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The Role of Glycogen Synthase Kinase 3 Beta in Neuroinflammation and Pain

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Abstract

Neuroinflammation is a crucial mechanism related to many neurological diseases. Extensive studies in recent years have indicated that dysregulation of Glycogen Synthase Kinase 3 Beta (GSK3β) contributes to the development and progression of these disorders through regulating the neuroinflammation processes. Inhibitors of GSK3β have been shown to be beneficial in many neuroinflammatory disease models including Alzheimer's disease, multiple sclerosis and AIDS dementia complex. Glial activation and elevated pro-inflammation cytokines (signs of neuroinflammation) in the spinal cord have been widely recognized as a pivotal mechanism underlying the development and maintenance of many types of pathological pain. The role of GSK3β in the pathogenesis of pain has recently emerged. In this review, we will first assess the GSK3β structure, regulation, and mechanisms by which GSK3β regulates inflammation. We will then describe neuroinflammation in general and in specific types of neurological diseases and the potential beneficial effects induced by inhibiting GSK3β. Finally, we will provide new evidence linking aberrant levels of GSK3β in the development of pathological pain.

Introduction

Glycogen synthase kinase 3 (GSK3) is a serine/threonine protein kinase, which is part of the mitogen activated protein (MAP) kinase family and is pivotal in many signaling cascades [1]. GSK3 is important in metabolism and signaling in development. The role of GSK3β in mediating peripheral and central nervous system inflammation in a multitude of neurological disorders has been extensively studied [2-6]. Studies of the role of GSK3β in pathological pain have recently just begun [5,7]. In the brain, GSK3β is localized primarily to neurons [8], but has also been shown to be in glial cells [9].

Inflammation of the brain has become recognized as a common feature shared by many neurological disorders like Alzheimer's disease[10-12], schizophrenia [13,14], multiple sclerosis [15,16], and HIV induced dementia [17,18]. Aberrant levels or activities of GSK3 play a critical role in the development of these diseases and pharmacological inhibition of GSK3β ameliorates these diseases [19-23]. Inflammation is also a critical component contributing to the development and maintenance of pathological pain induced by peripheral tissue or nerve injury. Accumulation of inflammatory cells including macrophages, neutrophils at the peripheral injury site and the dorsal root ganglion, proliferation and activation of microglia and astrocytes in the spinal dorsal horn, as well as the release of pro-inflammatory cytokines and other pro-inflammatory mediators in the injury site, the dorsal root ganglion and the spinal dorsal horn have all been shown to contribute to the development and maintenance of pathological pain [24-27]. Similarly, pharmacological inhibition of GSK3β has been recently shown to attenuate pathological pain induced by nerve injury or formalin injection [5,7].

In this review, we will first briefly discuss the history, structure, regulation, and pharmacology of GSK3β. We will then provide an overview of neurological diseases including pathological pain where neuroinflammation plays a crucial role and how GSK3β may play a role in the progression of these diseases.

Brief History, Functional Properties, and Structural Insights of GSK3

Glycogen Synthase Kinase 3 (GSK3) was first purified from rabbit skeletal muscle in 1980 and subsequently classified as a kinase based on its ability to phosphorylate and inactivate Glycogen Synthase, acting as a regulator in Glycogen synthesis [28]. However, Glycogen Synthase was thought to exist as early as the 1960s [29]. This kinase was later isolated and characterized from rat skeletal muscle [30]. Three forms of Glycogen Synthase Kinase were further identified that are referred to as Glycogen Synthase Kinase 3, Glycogen Synthase Kinase 4, and Glycogen Synthase Kinase 5, which regulates Glycogen Synthase by producing different levels of phosphorylation [31]. Glycogen Synthase Kinase 5 is referred to as Casein Kinase-2 (CK2), which is a primer of Glycogen Synthase that is phosphorylated by GSK3 [32,33]. In the early 1990s, it was shown that there are two similar forms of GSK3, GSK3-alpha (GSK-3α) and GSK3-Beta (GSK-3β)[8,34]. GSK3α and GSK3β differ in their C and N terminals, however, they share 98% sequence homology in their catalytic domains resulting in 84% overall sequence homology [8]. GSK3 is a serine/threonine kinase which is constitutively active in resting cells from a variety of tissues [35,36]. GSK3 has been implicated in many cellular processes and is thought to phosphorylate over 50 substrates [6]. In the following sections, we will mainly focus on GSK3β.

Through recent advances in bioinformatic approaches,

we have used the web service software from pathwayLinker to produce a link between GSK3β and its signaling pathways (Figure 1) [37]. In addition to the protein signaling pathways, Table 1 represents signaling pathways where GSK3β is significantly involved [37]. As can be seen in Figure 1 and Table 1, GSK3β is involved in a diverse range of signaling pathways. Some of the classic pathways involved in inflammation and pain which are represented in Table 1 are the chemokine, B cell, opioid, leukocyte, and toll-like receptor signaling pathways.

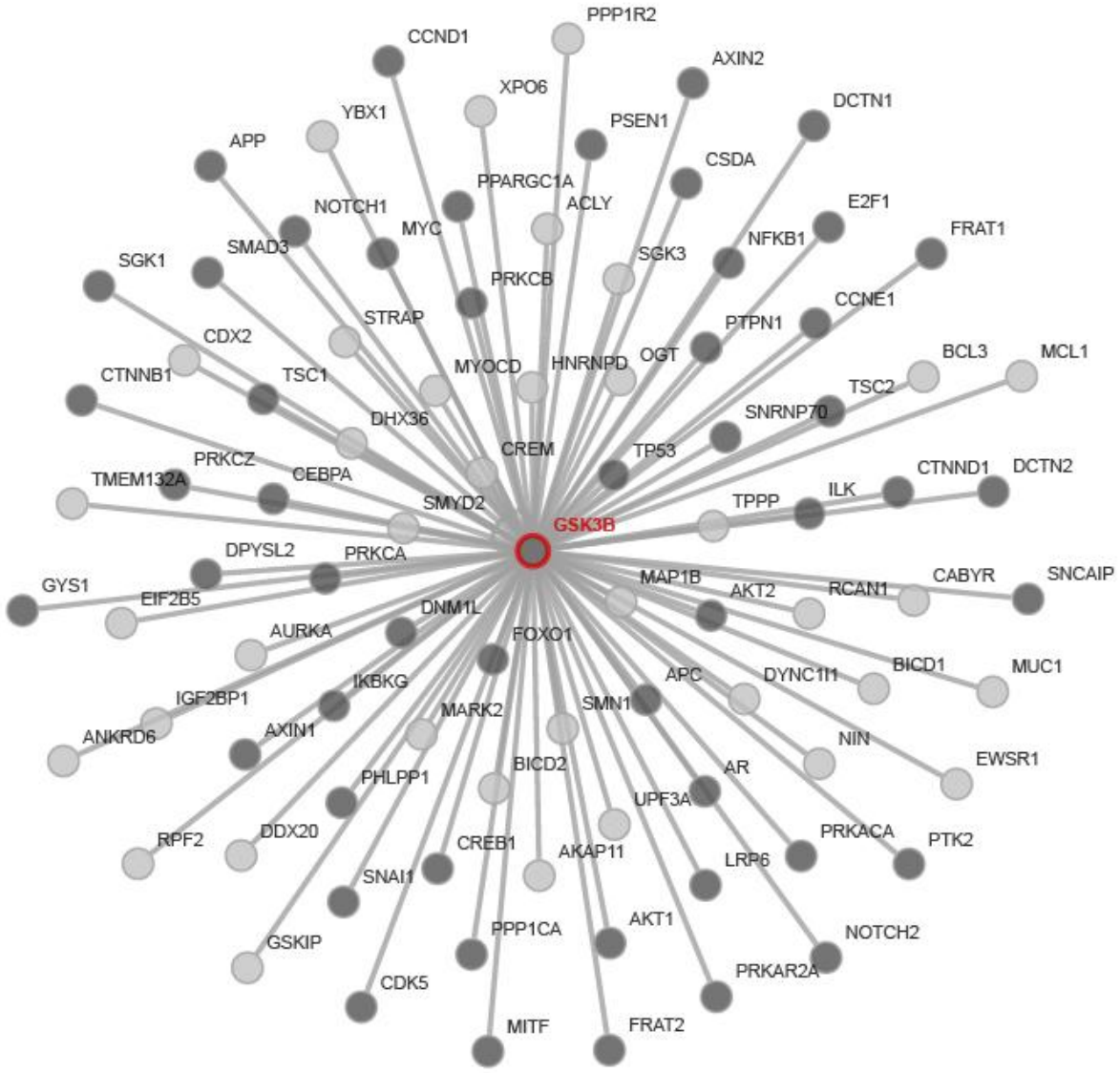

Figure 1: GSK3β and first neighbor interactions in *Homo sapiens*. The dark gray nodes represent interactions involved with proteins in non-signaling pathways. The light gray nodes represent interactions with proteins in signaling pathways. Schematic and interactions of GSK3β were produced using PathwayLinker [37].

Signaling pathway	Displayed proteins	All proteins of pathway	P-value
Cancer (KEGG)	22	502	1.6×10^{-30}
WNT (KEGG)	16	229	1.2×10^{-25}
Prostate cancer (KEGG)	13	127	3.1×10^{-23}
Endometrial cancer (KEGG)	11	73	9×10^{-22}
INS (KEGG)	13	196	1×10^{-20}
Colorectal cancer (KEGG)	11	104	5.5×10^{-20}
SCLC (KEGG)	10	128	6.6×10^{-17}
NGF (Reactome)	11	211	1.6×10^{-16}
CML (KEGG)	9	112	1.9×10^{-15}
WNT (SignaLink)	9	149	2.6×10^{-14}
Pancreatic cancer (KEGG)	8	111	1.8×10^{-13}
Adhesion (KEGG)	10	312	5.3×10^{-13}
NSCLC (KEGG)	7	80	1.5×10^{-12}
AML (KEGG)	7	84	2.2×10^{-12}
Chemokine (KEGG)	9	243	2.2×10^{-12}
Glioma (KEGG)	7	97	6.1×10^{-12}
Apoptosis (KEGG)	7	134	6.1×10^{-11}
ErbB (KEGG)	7	145	1.1×10^{-10}
Melanogenesis (KEGG)	7	151	1.4×10^{-10}
Basal carcinoma (KEGG)	6	79	1.5×10^{-10}
MAPK (KEGG)	9	393	1.6×10^{-10}
Cell cycle (KEGG)	7	173	3.6×10^{-10}
Melanoma (KEGG)	6	95	4.7×10^{-10}
Tight junction (KEGG)	7	180	4.8×10^{-10}
B cell (KEGG)	6	118	1.8×10^{-09}
Opioid (Reactome)	5	79	1.4×10^{-08}
NT (KEGG)	6	187	2.8×10^{-08}
Adipocytokine (KEGG)	5	95	3.5×10^{-08}
VEGF (KEGG)	5	106	6.1×10^{-08}
TGF-beta (SignaLink)	6	223	7.9×10^{-08}
Adherens junction (KEGG)	5	115	9.2×10^{-08}
Fc-gamma (KEGG)	5	124	1.3×10^{-07}
Wnt (Reactome)	4	56	2.6×10^{-07}
Bladder cancer (KEGG)	4	61	3.6×10^{-07}
Thyroid cancer (KEGG)	4	62	3.9×10^{-07}
Leukocyte (KEGG)	5	156	4.2×10^{-07}
Notch (Reactome)	3	16	4.5×10^{-07}
T cell (KEGG)	5	172	6.8×10^{-07}
MTOR (KEGG)	4	78	9.8×10^{-07}
Huntington (KEGG)	5	212	1.9×10^{-06}
P53 (KEGG)	4	93	2.0×10^{-06}

Fc-epsilon (KEGG)	4	101	$2.8x10^{-06}$
LTP (KEGG)	4	113	$4.3x10^{-06}$
Toll-like (KEGG)	4	145	$1.2x10^{-05}$
Smooth muscle (KEGG)	4	150	$1.3x10^{-05}$
Notch (KEGG)	3	62	$2.9x10^{-05}$
Notch (SignaLink)	3	64	$3.2x10^{-05}$
Immune (Reactome)	5	384	$3.3x10^{-05}$
EGF/MAPK (SignaLink)	5	388	$3.5x10^{-05}$
Axon (KEGG)	4	197	$3.9x10^{-05}$
JAK-STAT (KEGG)	4	203	$4.3x10^{-05}$
Ins receptor (Reactome)	3	76	$5.4x10^{-05}$
Alzheimer (KEGG)	4	227	$6.7x10^{-05}$
Hh (SignaLink)	3	85	$7.5x10^{-05}$
Cholerae infection (KEGG)	3	93	$9.8x10^{-05}$
IGF (SignaLink)	3	93	$9.8x10^{-05}$
Gap junction (KEGG)	3	115	$1.8x10^{-04}$
Oocyte (KEGG)	3	121	$2.1x10^{-04}$
GnRH (KEGG)	3	136	$3.0x10^{-04}$
Dorso-ventral (KEGG)	2	33	$4.5x10^{-04}$
Prion (KEGG)	2	45	$8.4x10^{-04}$
DNA-sensing (KEGG)	2	63	$1.6x10^{-03}$
E.coli infection (KEGG)	2	72	$2.1x10^{-03}$
Calcium (KEGG)	3	273	$2.2x10^{-03}$
Hedgehog (KEGG)	2	76	2.4×10^{-03}
Endocytosis (KEGG)	3	284	2.5×10^{-03}
NOD-like (KEGG)	2	83	2.8×10^{-03}
RIG-I-like (KEGG)	2	87	3.1×10^{-03}
Actin (KEGG)	3	317	3.4×10^{-03}
Renal cancer (KEGG)	2	99	4.0×10^{-03}
Depression (KEGG)	2	102	4.2×10^{-03}
Helicobacter infection (KEGG)	2	103	4.3×10^{-03}
PtdIns (KEGG)	2	111	5.0×10^{-03}
TGF (KEGG)	2	112	5.1×10^{-03}
NK cell (KEGG)	2	189	0.014
TGF beta (Reactome)	1	15	0.014

Table 1: GSK3β associated signaling pathways that are over expressed in *Homo sapiens*. Briefly, all first neighbor interactions of GSK3β and all proteins of *Homo sapiens* are queried against different signaling pathways. Overrepresentation is determined if members of the different signaling pathways are increased in the first neighbor interactions of GSK3β compared to all proteins. Signaling pathway indicates the signaling pathway along with the source. Displayed proteins indicate first neighbor interactions of GSK3β. All proteins of pathway represent the total number of proteins implicated in the signaling pathway. Pathway Linker was used to identify overexpressed signaling pathways [37].

Multiple groups have worked at idenfifying and characterizing the crystal structure of GSK3β. The first crystallized structure of GSK3β occurred in 2001 and provided insight into the mechanism by which the kinase is phosphorylated. The initial crystallization occurred in the unphosphorylated (apo) form of GSK3β (PDB ID: 1I09) [38]. GSK3β has a two domain kinase fold where the N-terminal end has a β-strand domain composed of 7 anti-parallel beta strands and a C-terminal end with a conserved alpha helical domain (Figure 2A,2B). A few characteristics of the kinase that have been determined from the crystal structure are that the kinase has an ATP binding site, an activation loop, and a glycine-rich loop. However, the inhibitory phosphorylation of Serine-9 is not present due to the lack of electron density being visible on the residue. The structure of GSK3β (PBD ID: 1Q5K) was later co-crystallized with the GSK3β inhibitor AR-A014418 in 2003, which provided additional insight into the mechanism of inhibition [39]. The mechanism for inhibition is further discussed in the pharmacology of GSK3 selection.

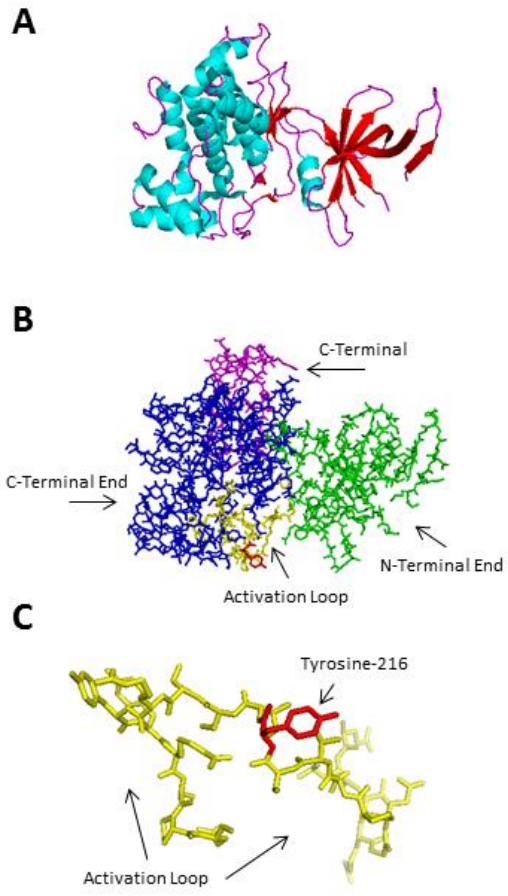

Figure 2: The crystal structure of GSK3β (Protein Data Bank PDBID: 1i09 [38]. A) The secondary structure of GSK3β showing seven anti-parallel β strands at the N-terminal end and an α-helical domain at the C-terminal end. Cyan indicates a helix, magenta indicates a loop, and red indicates a sheet. B) The overall structure of GSK3β. The N-terminal end is labeled green and encompasses residues 25-138, the C-terminal end is labeled blue and encompasses residues 139-343, and the activation loop is labeled yellow and encompasses residues 220-226 where Tyrosine-216 (red) is a phosphorylation site. C) A magnified view of Tyrosine-216, which is located in the activation loop. Figure was generated using PyMOL 1.4.1.

Regulation of GSK3

The regulation of GSK3β has been extensively studied and reviewed [40,41]. There are different ways of regulating GSK3β which occurs through phosphorylation of specific amino acid residues, by the formation of protein complexes with GSK3β, and with pharmacological interventions.

The regulation of GSK3β can occur through the phosphorylation of specific residues and the subsequent activation or inactivation of the kinase. Inactivation of GSK3β can occur through phosphorylation of Serine-9 [8,42], Serine-389 [43], Threonine-390 [43], or Threonine-43 [44], while activation can occur through the phosphorylation of Tyrosine-216 [45]. Phosphorylation of the Serine-9 residue has been extensively studied and is a major mechanism to regulate GSK3β activities by the inactivation of GSK3β. Activation of three upstream signaling pathways are known to inhibit GSK3β activity through phosphorylation of the Serine-9 residue including, the phosphatidylinositide 3-kinases (PI3K), the mammalian target of rapamycin (mTOR), and indirectly through the mitogen-activated protein kinase/extracellular signal-regulated kinases (MAPK/ERK) pathway [41]. The signals which lead to the downstream phosphorylation and inhibition of GSK3β occurs by way of a diverse group of substances including, but not limited to, amino acids, growth factors, esters, and insulin [41].

GSK3β pathways have drawn increased attention and the complexity of the pathways has been heavily studied in the field [3,46,47]. One of the first identified modulators of GSK3β phosphorylation was AKT, which is also termed Protein Kinase B (PKB) and is downstream of the PI3K pathway [48]. Initially, insulin was identified to inhibit GSK3β; however, the mechanism and specificity were unclear [48-50]. In studies that examined the effects of insulin on purified Glycogen Synthases, it was shown that the effect on glycogen metabolism was mediated by Glycogen Synthase 3 [35,48,51]. Glycogen synthase kinase serine residues were later identified to be dephosphorylated by insulin *in vivo* [51]. It was not until the identification of GSK3α and GSK3β when it was determined that insulin negatively regulates GSK3 [52]. The importance of the phosphotidylinositol (PI) 3-kinase in the signaling pathway was demonstrated by showing that inhibition of PI3K reverses insulin induced GSK3 inhibition and that incubation with serine/threonine phosphatases reverses insulin's inhibitor effect [53]. Soon after, serine residues were identified as phosphorylation targets that mediate the inactivation of GSK3 following insulin administration which is facilitated by PKB. The serine residues which are phosphorylated and inactivated following insulin inhibition are Serine-21 for GSK3α and Serine-9 for GSK3β [48].

Two signaling pathways of GSK3β that act as regulators of the kinase are mTOR and ERK. Glycogen synthase and Glycogen synthesis were first shown to be regulated through the mTOR pathway in human muscle cells. It was also shown that amino acid availability regulates glycogen synthesis through GSK3β Serine-9 phosphorylation independently in the mTOR pathway [54]. Following the identification of mTOR as a regulator of GSK3β, S6K was shown to be downstream of mTOR and to regulate GSK3β phosphorylation [55]. These studies show that GSK3β is regulated directly by S6K differently from how it is regulated in the PI3K pathway. Following the inhibition of AKT, S6K phosphorylates the Serine-9 residue of GSK-3β *in vivo* [55]. Another regulator of GSK3β which entails a different signaling pathway is the ERK pathway. This pathway is different from the PI3K and mTOR pathways in that ERK regulates GSK3β through a priming mechanism. In hepatocellular carcinoma, it has been shown that GSK3β is phosphorylated at the Threonine-43 residue following ERK

activation. In response to threonine-43 phosphorylation, MAPK-activated protein kinase 1 (MAPKAP-K1/p90RSK) acts to phosphorylate the primed GSK3β kinase at serine-9 residue rendering it inactive [44].

Another site of phosphorylation is the residue Tyrosine-216, which results in an increased activity of GSK3β [45] (Figure 2C). This was identified by the substitution of the tyrosine site with a phenylalanine residue showing that only Tyrosine-216 is phosphorylated under native conditions. In the catalytic domain, Tyrosine-216 lies in the activation loop and phosphorylation is needed for activity [45]. The activation loop, where catalytic activity increases following phosphorylation of Tyrosine-216, is highlighted in Figure 2B. In Dictyostelium, it has been shown that ZAK1, a tyrosine kinase, activates and phosphorylates GSK3β *in vitro* [56]. Phosphorylation of Tyrosine-216 has been shown to be increased following nerve growth factor (NGF) withdrawal and protein kinase C inhibition with staurosporine *in vitro* [57]. In a ratischemic model, Tyrosine-216 phosphorylation is increased [57]. Lysophosphatidic acid (LPA) has been shown to phosphorylate Tyrosine-216 during neurite retraction [58]. Tyrosine-216 phosphorylation has been shown to occur through phospholipase C activation of Protein Tyrosine Kinase 2 Beta (Pyk2), which phosphorylates the tyrosine on GSK3β and microtubule-associated proteins [59]. The upstream signaling pathways of Tyrosine-216 phosphorylation have been minimally studied compared to Serine-9 inhibition, and these studies indicate that tyrosine activation may play a role in the regulation of GSK3β; however, further studies are needed to examine the role of tyrosine phosphorylation in disease progression.

GSK3β can also be regulated through the formation of protein complexes which occurs in both the Wnt and Hedgehog pathways [60, 61]. The Wnt pathway has been shown to play a role in tissue maintenance, cell-cell interactions, and dysfunction in signaling may lead to degenerative diseases [46]. Proteins which bind with GSK3β to form an active or inactive complex are Axin and GSK3 binding protein (GBP), respectively [62]. In Xenopus embryos, it has been shown that the Wnt pathway is negatively regulated by the presence of Axin [63]. Through protein isolated from a rat cDNA library, it has been determined, *in vitro*, that the interaction between GSK3β and beta-catenin is negatively regulated through Axin binding to GSK3β in the Wnt pathway [62]. Axin is stabilized by the phosphorylation of GSK3β, which causes an increase of Axin [64]. Briefly, GSK3 forms a complex with Axin and adenomatous polyposis coli (APC), both substrates of GSK3, which phosphorylate beta-catenin leading to degradation in the absence of an initial Wnt ligand [65-67]. However, when a Wnt ligand is present, GSK3 does not form a complex with Axin and active GSK3 inhibits phosphorylation of beta-catenin preventing degradation of beta-catenin through the proteasome pathway [68,69]. These studies indicate the importance of GSK3β complex formation in the Wnt pathway and the role of GSK3β inhibition on signaling [70].

The Hedgehog pathway is important in both normal development and the production of cancers [71,72]. GSK3 has been shown to be a negative regulator of the Hedgehog pathway in Drosophila through forming a complex with Protein Kinase A (PKA) for proteolytic processing of the DNA-binding protein, Cubutis interruptus (Ci) [61]. Briefly, a complex forms between PKA, GSK3, and casein kinase 1 (CK1) which binds to costal-2 (Cos2). Following binding to Cos2, the complex phosphorylates Ci causing an inhibitory effect on the pathway due to the phosphorylation [72]. The phosphorylation of Ci causes the proteolytic degradation from an active form to an inactive form [73]. The decrease in phosphorylation of Ci leads to a decrease in proteolytic processing producing an opposite effect [61]. These studies indicate the relevance of GSK3β in

complex formations and further work is needed to study the role in disease progression.

Pharmacology of GSK3

Aberrant regulation of GSK3β has been implicated and studied in several disorders such as Alzheimer's disease, type 2 diabetes, and bipolar disorder [6,74-79]. Many pharmacological agents have been developed due to the involvement of GSK3β in the different diseases with different mechanisms of action [80]. There have been six completed clinical trials involving inhibitors of GSK3 in Alzheimer's disease [81], hair loss [82], progressive supranuclear palsy [83,84], and bipolar disorder[85]. There are currently three clinical trials which are recruiting for bipolar disorder [86,87], spinal cord injuries and muscle atrophy [88]. In addition, there is a suspended clinical trial in patients with gliomas [89]. The broad spectrum of clinical trials indicates the importance of GSK3 in many pathological processes.

GSK3β activity can be regulated by pharmacological interventions. The most extensively studied GSK3β inhibitor is lithium chloride [90]. Within this context, lithium has been extensively studied as a regulator of GSK3β signaling pathways in bipolar disorder [91]. While lithium chloride has been shown to be effective in treating bipolar disorder, high concentrations are needed for physiological and pharmacological effects [92]. Lithium was first shown to have an effect on GSK3 in the early 1990s [90]. GSK3 was identified as a target in intact cells and *in vitro* showing the potential role of GSK3 in development [92]. In the nervous system, axonal growth has also shown to be decreased by lithium administration [93] and in a mouse model of degeneration [20]. Compared to other inhibitors, lithium chloride is a noncompetitive ATP inhibitor which competes with Mg^{2+} for association with the kinase and is most effective *in vivo* [94]. Lithium also has a high inhibitor constant, Ki = 2mM [94,95]. Lithium compared to other inhibitors, at high doses can inhibit both GSK3β and GSK3α [96]. Also, lithium has been shown to indirectly activate AKT in neuronal cells which can consequently phosphorylate kinases other than GSK3β and has also shown to be protective against glutamate excitotoxicity *in vitro* [97]. These studies point to the potential clinical and therapeutic effects of lithium and the lack of specificity for GSK3β.

Two GSK3β inhibitors of interest that utilize a different mechanism of inhibition other than lithium chloride are SB216763 and AR-A014418 (Figure 3A and Figure 3B). For both inhibitors, their mechanism of action is through binding in the ATP pocket of GSK3β [98]. SB216763 is an anilinomaleimide with a Ki ranging from 10nM to 30nM for GSK3α and in the presence of ATP inhibits GSK3β activity by up to 96% [99]. AR-A014418 is a thiazole with a Ki of 38nM and inhibits GSK3β with an IC_{50} = 104+/-27nM. The inhibitor binds within the ATP pocket along the hinge/linker region where the nitro group engages the ATP pocket [39]. GSK3α is also inhibited by these small molecule inhibitors; however the focus of this review is on the inhibition of GSK3β. Both of these small molecule inhibitors are more selective and potent compared to lithium chloride, which may help in identifying the specific role of GSK3β in different disease pathologies.

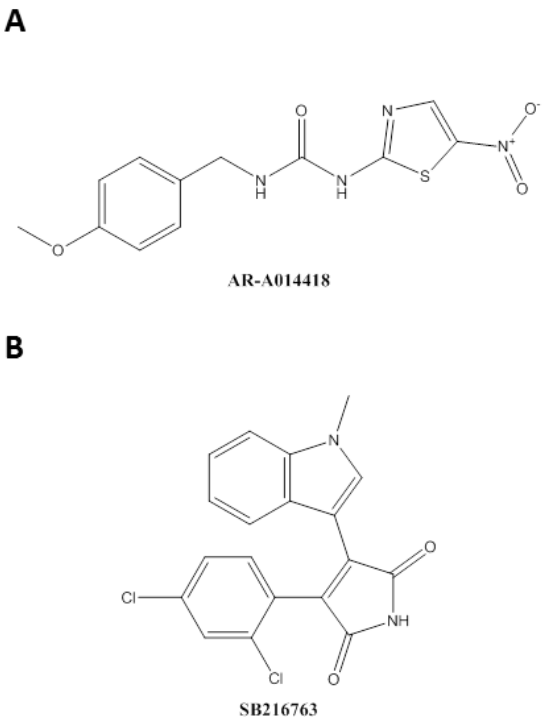

Figure 3: The chemical structure of two GSK3β inhibitors. A) The chemical structure of 1-(4-methoxybenzyl)-3-(5-nitrothiazol-2-yl) urea (AR-A014418) with the MW=308.31. B) The chemical structure of 3-(2,4-dichlorophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione (SB216763) with the MW=371.22 [39,99].

GSK3β Inflammatory Signaling Pathways

Glycogen Synthase Kinase 3 has been identified as a target for inflammatory mediated diseases [6] and plays a key role in mediating inflammatory responses (see Table 1 for signaling pathways). The role of GSK3β in inflammation was first shown by Martins and coworkers in 2005 [100]. They determined that following the development of inflammation, the kinase acts as a modulator for the expression of key pro-inflammatory and anti-inflammatory cytokines derived from monocytes and other peripheral blood cells to dampen inflammatory responses [100]. The mechanism by which GSK3β attenuates inflammation has been hypothesized to be regulated, in part, through the nuclear translocation of the transcriptional factor CREB (cAMP Response Element-Binding Protein) [100]. GSK3 has been shown to have an inhibitory effect on CREB regulation resulting in decreased nuclear translocation of CREB [101]. The decreased translocation of CREB into the nucleus increases the expression of pro-inflammatory cytokines such as Interleukin-1-Beta (IL-1β) and Tumor Necrosis Factor -1 alpha (TNF-α). Inhibition of GSK3β increases CREB DNA binding activity, which increases the transcription and expression of anti-inflammatory cytokines (IL-10) [100,102,103]. In dendritic cells, GSK3β is involved in TNF-α and IL-6 secretion [104]. These studies provide evidence for the importance of GSK3 activity in regulating pro- and anti-inflammatory response where by an increase in GSK3β activity increases the production of pro-inflammatory cytokines while a decrease in GSK3β activity results in the production of anti-inflammatory cytokines.

The mechanism by which of GSK3 regulates CREB translocation has been further elucidated in a model for

intestinal inflammation. Following the development of inflammation, inhibition of GSK3 reduces the pro-inflammatory phenotype of Toll-like receptors [100]. In addition, inhibition of GSK3 modulates the transcriptional activity of both CREB and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a master regulator of inflammation, in the intestinal immune cells [105]. The translocation of NF-κB from the cytoplasm to the nucleus results in the transcription of pro-inflammatory genes such as IL-1β, TNF-α, and IL-6 [106]. In a model of acute inflammation, inhibition of NF-κB in leukocytes decreases inflammation [107] and adenoviral infection of human macrophages, which inhibits NF-κB activation, decreases the production of TNF-α [108]. Interleukin-10, an anti-inflammatory cytokine, decreases the transcription of NF-κB resulting in a decrease in the expression of pro-inflammatory cytokines [109]. The activation of GSK3β modulates the nuclear translocation of both NF-κB and CREB by enabling CREB Binding Protein (CBP) to bind both transcriptional factors, which facilitates nuclear translocation and increases the production and transcription of pro-inflammatory cytokines. When GSK3 is inactivated, NF-kB translocation is decreased while CREB transcription increases [101]. This result in a relative increase of CREB in the nucleus compared to NF-κB and increases the binding of CBP to CREB. The increase of CBP binding to CREB produces an increase in the transcription of anti-inflammatory cytokines (e.g., IL-10) [100,103]. The importance of GSK3 in regulating anti-inflammatory cytokines through the NF-κB pathway has been shown in GSK3 mouse knockouts [110]. In addition, it has been shown that inhibition of GSK3 can decrease NF-κB activation in hepatocytes [111]. It has also been shown in hepatocytes that the inhibition of GSK3β reduces NF-κB activity, increases CREB transcription factor, and attenuates TNF-α mediated apoptosis [101]. These studies indicate the importance of CREB and NF-κB transactivation through GSK3 in regulating the release of pro-inflammatory and anti-inflammatory cytokines (Figure 4).

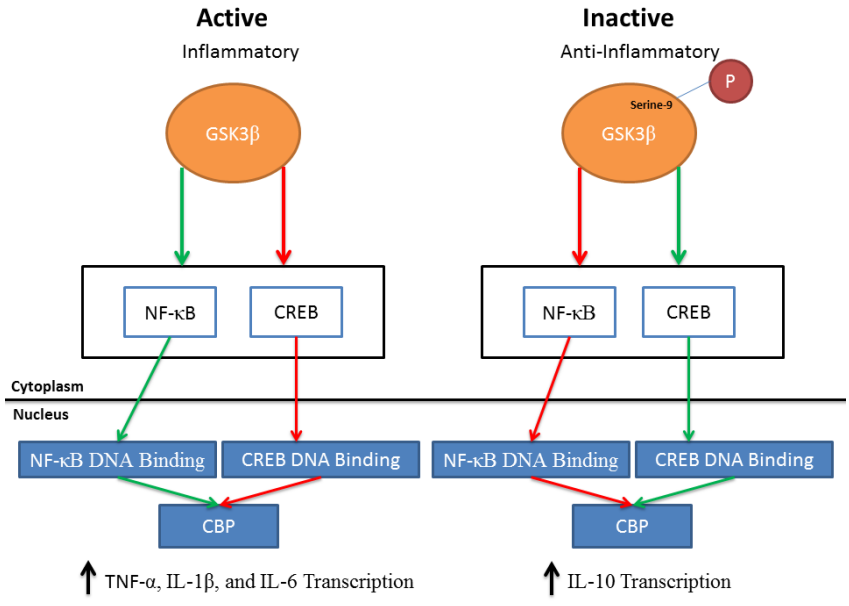

Figure 4: Putative downstream pathways of GSK3β that modulate the expression of pro-inflammatory and anti-inflammatory cytokines. Following GSK3β activation, NF-κB istranslocated from the cytoplasm to the nucleus and binds transcriptional sites with CBP leading to an increase in the transcription of pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6). An increase in GSK3β phosphorylation (inhibition of GSK3β) results in an increase of CREB being translocated from the cytoplasm to the nucleus. This results in an increase in CBP binding with CREB at transcriptional sites, which increases the transcription of anti-inflammatory cytokine (IL-10). Green lines indicate an increase in activation and red lines indicate a decrease in activation.

Neuroinflammation and GSK3

Neuroinflammation is an inflammatory response that is characterized by glial activation and the production of inflammatory cytokines [112]. There are many diseases which are linked to the increased activation of glial cells and elevated pro-inflammatory cytokines.

Neuroinflammation has been implicated in multiple neurological disorders of the central nervous system (CNS) such as acquired immune deficiency syndrome (AIDS), stroke, and multiple sclerosis [113]. These disorders all share a common neuroinflammatory response; however, the etiologies of these disorders vary. GSK3β has recently been shown to be involved in the activation of glial cells. In rat cortical glia, GSK3β is expressed in both astrocytes and microglia and is activated following exposure to lipopolysaccharide (LPS) [114]. Following stimulation, pro-inflammatory cytokines are increased and inhibition of GSK3β attenuate the production of pro-inflammatory cytokines (IL-1β and TNF-α) and augments the production of anti-inflammatory cytokines (IL-10) *in vitro* [114]. In addition, GSK3 activation has been linked to the increase in glial cell proliferation [115].

Inflammation of the brain is isolated from the body through the blood brain barrier (BBB), where an increase in immune cell trafficking occurs following injury [116,117]. Originally, the CNS was thought to be protected from systematic inflammation, however, it is now established that multiple factors can contribute to a neuroinflammatory response [118]. The inflammatory response of the brain appears to be more tightly regulated than peripheral inflammation and regulation occurs through the activation of glial cells [119]. In the CNS, glial cells encompass nearly 75% of the overall cells where the majority of glial cells are astrocytes and oligodendrocytes while the rest are microglia cells [120]. These three types of glial cells, particularly microglia and astrocytes act as immune responders in the CNS.

Microglia cells were first established to be a different cell type compared to astrocytes and oligodendrocytes in the early 1900s [121]. Microglia cells act as macrophages of the nervous system in normal conditions [122]. They are considered to be resident macrophages even though they only consist of 15% of the cells in the CNS [123,124]. In the CNS, it has been shown that microglia cells become activated in different pathological conditions and release pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) and chemokines [125]. The role of microglia cells in facilitating the proliferation of immune cells has also been thoroughly studied [126,127].

Astrocytes, in regards to neuroinflammation, act as scavengers of the CNS and recycle excess neurotransmitters and ions. Dysregulation of astrocytes has been shown to play a role in the development of neurological diseases [128]. Under normal conditions, astrocytes regulate excess glutamate in the synaptic cleft preventing neurotoxicity [129,130]. Following the accumulation of pro-inflammatory cytokines in the CNS, astrocytes become activated and proliferate. The increase in astrocytic activation causes an increase in proliferation, which can be identified with the increase in glial fibrillary acidic protein (GFAP) [131]. Although GFAP is currently used for immunostaining and Western blotting of astrocytes, it only identifies ~15% of the total volume of the astrocyte [132]. An in vitro model of metabolic injury shows that astrocytes release interleukin 1 (IL-1), IL-6, TNF-α and interferon gamma (IFN-g) [133]. When a neuroinflammatory response ensues, glial cells become activated and produce pro-inflammatory cytokines such a TNF-α, IL-1β, and IL-6 and chemokines [134,135].

Microglia cells act as the first responders to injury while astrocytes sustain the inflammatory response.

Neuroinflammation can be seen as both beneficial and detrimental. Some of the common themes involved in the beneficial aspects of neuroinflammation occur through neuroprotection and axonal regeneration. Following stroke, microglial cells act in response to injury to scavenge necrotic debris in the CNS [136]. This occurs from the release of pro-inflammatory cytokines almost immediately [112] and activated glial cells, which sense and respond to ATP gradients, act to scavenge excess neurotransmittors and dead cells [137]. The increase in astrocyte activation and proliferation produces the formation of a glial scar, isolating the damaged area from the rest of the CNS [118]. Acute inflammation is advantageous due to the short lived nature and minimization of neuronal damage and toxicity resulting from the release of inflammatory cytokines and chemokines [138]. In a mouse genetic model for Alzheimer's disease, it has been shown that neurotoxicity of β-Amyloid deposits is reduced through bone marrow derived microglia activation [139]. In addition, Nitric Oxide-induced neuronal damage is increased in mice deficient for TNF-α and TNF-α is required for microglia activation following injury [140]. In addition, in mice IL-6 is important for glial cell activation and is important for neuronal protection following injury to the CNS [141]. Following spinal cord injury, it has been shown that the injection of microglial cells into the site of injury is associated with the regrowth of axons through immune cytochemical detection [142]. In a double blind study with patients with MS, blocking of TNF-α produces an exacerbation of the condition [143]. These studies indicate that cytokine production and glial cell activation and proliferation following injury can act beneficially.

However, dysfunctional regulation of neuroinflammation can can also be detrimental. The chronic activation of glial cells and elevation of pro-inflammatory cytokines have been linked to multiple disorders [144]. The development of neuroinflammation can occur multiples ways with different disease etiologies. The inflammatory response can be induced from infection, injury, or an autoimmune disorder. Following the infection of human immunodeficiency virus type 1 (HIV), the disease progresses to Acquired Immune Deficiency Syndrome(AIDS) with the late onset of neurological dysfunction termed AIDS dementia complex (ADC) [145]. ADC is shown to affect roughly 80% of individuals who progress from HIV to AIDS [146]. The progression to ADC has similar mechanisms of other neurological disease through means of neuroinflammation mainly from the activation of glial cells in the CNS [147]. Microglial cells are the primary targets of HIV infection [148] and increases in microglial cells appear to associate with increased viral infection. Histological studies in the CNS from individuals with AIDS show an increase in the proliferation and activation of astrocytes and microglial cells leads to the increased release of IL-1β, TNF-α, and IL-6, which promotes the activation and proliferation of the virus [149]. Through *in vitro* HIV exposure to human and rat astrocytes, it has been determined that astrocytes are involved through voltage gate and NMDA calcium channels in the progression to ADC [150]. It has further been shown that following ADC, nonviral cells produce proinflammatory cytokines in addition to the viral production of cells in the CNS [151].

GSK3β has been studied in the context of ADC. Through genome wide mRNA and microRNA expression profiles of HIV patients with and without dementia, GSK3β expression is down regulated in patients with dementia [152]. Lithium

treatment of mice, *in vivo*, has been shown to protect the hippocampus from HIV induced neurotoxicity and *in vitro* lithium exposure prior to infection has shown to reduce HIV induced neurotoxicity [153]. In addition, low dose administration of lithium has been shown to improve the psychological impairment produced from HIV progression [154].

Injury, another inducer of neuroinflammation, can produce strokes (i.e., CNS ischemia), which is a common type of acute brain injury caused by an interruption in the blood supply to the brain where intracellular mechanisms such as inflammation can cause neuronal damage [155]. After the initial injury, macrophages infiltrate the BBB causing an additional inflammatory response to occur [113]. The inflammatory response causes the proliferation and activation of glial cells in the CNS leading to the production of cytokines [136]. Following a stroke, elevated levels of IL-6 and TNF-α in blood or cerebrospinal fluid occur with early clinical symptoms of deterioration [156]. In addition, it has been shown in stroke patients that ischemia causes an abundance of the proinflammatory cytokines IL-6 and TNF-α in peripheral blood cells [157]. Stroke patients have an elevated intrathecal production of proinflammatory cytokines and chemokines indicating there is an increase in glial cell activation [158].

GSK3β has also been studied in the context of stroke. *In vivo*, following focal cerebral ischemia, GSK3β inhibition has been shown to reduce infarct size [159]. In a rat transient middle cerebral artery occlusion model, pretreatment with lithium decreases infarct size and improves recovery through the increased migration of mesenchymal stem cells [160]. The mechanism by which lithium is beneficial to the treatment of stroke has been thought to occur through activation of the AKT pathway and subsequent phosphorylation of GSK3β, resulting in the attenuation of glutamate excitotoxicity [97]. In addition, pretreatment with lithium has shown to attenuate the dephosphorylation of GSK3β induced through hypoxia *in vivo* [161].

Multiple Sclerosis (MS) is an autoimmune disorder of the CNS that leads to neurological disability due to axonal deterioration and the pathogenesis of the disorder has been linked to inflammatory elements of MS plaques [162,163]. Briefly, leukocytes infiltrate the CNS through the BBB causing the migration of microglial cells leading towards axonal dysfunction [164]. In post-mortem human brain samples of patients with MS, it has been shown that the cytokines and chemokines RANTES, MCP-1, MIP-1α, and MIP1-β are differentially expressed in glial cells to increase inflammation [165]. TNF has also been shown to be in the lesions of patients with MS indicating an increase of proinflammatory cytokines [166]. Inflammation of the CNS causes the activation of astrocytes and microglial cells which have been targeted in MS. In Act1 deficient mice, it has been shown that astrocytes are necessary for leukocyte recruitment in the CNS in an autoimmune encephalomyelitis model, a model of MS [167]. Inflammatory genes have also been shown to be up regulated in MS plaques [168]. MS is different from other neuroinflammatory mediated diseases in that it originates from immune dysregulation compared to insult/injury of the CNS.

GSK3β has also been studied in the context of MS. In experimental autoimmune encephalomyelitis (EAE), over expression of GSK3β increases disease severity, and lithium pretreatment suppresses clinical symptoms and microglial activation in the spinal cord and post treatment promoted partial recovery [169]. In individuals with chronic progressive MS, GSK3β has been shown to be over expressed [170]. Interleukin-17 producing cells, which have been shown to be increased in MS, are inhibited following GSK3 inhibition in an EAE model in the spinal cord [171]. These studies provide

evidence that GSK3β is important in disease progression and inhibition of the kinase may play a role in a potential therapeutic.

Alzheimer's disease (AD) is a neurodegenerative disease involving inflammation of the CNS leading to memory loss followed by dementia. It has been suggested that the main cause of AD is due to an abundance of β-Amyloid in the brain [172]. β-Amyloid plaques have been shown to increase the inflammatory response in the brain of Alzheimer's patients. Glial cells are known to surround Alzheimer's plaques and *in vitro* it has been shown that they release IL-1 and growth factors which are components of plaques [173,174]. In addition, astrocytes have also been shown to surround neuritic plaques in AD [175]. In an age-matched study, Alzheimer's patients have higher levels of TNF-α in sera indicating an inflammatory response [176].Using a mouse model for AD, it has been shown that β-Amyloid plaques cause an inflammatory response and LPS stimulation further enhances the production of the pro-inflammatory cytokines IL-1β, TNF-α and monocyte chemo attractant protein-1 [177].

GSK3β has been extensively studied in the context of Alzheimer's disease. AD patients have been shown to have an increase in GSK3β activity that correlates with an increase in neuronal death [178]. It has been shown, *in vitro*, that GSK3β inhibition with lithium treatment blocks the production of β-Amyloid [179]. In cultured rat cortical neurons, lithium protects cells from β-Amyloid induced death and there is a decrease in tau phosphorylation, a process that occurs in response to β-Amyloid accumilation [180]. This was further validated with chronic lithium pretreatment on β-Amyloid induced cerebellar cell death [181]. Clinical studies have also been conducted to determine the benefits of lithium treatment in patients with AD. In elderly patients with both AD and bipolar disorder, chronic lithium treatment has shown to decrease AD symptoms to that seen in the general population [182]. Furthermore, it has been shown that chronic lithium treatment reduces the rate of dementia in a nationwide study [183]. These studies indicate the importance of GSK3β in cognition and neuroinflammation.

Neuroinflammation and Pathological Pain

Glia activation and the subsequent release of proinflammatory cytokines play crucial roles in the development and maintenance of pathological pain [184-186]. Microglia and astrocytes are reactivated in almost every animal model of pathological pain [187,188], including neuropathic pain induced by nerve injury [189,190], inflammation induced by complete Freund's adjuvant [185], surgical incision[191], and morphine tolerance [192]. These are accompanied with elevated levels of proinflammatory cytokines [193,194] and an increased expression of proinflammatory cytokines in microglia and astrocytes in the spinal dorsal horn [195-197], suggesting that the increased proinflammatory cytokines come from glial cells. Intrathecal administration of IL-1β and TNF-α in normal rats enhances both the acute response and the wind-up activity of dorsal horn neurons and mechanical allodynia and hyperalgesia [198,199]. Suppression of astrocyte and microglial activation with the glial inhibitor, propentofylline, or inhibition of microglia activation by minocycline, results in attenuation of hyperalgesia induced by nerve injury, which is associated with decreased expression of the cytokines IL-1β, IL-6 and TNF-α *in vivo* [194,200,201]. Similarly, treatments with antagonists of IL- 1β, IL-6 and TNF-α reduced hypersensitivity induced by inflammation, nerve injury or morphine tolerance. Besides the release pro-inflammatory cytokines, we and other have shown that activation of astrocytes is associated with dysfunction of glial glutamate transporters [202-204]. Down regulation of glial glutamate transporters in the spinal dorsal horn contributes to

the genesis of many types of pathological pain including neuropathic pain induced by nerve injury [202,205,206], chemotherapy [207,208] and morphine tolerance [202,209]. We have demonstrated that impaired glutamate uptake by glial glutamate transporters is a key contributing factor to strengthening AMPA and NMDA receptor activation in the spinal sensory neurons [206,210-213]. Deficiency in glial glutamate uptake results in decreases in GABAergic synaptic strength due to impairment in the GABA synthesis through the glutamate-glutamine cycle [214]. Hence, the integrity of glial GTs is critical to maintain synaptic excitatory-inhibitory homeostasis and normal nociception in the spinal dorsal horn. Glial glutamate transporters appear to be regulated by neuroinflammation processes induced by nerve injury. Suppression of glial activation and pro-inflammatory cytokine production with propentofylline or minocycline up-regulates mRNA and protein expression of glial glutamate transporters in the spinal dorsal horn and ameliorates the nerve-injury-induced allodynia [201,204,215,216]. Therefore, identifying molecules that can suppress neuroinflammation has a great potential to open a new door to alleviate pathological pain.

Given the fact that the role of GSK3 in neuroinflammatory diseases in the CNS has been extensively studied and neuroinflammation is a crucial mechanism underlying the genesis of pathological pain, it is surprising that there are only a handful of papers reporting the effects of pharmacological inhibition of GSK3β on spinal nociceptive processing.

The first report of GSK3β inhibition on spinal nociceptive processing has been shown in morphine-tolerant rats [217]. Parkitina and coworkers (2006) have shown that GSK3β inhibition alters the tolerance to morphine in the dorsal lumbar of the spinal cord. Intrathecal inhibition of GSK3β (SB216763) has shown to decrease pharmacological tolerance to morphine in a dose-dependent manner whereas GSK3β inhibition of naïve rats has no effect determined from the tail flick test. Tolerance to morphine is associated with an increase in active GSK3β where chronic intrathecal inhibitor administration increases phosphorylation at the Serine-9 residue of GSK3β and decreases tolerance. Intrathecal inhibitor administration has shown to have no effect on increasing the phosphorylation of the Serine-9 residue or have analgesic effects in naïve rats [217].

The next report of GSK3β inhibition on pain processing has been shown in mouse models of acute nociception through acetic acid induced abdominal constrictions and formalin induced nociception [7]. Martins and coworkers (2009) have shown that pharmacological inhibition of GSK3β can have antinociceptive effects. They have shown that intraperitoneal pretreatment of a GSK3β inhibitor (AR-A014418) prior to acetic-acid induced abdominal constrictions reduces writhings. They have also shown that pretreatment with a GSK3β inhibitor by intraperitoneal, intraplantar, and intrathecal injection reduces licking frequency, a measure of nociception, following formalin induced nociception. Lastly, through intrathecalco-administration of the GSK3β inhibitor with glutamate, NMDA, trans-ACPD, TNF-α, or IL-1β there is a decrease in cytokine induced biting [7]. This study indicates that pharmacological inhibition of GSK3β may play a role in nociception and pain.

The most recent report of GSK3β inhibition on pain processing has been shown in a mouse model of neuropathic pain through peripheral nerve injury [5]. Martins and coworkers (2012) have shown that intraperitoneal administration of a GSK3β inhibitor (AR-A014418) produces antihyperalgesic effects and decreases the proinflammatory cytokines IL-1β and TNF-α in the lumbar portion of the spinal cord (Lumbar 1 to Lumbar 6). They have also shown that following the development of mechanical hyperalgesia, a single

intraperitoneal administration of a GSK3β inhibitor attenuates mechanical and thermal hyperalgesia. In addition, they have shown that chronic inhibition of serotonin synthesis prior to GSK3β inhibition prevents the decrease in mechanical allodynia. While pharmacological inhibition of GSK3β has been shown to reduce hyperalgesia induced by nerve injury [5], it remains unknown if inhibition of GSK3β can prevent the development of allodynia following nerve injury. In an adult rat (Sprague Dawley) model of neuropathic pain induced by partial sciatic nerve ligation [206], with the same inhibitor (AR-A014418) and concentration (0.3 mg/kg), we found that chronic intraperitoneal administration from the day of surgery (1 injection/day) for 8 days significantly attenuates the development of mechanical allodynia induced by partial sciatic nerve ligation (Figure 5). These data suggest that altered GSK3β activities may contribute to the development of neuropathic pain.

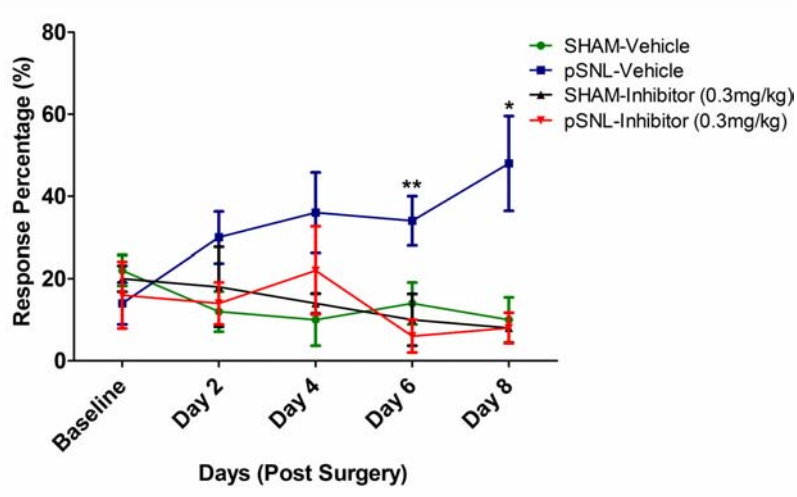

Figure 5: Chronic intraperitoneal injections (IP) of a GSK3β inhibitor prevent the development of allodynia in a model of neuropathic pain. Rats were injected with the GSK3β inhibitor, AR-014418 (0.3mg/kg), or vehicle (saline) 1 hr prior to surgery and then daily for 8 days following surgery. A von Frey filament with 1.5g force was used to stimulate the plantar region of the hindpaw 10 times and the percentage of hindpaw withdrawal response to 10 time stimulations was used to indicate changes in nociceptive behaviors. Behavior tests wereperformed before surgery and then prior tothe daily drug administration where the examiner was blinded to the types of treatment given to the rats. SHAM rats represent animals where the sciatic nerve was exposed but not ligated. Green represents the SHAM rats receiving vehicle treatment. Blue represents the pSNL group receiving vehicle treatment. Black represents the SHAM rats receiving the GSK3βinhibitor treatment. Red represents the pSNL rats receiving the GSK3β inhibitor. The percentage of hindpaw withdrawal response to the von Frey filament stimulation in pSNL rats receiving the GSK3β inhibitor is significantly lower than that in pSNL rats receiving vehicle treatment, indicating that suppressing GSK3β activities prevents the development of allodynia induced by nerve injury. The asterisks denote a significant difference between the AR-014418 treated pSNL group to the vehicle treated pSNL group. (n=5 per group, Unpaired students t-test. *P <0.05 **P<0.01).

We further investigated possible changes of GSK3β activities in the spinal dorsal horn in neuropathic rats induced by partial sciatic nerve ligation through immune histochemical techniques. Eight days following peripheral nerve injury, we found that there is a decrease in phosphorylated GSK3β on the lesion side compared to the uninjured side and sham operated rat (Figure 6). We also demonstrated that phosphorylated and total GSK3β is localized to both astrocytes and neuronal cells (Figure 7). These data indicate that increased GSK3β activities in the spinal dorsal horn may contribute to the genesis of neuropathic pain induced by sciatic nerve injury. Together with findings by others, these data suggest that GSK3β may be a potential target for the development of analgesics for the treatment of neuropathic pain.

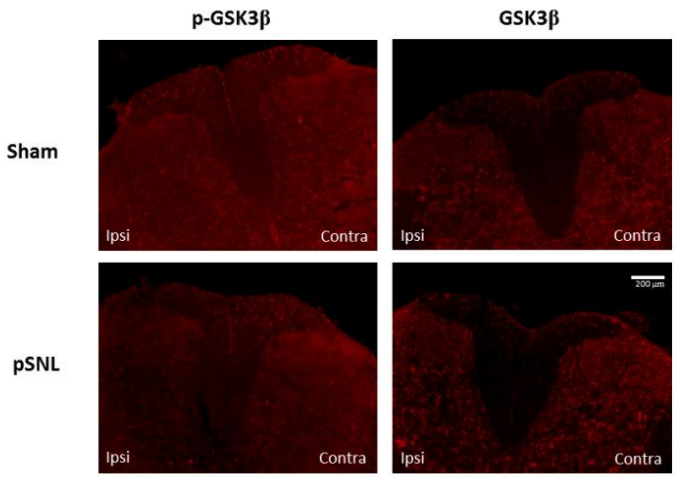

Figure 6: Partial sciatic nerve ligation results in a decrease in phosphorylated GSK3β expression in the injured side of the dorsal horn 8 days following surgery.There was no significant difference in total expression of GSK3β between the pSNL and sham operated rats. Eight days following surgery, rats were deeply anesthetized with pentobarbital (60 mg/kg, IP) and perfused intracardially with 200mL of heparinized 0.1 M PBS (pH=7.35) followed by 300mL of a solution containing 4% formaldehyde in 0.1 M PBS (pH=7.35). L4 and L5 spinal segment tissues were then fixed for 48 h at 4°C in the same fixative and cryoprotected for at least 24 h at 4°C in 30% sucrose in 0.1 M PBS (pH=7.35). Serial transverse sections, 30-µm thick, were cut on a freezing microtome at -20°C and collected in 0.1M PBS and processed while free floating. The sections were then washed three times in 0.1 M PBS and then blocked with 10% normal goat serum plus 0.3% Triton X-100 in 0.1 M PBS (pH=7.35) for 1 h at room temperature. Sections were then incubated for 24 h at 4°C in rabbit anti-pGSK3β (1:100) or anti-GSK3β(1:200) in 4% normal goat serum plus 0.3% Triton X-100 in 0.1M PBS (pH=7.35). Sections were then washed 3 times and incubated with with Texas Red goat anti-rabbit antibody (1:500) in 4% normal goat serum plus 0.3% Triton X-100 in 0.1M PBS (pH=7.35) for 2 h at room temperature. After rinsing in 0.1 M PBS, sections were mounted onto double frosted pre-cleaned microscope slides, air-dried, and cover slipped with UltraCruz Mounting Medium. Slides were imaged using an Olympus 1x71 Inverted Microscope with an Olympus DP72 Microscope Digital Camera. Images were processed using ImageJ (NIH) [218].

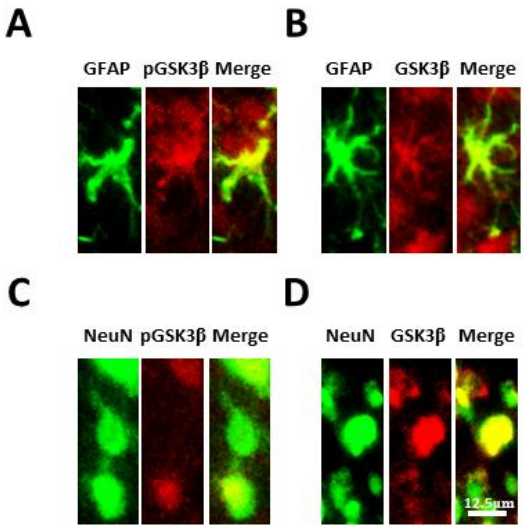

Figure 7: Phosphorylated and total GSK3β are colocalized with neurons and astrocytes in the spinal dorsal horn. GFAP is a marker for astrocytes and NeuN is a marker for neuronal cells. Sections were incubated for 24 h at 4°C in rabbit anti-pGSK3β (1:100) or anti-GSK3β (1:200) in 4% normal goat serum plus 0.3% Triton X-100 in 0.1M PBS (pH=7.35). Sections were then washed 3 times and incubated with a combination of either mouse anti-NeuN Alexa Fluor 488 conjugated antibody (1:200) or mouse anti-GFAP Alexa Fluor 488 conjugated antibody (1:200) with Texas Red goat anti-rabbit antibody (1:500) in 4% normal goat serum plus 0.3% Triton X-100 in 0.1M PBS (pH=7.35) for 2 h at room temperature. After rinsing in 0.1 M PBS, sections were mounted, air-dried, cover slipped, and images mentioned in Figure 6.

Conclusion

Glycogen Synthase Kinase 3β has been linked to the development and progression of multiple disease entities. Following the initial identification of GSK3β, significant strides have been made in understanding the structure, regulation, pharmacology, and diseases linked with the kinase. GSK3β is a common target in inflammation of the CNS which has been associated with many diseases such as Alzheimer's disease, AIDS dementia complex, and stroke. Inhibition of GSK3β has been shown to alleviate multiple symptoms and the progression of these diseases.

The role of GSK3β in pathological pain has recently emerged. The development and maintenance of pathological pain is associated with changes in GSK3β activities. Inhibition of GSK3β activities can prevent the development and reverse the existence of neuropathic pain. Further studies are needed to understand the upstream pathways regulating GSK3β activities and the downstream signaling pathways used by GSK3β to alter spinal sensory processing, which will potentially lead to the use of GSK3β as a novel target for the development of analgesics.

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