lipid rafts

The finding of ERs in neuronal lipid rafts suggests that the receptor may be part of dynamic structures formed by interactive lipid and protein associations in these membrane compartments. Coordination of estrogen signalling in lipid rafts has already been demonstrated in cardiomyocytes, breast cancer and platelet regulation (98-100), but there is still very little information related to neuronal bioactivities. Since raft platforms are formed from fluctuating assemblies of lipid and protein oligomers that interact in response to extracellular signals (101), then signalling platforms integrated in lipid rafts may be crucial for the development of neuronal physiological activities related to neuroprotection. Therefore, a key to understand the motility, modulation and activities of mERs in neurons is the identification of partners associated with these receptors that may contribute to estrogen coupling to signal transduction.

Raft-located ER has been evidenced in cerebral cognitive areas, such as human frontal cortex and hippocampus, and in murine septal and hippocampal immortalized neurons, where a mER similar to ERalpha was found to participate in prevention of cell death following Abeta treatment (44, 55-56). In raft human and murine membrane fractions as well as in microsomal fractions from different mouse brain areas (42), mER physically interacts with caveolin-1, which may serve as a docking point to recruit the receptor to this microdomain. In addition, this association appears to be necessary for steroid rapid signalling in neurons (85, 86), and it has been demonstrated that caveolin expression is also a requirement to compartmentalize, both, ERs and membrane glutamate receptors into functional signalling microstructures (39).

Some bioinformatic studies have indicated that a plausible possibility is that interaction of the receptor with

caveolin-1 may take place at the caveolar scaffolding domain (CSD), a sequence motif present in numerous signalling proteins that has also been found in the ligand binding domain (LBD) of ERalpha (42). In addition, this domain is the place where ERalpha is reversibly palmitoylated in Cys447 residue, a requirement for the receptor to locate within membrane subdomains (74, 102).

Furthermore, other identified molecules forming part of a complex with mER in lipid rafts are the insulin growth factor-1 receptor (IGF-1R), recently identified in lipid rafts from the human frontal cortex in association with caveolin-1 and ERalpha (103), and plasmalemmal voltage-dependent anion channel (pl-VDAC) (55, 104). IGF-1R activation is essential for different actions of estradiol in the brain, including neuronal survival and differentiation, synaptic plasticity, the regulation of ER mediated gene expression, and the control of cholesterol homeostasis (105-106). It is important to underline that ER and IGF-1R pathways cross-talk with each other to promote neuroprotective events, and that this association may be affected with aging (107-108). Then, it is plausible that ERalpha/IGF-1R interaction in membrane subdomains may be differentially activated by the extracellular availability of distinct ligands of these receptors, therefore adapting the neuronal response through their interactive signalling machinery (105).

4.2. Relevance of mER association with a voltage-dependent anion channel to palliate Abeta-induced toxicity

A recent finding is the association of VDAC with mER in lipid rafts, a phenomenon that has been observed in both cultured neurons and extracts from different murine and human brain areas (55, 56). In this channel, a CBD susceptible of binding to caveolin-1 has been identified in the second intracellular loop of its structure (42), thereby reinforcing the participation of VDAC in this raft signalling platform. VDAC is a porin located at the mitochondrial membrane, where its role has been related to intrinsic apoptotic pathway (109). The porin is also found at the plasma membrane of many cell species (named pl-VDAC) where it is involved in cellular ATP release and volume control, NADH:ferricyanide reductase activity, tumoral processes and apoptosis (reviewed in 110). In neurons, pl-VDAC plays a role in redox homeostasis and initiation of extrinsic apoptosis pathway (111, 112) that can be induced by different injuries including excessive glutamate release (113), and Abeta-induced toxicity (55). These latter observations suggest that VDAC participation is cardinal in the mechanisms related to AD pathology. In support of this, VDAC highly accumulates surrounding the main hallmarks of AD neuropathology, i.e. senile plaques and neurofibrillary tangles, in murine and human brains affected by this disease (56, 114). In addition, the channel increases its expression in either murine amyloidogenic models of AD or following Abeta exposure in cultured neurons (115). VDAC is also overexpressed in mitochondria related to autophagic processes enhanced during AD (116). Since the foregoing data indicate that pl-VDAC activation may contribute to AD development, then it is plausible that pl-VDAC/ER interaction in lipid rafts may be important for the rapid estrogen mechanisms to palliate neuronal death against Abeta toxicity (103).

Overall, these findings indicate that ER in neuronal lipid rafts may be part of macromolecular complexes formed by several signalling molecules involved in the control of neuronal maintenance, whose dynamic interaction may be at the basis of the complex neuroprotective responses against different toxicities. In these signalosomes, anchoring proteins such as caveolin-1 and different lipid-lipid, lipid-protein and protein-protein interactions may supply stability for the integration and functionality of ERalpha, thus facilitating its associations with other signalling proteins in the raft microstructure. These interactions may be also affected by the availability of extracellular ligands binding to the different components of these platforms. In agreement with this hypothesis, emerging data suggests that estrogens modulate pl-VDAC activation, as discussed in the following section.

5. ESTROGEN SIGNALLING PATHWAYS AGAINST ALZHEIMER'S DISEASE NEUROPATHOLOGY

5.1. Membrane estrogen strategies to palliate AD parameters of neurotoxicity

Estrogens can mitigate important events related to AD neuropathology acting at different intracellular levels. In this order of ideas, numerous studies have demonstrated that estrogens exert their neuroprotective actions through different mechanisms leading to modulation of amyloid precursor protein (APP), regulation of Abeta formation and clearance, reduction of tau hyperphosphorylation, modulation of anti-apoptotic agents and preservation of mitochondrial integrity, among others (37, 117). Some of these estrogen actions take place via the activation of different signal transduction pathways. At this level, one of the most studied effects is related to estrogen protective role against Abeta-induced toxicity, with the participation of several kinase cascades. In this sense, two main pathways have been associated with these actions, MAPK signalling (through Raf/MEK/ERK activation), and PI3-K signalling (through PI3-K/Akt/GSK3 activation) (17, 26, 37). These pathways, triggered by estrogens within minutes, have been shown to be important in palliating Abeta neurotoxicity in neurons from different brain areas, such as hippocampus, septum, cortex and hypothalamus (26). Moreover, MAPK signalling is involved in APP processing promoting non-amyloidogenic products (37) although alternative pathways, such as PKC signalling, also appear to be part of this process (118, 119). Other data has demonstrated that estrogens binding to ER can reduce Abeta-induced neuronal apoptosis through the inhibition of c-Jun N-terminal kinase (JNK) pathway, which subsequently attenuates pro-apoptotic agents and upregulates anti-apoptotic agents (120).

Some particularly relevant results are related to the inhibition of tau hyperphosphorylation promoted by estrogens through the modulation of GSK3 activity (121). Tau is a microtubule-associated protein that is aberrantly hyperphosphorylated by kinases such as GSK3, then

provoking microtubule destabilization and neurofibrillary tangle formation during AD. It appears that the hormone regulates the interaction of tau with different components of PI3-K pathway, including GSK3 and beta-catenin (122, 123). Also, modulation of GSK3 by estrogens may be related to ER activation (124). Furthermore, estrogen attenuates elevation of cAMP and overactivation of PKA, two relevant steps of tau hyperphosphorylation, thereby preventing phospho-Tau increase (125).

5.2. Modulation of pl-VDAC by estradiol and its relevance against Abeta-induced cell death

In addition to the rapid effects of estrogen involving the regulation of Abeta clearance, APP processing and tau phosphorylation, part of the strategies to palliate AD may involve the regulation of putative modulators of Abeta-induced toxicity, such as VDAC (127). Thus, some recent data have shown that estrogens regulate pl-VDAC activation through the control of post-translational modifications of the porin. In septal and hippocampal cells, short exposures to estradiol resulted in the maintenance of the pl-VDAC phosphorylation status (127), a fact that may preserve the inactivation and closing state of the channel. Similarly, VDAC phosphorylation has been observed at the neuronal membrane from mouse frontal and parietal cortices (128), and in human brains (unpublished results), suggesting that the presence of a phosphorylated VDAC isoform at the neuronal membrane may be a general phenomenon. Further analysis in cultured neurons has demonstrated that estradiol induces VDAC phosphorylation by the activation of, both, PKA and Src-kinase (127). In support of these observations, VDAC was also found phosphorylated by PKA in mitochondria of rat liver, thereby provoking the closure of the channel (129-130). Moreover, although still unexplored, GSK3 may also be a candidate to modulate VDAC at the plasma membrane since this kinase phosphorylates the mitochondrial form of the porin following inhibition of Akt (131).

Therefore, one could hypothesize that Abeta may physically interact with VDAC in lipid rafts thereby contributing to the channel opening, ultimately leading to intracellular apoptosis (126). In line with this possibility, binding of this peptide to gangliosides, lipid raft components, is thought to induce the assembly of Abeta proteins involved in the formation of senile plaques (93, 132-133). An alternative possibility is that VDAC might be activated by membrane depolarization as a result of intracellular Ca^{2+} levels increase provoked by Abeta interacting with the neuronal membrane. In agreement with this hypothesis, some data have reported that Abeta peptide may induce cellular toxicity by regulating Ca^{2+} homeostasis, based on its property to activate Ca^{2+} channels (134). It should also be mentioned that a large number of studies have proposed that cell exposure to Abeta peptide results in unregulated flux of Ca^{2+} through the plasma membrane, upon disruption of plasma membrane integrity (reviewed in 135). In addition, several studies have highlighted the importance of the specific interaction of Abeta (and other protein) amyloids with glutamate receptors (AMPA and NMDA). Notably, a rise in intracellular Ca^{2+} induced by Abeta decreases the availability of AMPA receptors at the synapses, thereby affecting synaptic plasticity (125, 136). Abeta also affects NMDA receptor activation culminating in intracellular Ca^{2+} overload, which disrupts neuronal transmission (137).

5.3. Antagonist effects of selective estrogen receptor modulators on pl-VDAC regulation

An interesting fact is that maintenance of pl-VDAC phosphorylation /inactivation may be specific for physiological estradiol concentrations. Indeed, tamoxifen, a selective estrogen receptor modulator (SERM), has been observed to provoke the antagonist effect on the channel, increasing its dephosphorylation through the activation of either serine-threonine protein phosphatase 2A (PP2A) or tyrosine phosphatases (127). These findings are in line with previous electrophysiological outcomes on neuroblastoma cells and NIH3T3 fibroblasts, where brief exposures to different SERMs (i.e. tamoxifen, toremifen) were found to open Maxi-Cl^- channel through a mechanism involving PP2A activation (138-139). It is worth mentioning that VDAC is considered the molecular correlate of the plasma membrane Maxi-Cl^- channel (140). On the contrary, another set of electrophysiological experiments in cells treated with estradiol resulted in the inactivation (closing) of this channel (138). Interestingly, the antagonist effects of estrogen and tamoxifen in pl-VDAC modulation correlates with the different efficacy of these molecules in neuronal defence against Abeta-induced neurotoxicity. Indeed, in septal and hippocampal neurons, a high degree of estrogen-induced cell survival has been observed following exposure to the amyloid, whereas no significant cell viability has been detected in the presence of tamoxifen (128).

These findings support the notion that a main non-genomic mechanism of estrogen to achieve neuroprotection may be through the modulation of VDAC phosphorylation to maintain the channel in a closing state. The preservation of phosphorylated VDAC may be a crucial parameter of neuronal survival, since data in the temporal, frontal and occipital cortex of AD brains have evidenced that changes in VDAC phosphorylation pattern may be related to synaptic loss (141). Indeed, this porin has been found in a nitrated form in hippocampus of AD brains, as an alternative isoform modification, and this may produce an irreversible dysfunction of the channel (142).

In summary, the reported data indicate that estrogens acting at the neuronal membrane may trigger different intracellular signalling pathways converging in neuronal survival. Adding more complexity to these mechanisms, it is probable that an additional parameter to develop the final cellular response may depend on the dynamic associations of ER and other estrogen targets, such as VDAC, in neuronal membrane subdomains.

5.4. Disruption of mER signalling complex in lipid rafts of AD brains

It has been speculated that changes in the lipid composition of lipid rafts may contribute to AD pathology (143). Thus, multiple lines of investigation have demonstrated the role of cholesterol, one of the major lipid constituent of lipid rafts, in amyloidogenic processing of

APP (67, 144), suggesting a dynamic interaction of APP with lipid rafts. Apart from cholesterol, gangliosides are other lipid raft components that appear to be involved in Abeta peptide formation and processing (87), and have also been observed to be modified in lipid rafts of AD patients (145). In addition, other lipid classes such as phospholipids have been claimed to either mediate or modulate key pathological processes associated with AD in relation to phospholipase D activity (146). Moreover, although less characterized, polyunsaturated fatty acids may also play an important role in lipid raft stability and its deficiency has been associated with AD pathology (147). In particular, docosahexanoic acid (DHA) has been shown to be highly enriched in neuronal membrane phospholipids (148). Indeed, we have recently demonstrated that AD lipid rafts obtained from brain cortex at late stages exhibited significant reductions in DHA when compared with age-matched controls (149). These abnormal low levels of PUFA are in consonance with previous observations in whole membranes from different brain areas of AD patients, and are correlated with reduced unsaturation and peroxidability indexes (149-150). Overall, these observations suggest that changes in brain lipid composition are important determinants of AD progression.

Taking into account these findings, it can be suggested that anomalies in lipid composition of lipid rafts may presumably result in a profound modification of the physico-chemical properties of these structures, such as increase in membrane viscosity and rigidity, which may largely affect the activities and interactions of raft resident proteins (149). This possibility is gaining considerable support as content alterations in lipid classes such as sterols, gangliosides and polyunsaturated fatty acids (PUFA) largely contribute to the development of AD and other neurological impairments (59, 151).

A support of signalling complex disfunction in neuronal lipid rafts have recently been demonstrated, observing the dissociation of pl-VDAC/ER/caveolin-1 complex in lipid rafts in cortical areas at late stages of this disease (56). In these membrane fractions, pl-VDAC increased its concentration and interaction with caveolin-1 in lipid rafts of AD patients, whereas ERalpha levels in these fractions was reduced. In fact, and in agreement with previous data (152), ERalpha was mostly observed in astrocytes, suggesting a role of this receptor in estrogen protective effects related to these glial cells. Therefore, it is conceivable that anomalies in the composition of lipid rafts may interfere in pl-VDAC/mERalpha interactions and consequent modulation (i.e. phosphorylation) of the porin by estrogens, thus contributing to reduce the defenses facing Abeta-induced toxicity. These disrupted interactions may also affect other proteins participating in this signalling complex, such as IGF-1R. Furthermore, an additional parameter to consider is the proper ability of estradiol to alter the fluidity of phospholipids in membrane bilayers, a fact that is directly related to hormone effects on integral proteins of this structure, although these effects have been observed at supraphysiological conditions (153).

Overall, these findings indicate that ER in neuronal lipid rafts may be part of macromolecular complexes formed by several signalling molecules involved in the control of neuronal maintenance, whose dynamic interaction may be at the basis of the complex neuroprotective responses against different toxicities. In these signalosomes, anchoring proteins such as caveolin-1 and different lipid-lipid, lipid-protein and protein-protein interactions may supply stability for the integration and functionality of ERalpha, thus facilitating its associations with other signalling proteins in the raft microstructure. These interactions may be also affected by the availability of extracellular ligands binding to the different components of these platforms.

6. PERSPECTIVES

There is a general agreement on the importance of lipid/protein and protein/protein interactions in lipid rafts as crucial parameters for the integrity of these membrane microstructures, which may be profoundly modified in different neuropathologies including Alzheimer's disease. Raft protein interactions in dynamic signalling platforms may have a pivotal role in the regulation of distinct cellular responses directed to neuronal preservation. Among the relevant protein complexes related to neuroprotection, emerging data suggest that ERs, through its binding to estradiol followed by interaction with proteins integrated in lipid rafts, may have a pivotal role in the regulation of distinct cellular responses directed to neuronal integrity. Thus, membrane compartmentalization related to neuroprotection against Abeta neurotoxicity may include mER, pl-VDAC and IGF-1R together with caveolin-1, as a part of a multimolecular signalling complex involved in neuronal survival. It is important to keep in mind that these molecular clusters may combine both, survival and apoptotic modulators, whose dynamic modulation in particular microenvironments may ultimately give rise to the final orchestrated response. Indeed, VDAC and caveolin-1 are known to participate in the mechanisms of Abeta generation and processing (55, 87, 127), suggesting a role of these raft proteins in brain degeneration. Undoubtedly, other participants of this macromolecular complex remain to be identified. In addition, mER involvement in other signalling platforms with significant effects on neurological processes is starting to be revealed.

Furthermore, mER-related actions of estradiol may play a crucial role in the maintenance of VDAC in a phosphorylated/inactivated state, a fact that may largely contribute to a reduction of apoptotic effects triggered by the amyloid. Therefore, the potential post-transductional modifications of this porin at the neuronal membrane may be at the basis of estrogen mechanisms leading to brain preservation. In line with this, it is enticing to speculate that disruption of VDAC association with mER observed in AD may induce irreversible post-transductional changes in this channel, such as nitration and carbonylation, thereby contributing to oxidative stress and lipid raft impairment.

In addition, lipid composition of membrane subdomains may have a pivotal role in the interactions and activities of mER at this level. In this order of ideas, it is known that palmitoylation of ER is a requirement for its

trafficking to the plasma membrane (102), and it has been assessed that this lipid modification may regulate raft affinity in integral proteins (154). In addition, modification of physico-chemical properties of lipid rafts as a result of alterations in lipid composition may profoundly affect the associations and functionality of proteins integrated in these structures that may underlie some of the neuropathological parameters. An example of this phenomenon is the observed disruption of ER/VDAC/caveolin-1 signalling complexes in the cortex of AD brains as a result of a reduction in PUFA levels (149). Therefore, identification of lipid markers as part of signalling platforms in lipid rafts may also give some hints to elucidate the parameters of neuronal impairments.

Future investigation on the participation of ERs in membrane microenvironments to initiate intracellular signalling may certainly provide some clues to elucidate the complex strategies of neuronal survival triggered by estrogens. Undoubtedly, understanding the complex dialogue of lipid-lipid, lipid-protein and protein-protein interactions occurring in signalling compartments involved in estrogen neuroprotective mechanisms will contribute to the development of novel strategies on early diagnoses to prevent from devastating brain impairments occurring in AD.

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Abbreviations: AD: Alzheimer's disease; ER: Estrogen receptor; GSK-3beta: Glycogen synthase kinase-3beta; IGF-1R: Insulin growth factor-1 receptor; JNK: c-Jun N-terminal kinase; MAPK: Mitogen-activated protein kinase;, PD: Parkinson's disease; PI3-K/Akt: Phosphatidylinositol-3 kinase/Akt; PKA: Protein kinase A; PKC: Protein kinase C; VDAC: voltage-dependent anion channel

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