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Estrogen-induced cell signalling in a cellular model of Alzheimer's disease☆

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Abstract

Alzheimer's disease (AD) is characterised by deposition of a 4 kDa amyloid-β peptide (Aβ) into senile plaques of the affected brain. Aβ is a proteolytic product of the membrane protein, amyloid precursor protein (APP). An alternative cleavage pathway involves α-secretase activity and results in secretion of a 100 kDa non-amyloidogenic APP (sAPPα) and therefore a potential reduction in Aβ secretion. We have shown that estrogen induces α-cleavage and therefore results in the secretion of sAPPα. This secretion is signalled via MAP-kinase and PI-3 kinase signal-transduction pathways. These pathways also have the potential to inhibit the activation of glycogen synthase kinase 3β (GSK), a protein involved in cell death. Therefore, the aim of this work was to further elucidate the estrogen-mediated signaling pathways involved in APP processing, with particular emphasis on GSK activity. By stimulating rat hypothalamic neuronal GT1-7 cells with estradiol, we found that estrogen decreases the activation state of GSK via the MAP kinase pathway. Moreover, the inhibition of GSK activity by LiCl causes enhanced sAPPα secretion in a pattern similar to that seen in response to estrogen, suggesting a pivotal role for this deactivation in APP processing. Further, inactivation of GSK by estrogen can be confirmed in an in vivo model. Elucidation of the signaling pathways involved in APP processing may help to understand the pathology of AD and may also prove beneficial in developing therapeutic strategies to combat AD.

Keywords: Estrogen; Alzheimer's disease; Glycogen synthase kinase-3β; Amyloid precursor protein

1. Introduction

Alzheimer's disease (AD) is manifested by a number of clinical features, including selective oxidative stress-induced neuronal cell death, the deposition of amyloid-β peptide (Aβ) into senile plaques in the extracellular space and the hyperphosphorylation of the tau protein causing neurofibrillary tangles. Of particular interest recently have been the signalling events that are responsible for these AD-associated features with the goal to design drug therapies that target these events in an attempt to alleviate some of the symptoms of AD. Here we present a summary of some of the signalling events we believe are associated with estrogen-induced APP processing that may prove to be neuroprotective in vivo.

2. What is APP?

APP is a 770 amino acid residue, transmembrane protein whose precise function in the brain is not clear. Recent studies have identified a number of proteins that can bind to APP, which may suggest a role for APP in their function. For example, Fe65 has been identified as having the ability to bind to APP and through this interaction possibly play a role in membrane extension and cellular motility [1]. However, what role any of the identified putative functions of APP have in AD pathology is completely unclear. What is strongly believed is that the specific type of proteolysis and secretion of the APP cleavage products is central to the pathogenesis of AD.

2.1. Cleavage of APP

There are two main secretase activities that are responsible for the processing of APP. These have been designated α- and β-secretase. APP can undergo α-secretase cleavage at residue 687 which allows the secretion of a 100 kDa non-amyloidogenic soluble APP (sAPPα) and a retention of an 83 residue C-terminal fragment in the membrane.

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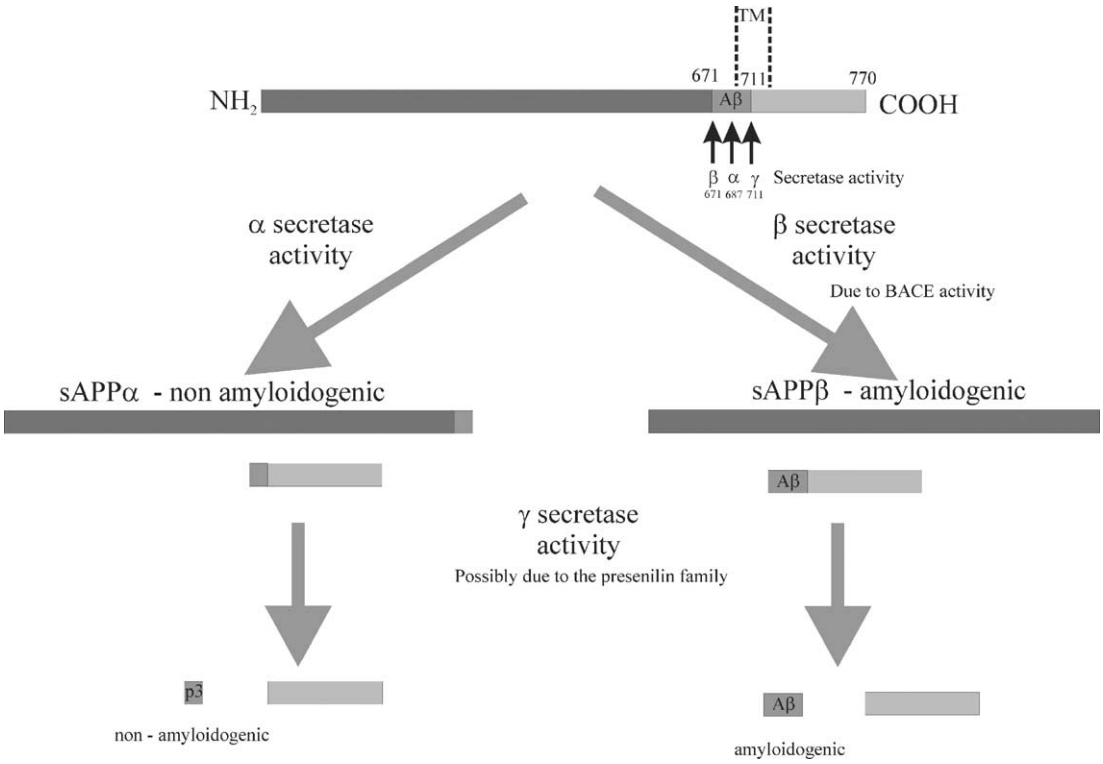

Fig. 1. Schematic diagram of amyloid precursor protein (APP) and its principal metabolic derivatives. APP is a membrane bound protein of 770 amino acid residues. A single membrane-spanning domain (TM) at amino acids 700–723 is indicated by the vertical dashed lines. APP can undergo α secretase cleavage at residue 687, which allows secretion of the large, soluble ectodomain (sAPPα) into the medium and retention of an 83 residue C-terminal fragment in the membrane. Alternatively, APP can undergo β cleavage at residue 671, which results in the slightly truncated sAPPβ and retention of a 99 residue C-terminal fragment. From here, both membrane bound species can undergo γ cleavage at residue 711 and results in the release of the non-amyloidogenic p3 or the insoluble, amyloidogenic Aβ, respectively.

Alternatively, APP can undergo β-cleavage at residue 671, which results in the slightly truncated sAPPβ and retention of a 99 residue C-terminal fragment in the membrane. From here, both membrane bound species undergo γ cleavage and results in the release of the non-amyloidogenic p3 (after α cleavage) or the insoluble, amyloidogenic Aβ (after β cleavage) (reviewed in [2]). A schematic representation of this can be seen in Fig. 1. It can be argued that any therapy that increases the activity of the α-secretase pathway could potentially decrease the amount of Aβ secreted and therefore may be beneficial in halting the progression of AD.

3. Estrogen in prevention and therapy of Alzheimer's disease

Estrogen is strongly suggested to be of preventive value against the development of AD. Clinically, hormone replacement therapy has been demonstrated to protect women against the development of AD, but is of little or no value once the disease has progressed to where it is clinically detectable [3]. Preclinically, it has been shown to be neuroprotective against oxidative stressors such as Aβ and H_2O_2 as well as stimulating the α-secretase pathway resulting in increased sAPPα secretion, thereby possibly affording neuroprotection indirectly [4]; for review see [5].

4. Estrogen affects APP processing and stimulates sAPPα release

Many factors that stimulate the α-secretase pathway have already been identified. Of particular note are the steroid sex hormones estrogen and testosterone. We and others have previously shown that estrogen and testosterone regulate APP processing [4,6,7]. More specifically estrogen has been shown to reduce neuronal generation of Aβ in vitro [8] and in vivo [7] and to stimulate the α-secretase pathway and therefore the release of sAPPα [4]. Further, a similar effect on APP processing can be seen in neuronal cells with testosterone [6,9]. We have also shown that the first step in this pathway is the conversion of testosterone to estrogen via aromatase activity [6]. The mechanism of action of estrogen in stimulating sAPPα release is very rapid (within 30 min) and occurs to a similar extent in neuronal cells either lacking or overexpressing estrogen receptors [4]. This suggests that this event is occurring through a receptor-independent

mechanism and therefore probably via various cell signalling pathways.

5. Signalling pathways involved in APP processing

Some of the members of the signalling pathways involved in this process have been identified. Protein kinase C (PKC) has long been thought to regulate the α-secretase pathway. Stimulation of PKC by phorbol esters results in a decrease in Aβ production and an increase in sAPPα in a variety of cell lines [10–13]. Further, this PKC-mediated regulation is via the activation of the MAP kinase pathways [11,14]. We have also shown a dependence of estrogen- and testosterone-mediated APP processing on the MAP kinase pathway in an estrogen-receptor-independent manner [4,6]. Another major signalling pathway involved is the PI-3-kinase pathway [15,16], however, the precise role of this pathway is unclear.

Interestingly, both the MAP kinase pathway and the PI-3-kinase pathway can converge to inhibit glycogen synthase kinase-3β (GSK-3β; [17–19]) via phosphorylation at the serine 9 residue of GSK-3β. Generally, GSK-3β can be activated by phosphorylation at the tyrosine 216 residue and deactivated either by dephosphorylation at this site or by phosphorylation at the serine 9 residue (reviewed in [20]). This enzyme has been shown to trigger cell death [21] as well as being responsible for the hyperphosphorylation of tau [22–25], which may result in the neurofibrillary tangles observed in AD brains. The effect of this GSK-3β activity on other processes of AD pathogenesis, however, remains unclear.

6. Estrogen-induced cell signalling inactivates GSK

Because GSK is the substrate of both the PI-3K and MAPK pathways, both of which are activated in the presence of estrogen, we have determined if estrogen treatment of GT1-7 cells has any effect on the phosphorylation state of GSK. Using phosphospecific antibodies in a Western blotting procedure it was observed that 60 min after physiological doses of estrogen there was a seven-fold increase in the phosphorylation of GSK at the serine 9 position which inhibits the activity of GSK. No change in total GSK levels was observed during this time.

To determine if any of the above mentioned pathways are responsible for the estrogen-induced phosphorylation of GSK, the effect of specific inhibitors of MAP kinase and Akt on this was performed. By preincubating the GT1-7 cells with PD98059 (MAP kinase inhibitor) for 2 h the basal level of phospho-serine GSK-3β was significantly reduced. Further, the subsequent increase in estrogen-stimulated serine 9 phosphorylation was abolished by inhibiting MAP kinase activity. No effect was observed when using a specific Akt inhibitor, suggesting that GSK-3β is phosphorylated by the MAP kinase pathway rather than the PI-3 kinase pathway after estrogen stimulation.

7. GSK inactivation can play a role in estrogen-induced sAPPα release

The question of whether the inactivation of GSK is responsible for estrogen-induced sAPPα release was addressed in our model system. Lithium chloride is a general inhibitor of GSK-3β activity with an IC_{50} of 1–2 mM [26]. By exposing GT1-7 cells to 10 mM LiCl to completely block GSK-3β activity, there should be a similar increase in sAPPα release as seen after estrogen. Indeed, LiCl also causes a rapid increase in sAPPα release with similar kinetics to that seen after estrogen [4]. Lithium, however, also has many other biochemical actions in the nervous system including modulation of neurotransmitters and effecting the signalling thought to be critical for neuroplasticity (reviewed in [27]). Therefore, it can not be excluded that the lithium effect on sAPPα release may be due to one of the other activities.

8. Estrogen inactivates GSK in vivo

In order to verify the link between estrogen and phosphorylation of GSK-3β, an in vivo model was employed whereby mice were exposed to estrogens prenatally and the effect on phosphoserine-GSK-3β immunoreactivity was determined 4 weeks after birth. The treatment regime used here caused a sustained increase in phosphoserine GSK immunoreactivity in the hippocampus when compared to vehicle injected controls. This increase was observed in all of the subfields of the hippocampus and dentate gyrus and was predominantly in the pyramidal cell layer. No obvious changes were observed in the cortex or the hypothalamic region. This supports the role of estrogen on the inactivation of GSK-3β and may also be a possible mechanism of the neuroprotective action of estrogen.

9. Conclusion

From the current literature and the data discussed in this paper, a schematic diagram concerning the estrogen-induced signalling pathways that are involved in APP processing can be drawn (Fig. 2). Estrogen (and testosterone via its conversion to estrogen as a first step) stimulates both the MAP kinase pathway and PI-3-kinase pathway via a receptor-independent mechanism. Both of these pathways have been shown to be responsible for signalling estrogen-induced sAPPα release and can also inactivate GSK. The estrogen-induced inactivation of GSK observed by us is via the MAP kinase pathway and not the PI-3 kinase pathway. Both the MAP kinase pathway (via GSK inactivation) and the PI-3

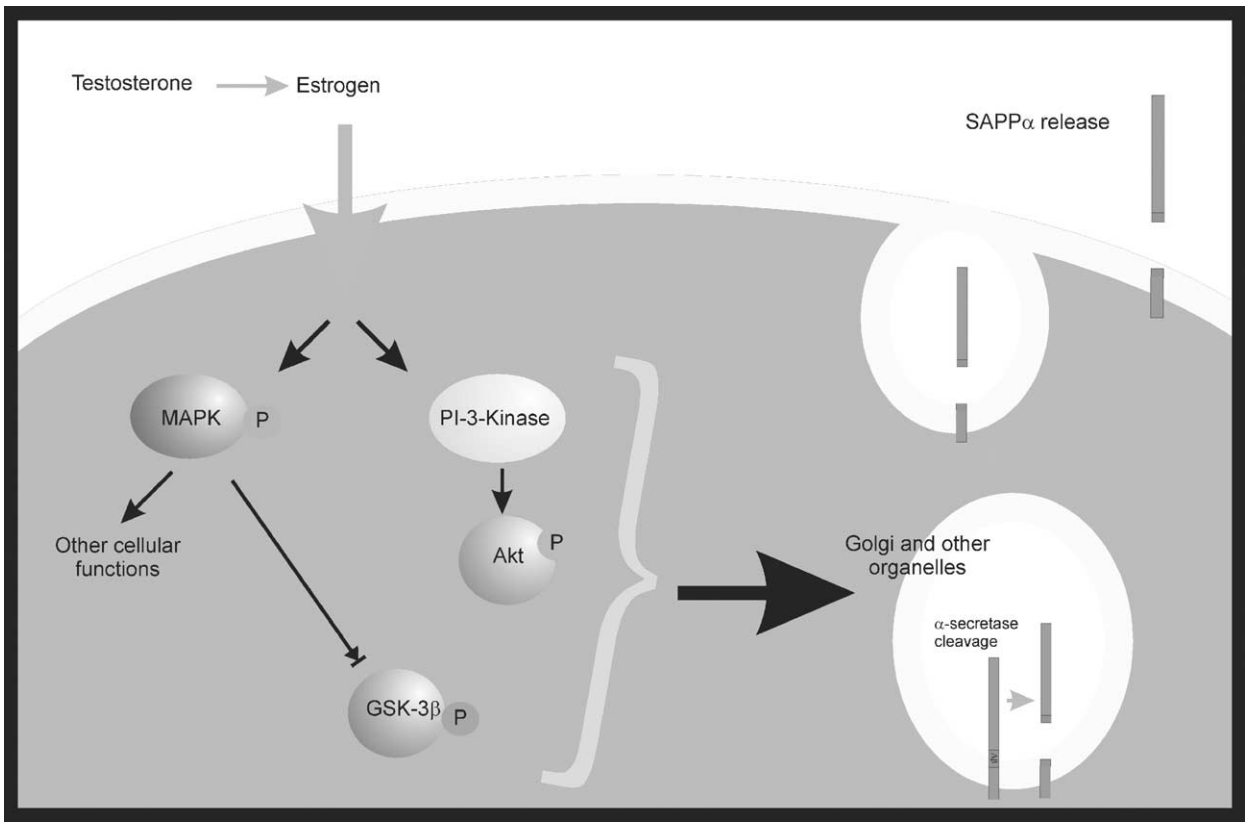

Fig. 2. Schematic representation of the cell signalling pathways associated with estrogen-induced sAPPα release. See text for details.

kinase pathway can stimulate the α-cleavage of APP and subsequent release of this into the extracellular space. Any stimuli that increases APP cleavage by the α-secretase logically would mean that there was less APP to be cleaved via the β-secretase pathway and therefore less Aβ generated. This suggests that any therapy that stimulates any of these signalling pathways may prove useful in inhibiting or reversing Aβ deposition seen in AD.

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