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Review Article

The Role of GSK3 in Presynaptic Function

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The past ten years of research have identified a number of key roles for glycogen synthase kinase 3 (GSK3) at the synapse. In terms of presynaptic physiology, critical roles for GSK3 have been revealed in the growth and maturation of the nerve terminal and more recently a key role in the control of activity-dependent bulk endocytosis of synaptic vesicles. This paper will summarise the major roles assigned to GSK3 in both immature and mature nerve terminals, the substrates GSK3 phosphorylates to exert its action, and how GSK3 activity is regulated by different presynaptic signalling cascades. The number of essential roles for GSK3, coupled with the numerous signalling cascades all converging to regulate its activity, suggests that GSK3 is a key integrator of multiple inputs to modulate the strength of neurotransmission. Modulation of these pathways may point to potential mechanisms to overcome synaptic failure in neurodegenerative disorders such as Alzheimer's disease.

1. Introduction

Glycogen synthase kinase 3 (GSK3) is a serine/threonine kinase that was originally identified as a regulator of cell metabolism but has a variety of roles in cellular function including cell survival, proliferation, neural development, and neurotransmission [1, 2]. GSK3 exists as two isoforms encoded by separate genes: GSK3α (51 kDa) and GSK3β (47 kDa) [3]. Both isoforms are ubiquitously expressed and, although structurally similar, perform overlapping but nonidentical functions [3]. GSK3 is constitutively active in most cells and is negatively regulated by phosphorylation at its N-terminus (Ser-21 for GSK3α and Ser-9 for GSK3β) by a variety of upstream signalling cascades [4]. GSK3 is an unusual kinase in that it generally only phosphorylates a substrate after a previous phosphorylation of the substrate by another protein kinase, an event called "priming" [1, 3]. GSK3 has been implicated in several neuronal disorders such as schizophrenia, bipolar disorder, and Alzheimer's disease [4, 5].

A number of the disorders mentioned above are part of a growing list of diseases called synaptopathies that have at their core a defect in synaptic communication [6]. GSK3 is expressed at the synapse and is found in both immature and mature nerve terminals (presynapses) [7–10]. This localisation suggested a role for GSK3 in presynaptic function and over the past 10 years this has been revealed, ranging from axonal growth and synaptogenesis to regulation of synaptic vesicle (SV) recycling in the mature synapse. This paper will summarise these studies and place the function of GSK3 in the context of nerve terminal physiology.

2. GSK3 Function in Immature Nerve Terminals

2.1. GSK3 Role in Axonal Growth and Polarity. GSK3 signalling is essential for multiple aspects of synaptogenesis: the formation of a functional synapse from nascent neurites. For the purposes of this paper, only the presynaptic contribution of GSK3 in synaptogenesis will be considered; however GSK3 also has multiple postsynaptic roles (for reviews see [11, 12]).

One of the first elements of synaptogenesis is axonal growth. There is an essential requirement for GSK3 activity in this process, with numerous studies demonstrating that an inhibition of GSK3 function greatly reduces axon elongation [13–15]. In addition to controlling growth, GSK3 has a key role in establishing neuronal polarity, specifically the differentiation of immature neurites into nascent axons. Inactive GSK3β (phosphorylated at Ser9) is localised at the

tip of all immature neurites before polarization; however, once polarization is triggered, it is restricted to only the single axonal tip [7, 13, 16]. Thus the localised inactivation of GSK3 seems to be critical for axonal polarity to occur. In agreement, inhibition of GSK3 function using pharmacological antagonists, peptide inhibitors or siRNA all induced the formation of multiple axons [7, 15, 16], whereas overexpression of a constitutively active GSK3β mutant (Ser9Ala) inhibited axon formation in primary neuronal culture [7, 16].

The control of localised microtubule dynamics is critical for neuronal polarization [17], and a number of downstream GSK3 substrates have microtubule organising activity. Collapsin response mediator protein 2 (CRMP2) promotes microtubule polymerization and when overexpressed in neurons produced multiple axons [18, 19]. Also, adenomatous polyposis coli (APC) protein stabilises microtubules in its nonphosphorylated form and is enriched in the nascent axon [20, 21]. GSK3 phosphorylates both CRMP2 and APC *in vitro* [16, 20, 22] and inhibition of GSK3 blocks CRMP2 phosphorylation in polarized axonal tips in culture [16]. Furthermore a non-phosphorylatable mutant of CRMP2 does not promote axon elongation but induces multiple axons when overexpressed in neurons [16, 22]. Thus phosphorylation of either CRMP2 or APC by GSK3 inhibits their binding to microtubules [16, 20]. This promotes microtubule polymerization, thus promoting growth and preventing axonal polarization.

The control of GSK3 activity in both axonal growth and polarization is regulated by an array of different growth factors. Classically, GSK3 is negatively regulated via downstream signalling cascades, involving action of phosphatidylinositol 3-kinase (PI3K) and Akt (also known as protein kinase B) [7, 13, 16, 23]. This is supported by studies showing that overexpression of constitutively active Akt or siRNA knockdown of PTEN (phosphatase and tensin homolog; which both inhibit GSK3) resulted in formation of multiple axons in culture [7]. In agreement these effects were prevented by the coexpression of constitutively active GSK3β (Ser9Ala) indicating that the same signalling pathway was involved [7]. Thus inactivation of GSK3 by growth factors is a critical event in axon formation via the prevention of phosphorylation of a number of key microtubule organising substrates (Figure 1).

2.2. GSK3 Role in Axonal Remodelling. GSK3 activity is also critical for the process of axonal remodelling. Axonal remodelling occurs when an axon meets its postsynaptic target and describes the process of decreased axonal growth, increased axon diameter and increased growth cone size. Axonal remodelling in mammalian neurons is controlled by the negative regulation of GSK3 via Wingless Integration (Wnt) proteins.

Wnts are secreted factors that initiate a downstream signalling cascade by binding to Frizzled receptors on the plasma membrane. The canonical Wnt signalling pathway prevents a protein complex of axin, APC, and GSK3 from targeting β-catenin for destruction in the proteasome. β-catenin phosphorylation by GSK3 is a key step in this
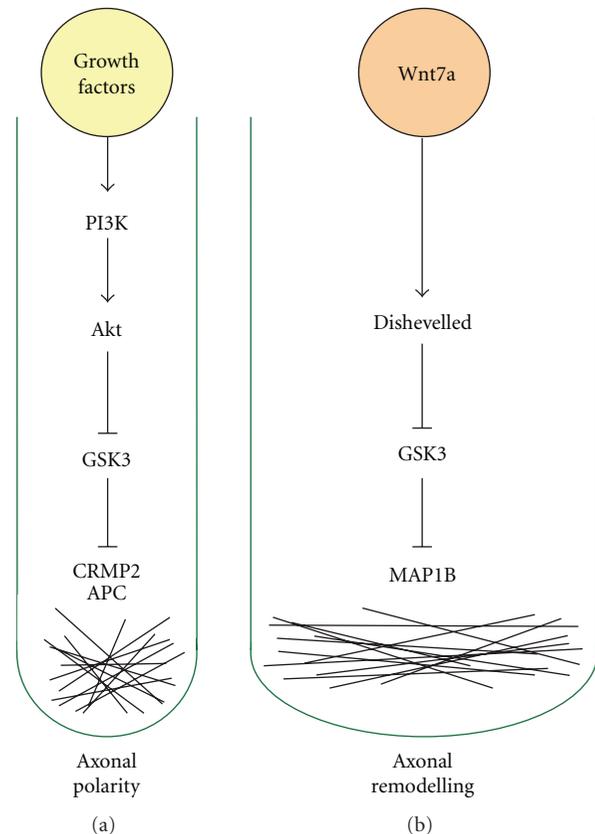
FIGURE 1: *GSK3 roles, substrates and signalling in immature nerve terminals.* (a) Inhibition of GSK3 activity is essential for the establishment of axonal polarity. GSK3 activity is inhibited by growth factors that signal through the PI3K/Akt signal transduction cascade. When GSK3 is active, it phosphorylates both CRMP-2 and APC, which prevents their interaction with microtubules, arresting microtubule polymerization. When GSK3 is inhibited by growth factors, it cannot phosphorylate CRMP-2 or APC; therefore microtubule polymerization is stimulated. (b) Axonal remodelling describes decreased axonal growth, increased axon diameter, and increased growth cone size when a nascent axon meets a postsynaptic target. Inhibition of GSK3 increases microtubule stability to allow axonal remodelling. GSK3 activity is inhibited by a divergent Wnt signalling cascade. When GSK3 is active, it phosphorylates MAP-1B; which results in increased microtubule instability. When GSK3 is inhibited by Wnts, it cannot phosphorylate MAP-1B therefore microtubules are stabilised.

targeting process. When Wnt binds to Frizzled receptors, this activates the Dishevelled protein to inhibit the function of the "destruction complex" resulting in an inability of GSK3 to phosphorylate β-catenin. Non-phosphorylated β-catenin then translocates to the nucleus to trigger the expression of a number of "Wnt" genes [24].

Axonal remodelling has been studied extensively at the mossy fibre-granule cell synapse in the cerebellum [11], where Wnt7a and Dishevelled both play a key role in this event [8, 25, 26]. Inhibition of GSK3 activity using the antagonist lithium mimics the effect of either Wnt7a or Dishevelled on axonal remodelling [27–29], suggesting

a key downstream role for GSK3. However a divergent pathway to the canonical Wnt route operates in axonal remodelling, where modulation of GSK3 activity results in dynamic changes to the microtubule cytoskeleton rather than alterations in gene expression [11, 12].

The link between the inhibition of GSK3 activity and changes in microtubule dynamics is the microtubule binding protein microtubule-associated protein-1B (MAP-1B). GSK3 phosphorylates MAP-1B both *in vitro* and *in vivo*, which results in altered microtubule organisation and increased microtubule instability within the axon [27, 30–32]. In growth cones the great majority of microtubules are dynamic, whereas on activation of the Wnt7a pathway both stable and dynamic microtubules are present, which form large loop-like structures [26]. This effect of Wnt7a on microtubule dynamics is mimicked by either overexpression of Dishevelled or inhibition of GSK3 [27, 29, 31]. Thus inhibition of GSK3 by a divergent Wnt signalling pathway increases microtubule stability by decreasing MAP-1B phosphorylation and thus allowing axonal remodelling (Figure 1).

2.3. GSK3 Role in Synaptogenesis. After axonal remodelling the growth cone has to differentiate into a nerve terminal. GSK3 again has key roles in this process, via the same divergent Wnt signalling pathway that controls axonal remodelling. For example, Wnt7a is a key retrograde messenger that acts to induce presynaptic maturation, as evidenced by the clustering of vesicle proteins at nascent synaptic sites [26]. Dishevelled is also essential for this process [8]. GSK3 is part of this maturation response too, since its inhibition mimics the effect of Wnt7a and Dishevelled on the clustering of synaptic proteins [26, 28]. The mechanism of GSK3 action is independent of transcription, since Wnt application caused an increase in the size and number of synaptic sites but not the expression of presynaptic proteins [8]. Similarly the Wnt7a knockout mouse displays defective localisation of presynaptic proteins but no decrease in their overall level [8]. β-catenin is also required for SV clustering, suggesting that its phosphorylation by GSK3 may control this event [33, 34]. This is unlikely however, since β-catenin operates independent of Wnt signalling in this process.

A very similar control of synaptogenesis is also observed in *Drosophila,* where the fly homologue of GSK3, *shaggy (Sgg),* is localised presynaptically. A hypomorphic mutation in *Sgg* resulted in an 80% increase in the number of boutons (nerve terminals), an effect rescued by expression of wild type *Sgg* [35]. Conversely increased expression of *Sgg* reduced the number of synaptic boutons below that seen with wild type [35, 36]. Finally a large increase in bouton number was observed when a dominant negative version of *Sgg* was expressed using a presynaptic driver [35].

Sgg is a likely downstream target of the *Wingless* pathway, since most components of the canonical pathway (*Wingless, Arrow,* and *Dishevelled*) are all expressed presynaptically [36]. In agreement, overexpression of dominant negative *Dishevelled* mimicked the reduction in bouton number observed with *Sgg* overexpression. Conversely *Wingless* overexpression mimicked the effects of presynaptically expressed dominant negative *Sgg* [36].

Interestingly an increase in microtubule loops was observed in flies with the hypomorphic mutation for *Sgg* [35]. This phenotype is very similar to that observed in mammalian axonal remodelling. In a further parallel, the MAP-1B homolog *Futsch* is required for this microtubule looping effect [35]. *Sgg* also phosphorylates *Futsch in vitro* on at least one conserved MAP-1B phosphorylation site [37]. This suggests that inhibition of *Sgg* by the *Wingless* signalling cascade stabilises the microtubule cytoskeleton by preventing the phosphorylation of *Futsch*. Since there are close parallels in the divergent Wnt/*Wingless* pathways in both mammals and *Drosophila,* it suggests that inhibition of GSK3 phosphorylation of MAP-1B may also be important for synaptogenesis in mammals.

In summary, in the immature nerve terminal GSK3 has key roles in axonal growth, polarity, remodelling, and differentiation. These effects are controlled by a range of different signalling cascades and in general are mediated by the phosphorylation-dependent control of the microtubule cytoskeleton.

3. GSK3 Function in Mature Nerve Terminals

Until recently GSK3 was thought to have a purely developmental role in presynaptic physiology; however, recent studies have suggested that it also performs key functions in adult brain, specifically in neurotransmission. In agreement, GSK3 is enriched in adult nerve terminals [8–10].

3.1. GSK3 Role in Neurotransmitter Release. The primary role of the nerve terminal is to release neurotransmitter, via the fusion of SVs by exocytosis. Initial studies that examined the role of GSK3 in neurotransmitter release suggested that it had no role in the process, since modulation of its activity had no effect on hippocampal neurotransmission [9, 10, 38]. Since these studies mainly investigated the postsynaptic response it could be argued that presynaptic effects were overlooked. However, when paired pulse facilitation (PPF) was examined (which is an indicator of the modulation of neurotransmitter release), a lack of role for GSK3 was again apparent. For example, there was no difference in PPF between wild type hippocampal slices and slices obtained from transgenic mice overexpressing GSK3β [9]. Furthermore PPF was unaltered in hippocampal slices exposed to bath application of GSK3 inhibitors [39]. One study has demonstrated an indirect effect of GSK3 inhibition on PPF, but this was only revealed after inhibition of upstream PI3K [40]. In the same study GSK3 inhibitors partially reversed an inhibition of KCl-evoked glutamate release from synaptosomes produced by PI3K antagonists [40]. Thus there is little evidence to date that GSK3 plays a direct role in neurotransmitter release from central nerve terminals.

Other studies have monitored the role of GSK3 in SV exocytosis rather than neurotransmitter release itself. In agreement with its lack of effect on neurotransmitter release, GSK3 antagonists had no effect on SV exocytosis in

cultured neurons monitored using action potential-evoked unloading of the fluorescent dye FM1-43 [39]. In contrast, overexpression of wild type GSK3β in primary hippocampal cultures retarded FM dye unloading evoked by elevated KCl [41]. This effect was proposed to be due to GSK3-dependent phosphorylation of an intracellular loop of a P/Q-type voltage-dependent calcium channel (VDCC). In support, GSK3β phosphorylated a recombinant GST-fusion protein containing this region *in vitro* and GSK3 antagonists reversed a phosphorylation of P/Q-type VDCCs in synaptosomes that was evoked by inhibition of PI3K [41]. It was proposed that GSK3 phosphorylation inhibits P/Q-type channel activity, since overexpression of wild type GSK3β reduced calcium influx in hippocampal neurons. The lack of an identified phosphorylation site did not allow this hypothesis to be tested directly; however, if true it would provide a mechanism by which the strength of neurotransmission could be regulated by GSK3 phosphorylation, since P/Q-type VDCCs are tightly coupled to neurotransmitter release [42]. Thus no direct role for GSK3 in neurotransmitter release has yet been shown; however, it may indirectly regulate this event via phosphorylation of VDCCs.

3.2. GSK3 Role in Activity-Dependent Bulk Endocytosis. In contrast to its, as yet undetermined, role in neurotransmitter release, GSK3 activity has a key role in SV endocytosis. Two major endocytosis modes exist in nerve terminals to retrieve SVs after their fusion by exocytosis. Clathrin-mediated endocytosis (CME) retrieves single SVs with a fixed rate and is dominant during low-intensity stimulation [43, 44]. In contrast activity-dependent bulk endocytosis (ADBE) is dominant during high-intensity stimulation [45] and generates large endosomes direct from the plasma membrane from which SVs can then bud [46]. Recent studies have shown that GSK3 activity is essential for ADBE but not CME during high-intensity stimulation and have also identified at least one GSK3 substrate whose phosphorylation is essential for the process.

The specific role for GSK3 in ADBE was discovered using a battery of different SV recycling assays in primary neuronal culture [39]. First, uptake of large fluorescent dextran molecules (too large to be accumulated inside single SVs) was inhibited with either GSK3 antagonists or by silencing GSK3β expression using shRNA. Second, pharmacological inhibition of GSK3 reduced the uptake of small fluid phase markers into bulk endosomes but not single SVs. Finally, GSK3 antagonists arrested uptake of FM1-43 (which labels both ADBE and CME modes [47]) but not FM2-10 (which only labels CME). There was an activity-dependent requirement for GSK3 in these events, with no role of GSK3 observed using identical assays during low-intensity stimulation. This agrees with a selective role for the enzyme in ADBE. One further key observation was that acute application of GSK3 antagonists had no effect on ADBE, whereas their continual application resulted in inhibition [39]. This indicated that GSK3 had no role during stimulation itself but was required to phosphorylate a substrate(s) after stimulation had terminated.

A number of endocytosis proteins are rephosphorylated after stimulation of the nerve terminal. They are called the dephosphins since they are co-ordinately dephosphorylated by the calcium-dependent protein phosphatase calcineurin on nerve terminal stimulation [48]. One of these proteins, the large GTPase dynamin I, is phosphorylated on two major sites on its C-terminus in resting nerve terminals (Ser-774 and Ser-778) [49]. The dephosphorylation of these sites by calcineurin recruits the endocytosis protein syndapin I to dynamin I [50]. The activity-dependent dephosphorylation of dynamin I and its interaction with syndapin I and syndapin I itself are all essential requirements for the triggering of ADBE [51].

Since dynamin I dephosphorylation is a critical event in the triggering of ADBE, its subsequent rephosphorylation after stimulation is also essential for the process. Dynamin I was originally thought to be rephosphorylated on both major sites by cyclin-dependent kinase 5 (cdk5, see [49]). In agreement with a key role for this rephosphorylation event, cdk5 activity is essential for ADBE but not CME [52]. However it was recently demonstrated that cdk5 is in fact a priming kinase for GSK3 on dynamin I, with cdk5 phosphorylating Ser-778, allowing GSK3-dependent phosphorylation of Ser-774 [39]. This was shown in both *in vitro* phosphorylation assays and also intact neuronal cultures using selective cdk5 and GSK3 antagonists [39]. A direct functional link between GSK3-dependent phosphorylation of dynamin I to ADBE was shown by the inhibition of dextran uptake by overexpression of dominant negative mutants encompassing mutations at Ser-774 [39]. Thus the rephosphorylation of Ser-774 on dynamin I by GSK3 is an essential requirement for the maintenance of ADBE during high-intensity stimulation. This is the first, and to date the only, demonstration that GSK3 activity controls SV recycling via direct phosphorylation of the SV trafficking machinery.

There are eight different dephosphin proteins, of which at least two are also rephosphorylated by cdk5. The fact that GSK3 acts in concert with cdk5 to phosphorylate dynamin I suggests that these proteins may also be essential for ADBE. These proteins are the lipid phosphatase synaptojanin and the lipid kinase phosphatidylinositol phosphate kinase type Iγ (PIPKIγ) which either break down or synthesise phosphatidylinositol (4,5) bisphosphate (PI(4,5)P$_2$), respectively [53, 54]. The activity of both of these enzymes is essential for SV endocytosis [55, 56], and importantly both enzymes are activated by their dephosphorylation by calcineurin and are inhibited by their rephosphorylation by cdk5 [57, 58]. Scans of the protein sequences of both synaptojanin and PIPKIγ using bioinformatic prediction software highlight multiple consensus sequences for GSK3 phosphorylation (S/T XXX S/TP, where GSK3 phosphorylates the first S/T and cdk5 the second). Thus it is eminently possible that a cdk5 rephosphorylation event also primes these enzymes for GSK3 phosphorylation after high-intensity stimulation. The determination of whether GSK3 both phosphorylates and/or regulates these enzymes' activity is imperative, since a tight temporal control of lipid metabolism is essential for ADBE [59].

3.3. Regulation of GSK3 in Mature Nerve Terminals. GSK3 is a highly regulated kinase, with its high basal activity negatively regulated by numerous signal transduction cascades [1, 3]. Since GSK3 activity is a key requirement for ADBE, it suggests that activation of a number of these signal transduction cascades may also regulate ADBE. GSK3 regulation may occur in two different instances, either acute control driven by action potential firing or longer-term control driven by activation of signalling cascades by growth factors/hormones.

Depolarization of neurons by prolonged stimulation results in GSK3 phosphorylation and thus its inactivation [60, 61]. This suggests that GSK3 activity could be acutely regulated during transient depolarization by action potential stimulation. This is indeed the case, with phosphorylation of GSK3 being triggered during intense, but not mild action potential stimulation in primary neuronal cultures (Smillie unpublished). Interestingly dynamin I is only dephosphorylated by calcineurin during high-, but not low-intensity stimulation [51]. Thus when dynamin I and the dephosphins are being dephosphorylated by calcineurin, GSK3 is simultaneously inactivated allowing maximal dephosphorylation to occur.

There are a number of possible candidates for the protein kinase that mediates the acute activity-dependent phosphorylation of GSK3. For example, the calcium-dependent protein kinase CaMKII phosphorylates GSK3 during chronic depolarization [62]. Also the activity of Akt is increased by prolonged membrane depolarization [63–65], suggesting that it may also phosphorylate GSK3 under these conditions. Preliminary findings from our laboratory suggest that Akt may be the activity-dependent GSK3 kinase, since Akt activation only occurred during intense, but not mild, neuronal stimulation (Smillie, unpublished). Thus an activity-dependent signal transduction cascade may activate Akt to inhibit GSK3 in nerve terminals, aiding the triggering of ADBE (Figure 2). The identity of the upstream components of this cascade will be of great interest and is currently under investigation.

GSK3 activity in mature nerve terminals can also be controlled in the longer term by classical signalling cascades. To date there are three major pathways identified which converge on GSK3 and all result in its phosphorylation and inhibition. Amino acids signal through the mTOR/S6K pathway, insulin and growth factors signal via a PI3K/Akt pathway, and growth factors also act through activation of MAPK and MAPKAP-K1 [1]. Considering the key role for GSK3 in ADBE, longer-term inhibition by any of these cascades should greatly reduce the functionality of this SV endocytosis mode (Figure 2). In agreement, overexpression of a constitutively active form of Akt results in the arrest of ADBE but not CME in primary neuronal cultures (Smille unpublished). It will be of great interest to identify the ligands that trigger these signal transduction cascades to determine how they control both GSK3 activity and modulate ADBE.

The converse of regulated inhibition of GSK3 via its phosphorylation is its activation by its dephosphorylation [1]. This will be critical for the activity-dependent inhibition
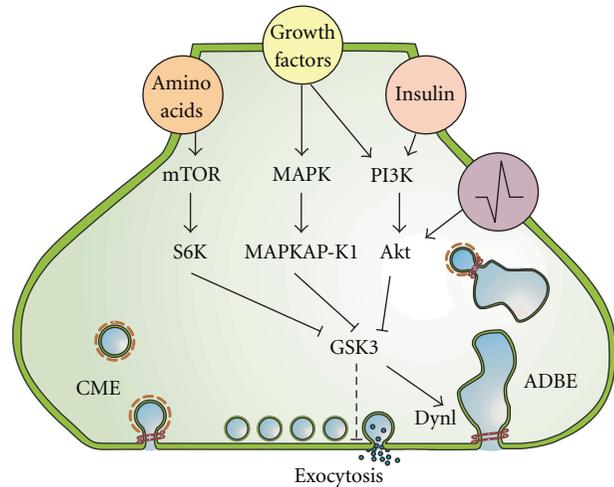
FIGURE 2: *GSK3-dependent control of SV recycling in mature nerve terminals.* When GSK3 is active, it phosphorylates Ser774 on dynamin I, which inhibits the interaction between dynamin I and syndapin I. This interaction is essential for the triggering of activity-dependent bulk endocytosis (ADBE) and thus during high-intensity stimulation GSK3 is inhibited in an activity-dependent manner by Akt (action potential symbol). After stimulation, GSK3 is active and can rephosphorylate dynamin I. If the rephosphorylation of dynamin I by GSK3 is inhibited, the subsequent cycle of ADBE is arrested. Signal transduction cascades that may regulate GSK3 activity in the longer term are: a mammalian target of rapamycin (mTOR)/S6 kinase pathway (activated by amino acids), a PI3K/Akt pathway (activated by either insulin or growth factors), or a mitogen-activated protein kinase (MAPK)/MAPKAP-K1 pathway (activated by growth factors). GSK3 has no role in clathrin-mediated endocytosis (CME). GSK3 may perform an additional role in SV recycling by negatively regulating neurotransmitter release (dotted line). This is because GSK3 is proposed to phosphorylate P/Q-type calcium channels and inhibit calcium influx into the nerve terminal.

of GSK3, since it must then be rapidly dephosphorylated after stimulation to rephosphorylate dynamin I. GSK3 can be dephosphorylated by either PP1 or PP2A protein phosphatases [66]. In agreement, the PP1 and PP2A antagonist okadaic acid increases the basal phosphorylation of GSK3 in both hippocampal slices and primary neuronal cultures [10, 67]. Another possible level of complexity has recently been reported with possible differential dephosphorylation of GSK3α and GSK3β by PP2A and PP1, respectively [68].

In ADBE, GSK3 acts in concert with the calcium-dependent protein phosphatase calcineurin. Interestingly these two enzymes are related on a number of different functional levels. For example, calcineurin activity can be arrested by an endogenous inhibitor called regulator of calcineurin 1 (RCAN1) [69]. Both RCAN1 activity and its degradation are regulated by GSK3-dependent phosphorylation [70–72]. In addition, RCAN1 activity also increases expression of GSK3 [73]. Thus the function and regulation of both calcineurin and GSK3 are tightly controlled by a number of intricate feedback pathways, highlighting the key roles their activities play in nerve terminal function. Thus control of GSK3

dephosphorylation, and indeed the control of calcineurin by GSK3, may be critical for the rephosphorylation of dynamin I and control ADBE in nerve terminals.

In summary GSK3 has one recently identified key role in the function of the mature nerve terminal, an essential requirement in ADBE. It remains to be seen whether this is its only role in the SV life cycle or whether this is the first of many to be revealed.

4. Presynaptic Function of GSK3 in Alzheimer's Disease

As discussed above, GSK3 activity plays key roles in shaping nerve terminal development and function. Thus regulation of its activity may provide a mechanism to increase synaptic function, especially in neurodegenerative disorders such as Alzheimer's disease. In Alzheimer's disease, synapse loss often precedes neuronal death [74], therefore modulation of the key identified roles of GSK3 in synaptogenesis offers the possibility of either generating new synaptic contacts or at least stabilising existing synapses before irreversible neuronal loss occurs.

Similarly, synaptic failure is one of the first events observed in patients with mild cognitive impairment, a prodromal state of Alzheimer's disease [74]; The essential role for GSK3 in ADBE suggests that its modulation may impact on neurotransmission during periods of intense neuronal activity such as during learning and memory generation. In agreement, inhibition of GSK3 results in increased neurotransmission during high-intensity stimulation [39]. Thus modulation of presynaptic GSK3 activity may either increase synaptic function or at least slow synaptic failure. This is especially interesting since GSK3 activity can be regulated by a wide variety of signal transduction cascades, providing possible entry points for pharmacological intervention in the disease.

In summary, the past 10 years of research have revealed that GSK3 is a key integrator of presynaptic signalling whose output determines the strength of neurotransmission via synaptic remodelling, synaptic strength, or synaptic capacity. The next 10 years will determine whether modulation of any of these key functional roles will facilitate the treatment of neurodegenerative disorders such as Alzheimer's disease.

Acknowledgment

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