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Alteration of GSK-3β in the hippocampus and other brain structures after chronic paraquat administration in rats

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Abstract

Systemic exposure of rodents to the herbicide paraquat (PQ) was suggested to reproduce pathological features of Parkinson's disease. Our recent data showed that long-term PQ administration influenced levels of glycogen synthase kinase 3β (GSK-3β) and its active form phosphorylated on tyrosine 216 in the nigrostriatal system, which may be related to its vulnerability to PQ toxicity.

The aim of this study was to analyse selectivity of the toxic effect of PQ after its systemic administration in rats. PQ was administered for 37 weeks and the protein level of total GSK-3β and its active GSK-3β (pY216) form in subcellular fractions of hippocampus, brain cortex and cerebellum was examined.

Our data indicated that the long-term administration of PQ significantly decreased the level of both GSK-3β forms in nuclear and cytosolic fractions of hippocampus in rats. In the brain cortex and cerebellum PQ decreased the level of both forms of GSK-3β in the nuclear fraction but increased their levels in mitochondria and in some cases also in the cytosol.

The results of the present study indicate that PQ influenced levels and activation of GSK-3β in different brain structures, which may contribute to its toxicity, but on the other hand may suggest development of adaptive, protective mechanisms.

Key words: *glycogen synthase kinase-3β (GSK-3β), paraquat, Wistar rats, Parkinson's disease.*

Introduction

Glycogen synthase kinase-3 (GSK-3) belongs to a family of conserved serine/threonine kinases present in all eukaryotic groups [17]. GSK-3, originally identified as a kinase that phosphorylates glycogen synthase, is rapidly becoming recognized as a central regulator of intracellular signalling cascades because of the wide array of substrates that it phosphorylates [15]. GSK-3 phosphorylates over 50 proteins and is involved in regulation of the cell cycle, cell polarity, migration, apoptosis and neuroinflammation

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[21,24,29,63]. GSK-3 has been implicated in the control of cytoskeletal proteins and several transcription factors [52]. The number of cell functions regulated by GSK-3 suggests that the activity of GSK-3 must be tightly regulated. Although the mechanisms regulating GSK-3 are not fully understood, precise control appears to be achieved by a combination of phosphorylation, localization, and interactions with GSK-3-binding proteins [23,29]. GSK-3 consists of two isoforms in humans, namely, GSK-3α (51 kDa) and GSK-3β (47 kDa). GSK-3α and GSK-3β have 97% sequence homology [10]. In this study our attention was concentrated on GSK-3β. It is known that GSK-3β activity is significantly reduced by phosphorylation of an N-terminal serine, Ser9 in GSK-3β and Ser21 in GSK-3α [52]. Several kinases can phosphorylate these serines, including Akt, protein kinase A (PKA), protein kinase C and p90Rsk [11,14]. In opposition to inhibitory regulation by serine phosphorylation, GSK-3 activity is facilitated by phosphorylation of tyrosine 216 in GSK-3β [20]. This might occur by autophosphorylation or by other tyrosine kinases but little is known about regulation of the tyrosine phosphorylation of GSK-3 [4].

The pivotal significance of GSK-3β was justified by previous experimental studies which demonstrated that deletion of the GSK-3β gene in mice is lethal [19,34,59]. The other studies showed that GSK-3β heterozygous (+/–) mice are viable and demonstrated a lot of neurological disorders, for example reduced exploratory activity, memory consolidation, aggression responsiveness to amphetamine and increased anxiety [2,26,41].

Activation of GSK-3β has also been suggested to be involved in a number of degenerative diseases, including Alzheimer's disease, Parkinson disease (PD) and others, as well as in diabetes type II [23]. Recent studies have pointed to a putative role of GSK-3β in PD [29,58]. Toxins which destroy dopaminergic neurons selectively, such as 6-OHDA and MPTP, have been found to activate GSK-3β [8,64]. Little is known, however, about the role of GSK-3β in PQ-induced parkinsonism. The controversial non-selective herbicide paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride, PQ), along with other pesticides, seems to play an important role in aetiopathogenesis of PD. Studies on the relationship between PD and pesticides began in the early 1980s, when it was discovered that users of the heroin substitute MPTP, which is chemically similar to paraquat, developed PD. A potential role of PQ in pathogenesis of PD has been suggested because an epidemiological association has been found between its application in agriculture and incidence of this disease [18,32]. The cytotoxic action of PQ clearly involved reactive oxygen species (ROS), an energy crisis, ER stress and neuroinflammation, but its specificity for dopaminergic neurons and possible involvement of the intrinsic cell death pathway are unclear [7,12,16,35,48,55,65-67]. PQ administered in rats acutely induced an increase in extracellular dopamine metabolism, which suggested an increase in dopamine release [43]. When injected subchronically it reduced the number of dopaminergic neurons in the substantia nigra pars compacta and inhibited dopaminergic transmission [28]. Our last data indicated that long-term PQ administration diversely alters levels of GSK-3β and its active phosphorylated form (pY216) in the midbrain with the pons and striatum, which may be connected with their different vulnerability to PQ toxicity.

Therefore, the aim of this study was to examine the influence of long-term (37 weeks) PQ administration in rats on the levels of total GSK-3β and its active form, phosphorylated on tyrosine 216 (pY216) in the hippocampus, brain cortex and cerebellum. Since GSK-3β has been shown to be located in several cell compartments such as cytosol, nucleus and mitochondria (using microscopy and immunoblotting experiments) [5,42,46,49,58], the subcellular localization of this kinase was analysed.

Material and methods

Animals

The experiments were carried out in compliance with the Animal Protection Bill of August 21, 1997 (published in Journal of Laws no. 111/1997 item 724), and according to the NIH Guide for the Care and Use of Laboratory Animals. They also received the approval of the Local Ethical Committee. All efforts were made to minimize the number and suffering of animals used. Male Wistar rats 3 months old weighing 200-250 g at the beginning of experiments were kept on a light/dark cycle (12/12 h; the light on from 7 am to 7 pm) with free access to food and water.

Drug

Paraquat dichloride (Sigma-Aldrich, Germany) was dissolved in sterile water and administered at a dose of 10 mg/kg/2 ml i.p. once a week for 37 weeks. The dose of paraquat was chosen according to earlier papers [28,43-45,58]. Animals were killed by decapitation 7 days after the last injection. Control animals were treated with physiological saline i.p. once a week.

This model was described previously by Songin *et al.*, 2011 [58].

Tissue sectioning

After decapitation, brains were rapidly removed and sectioned on an ice-cold Petri dish. Hippocampus, brain cortex and cerebellum were analysed by Western blotting (GSK-3β).

Isolation of subcellular fraction

After dissection, the hippocampus, brain cortex and cerebellum were immediately frozen and stored at –80°C until further procedures were applied. The structures were homogenized in 0.32 M sucrose with 10 mM Tris-HCl, pH 7.4, 1 mM EDTA (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) and with Complete™ protease inhibitors cocktail (Roche Applied Science, Indianapolis, IN, USA)]. The 10% homogenates were centrifuged at 900 g for 3 min at 4°C. The pellet (P1)-crude nuclear fraction was resuspended in the above described solution. The supernatant (S1) was centrifuged at 15,000 g for 10 min at 4°C to separate the crude mitochondrial fraction (P2) and cytosolic fraction (S2). The pellets (P1, P2) were resuspended in 10 mM Tris-HCl (pH 7.4) buffer with protease inhibitors. Protein concentration was determined by protein dye-binding with a commercial reagent (Bio-Rad Laboratories, Hercules, CA, USA). Then crude nuclear, mitochondrial and cytosolic fractions were obtained from each brain part and mixed with 5 × Laemmli sample buffer and denatured for 5 min at 95°C. After standard SDS-PAGE on 10% polyacrylamide gel, proteins were transferred onto PVDF membranes, then the membranes were washed for 5 min in TBS-T buffer (20 mM Tris, 500 mM NaCl, pH 7.5, TBS containing 0.5 ml/L Tween 20). After incubations with antibodies and autoradiography, densitometric analysis and marker size-based verification were performed with TotalLab software.

The immunochemical determination of a typical mitochondrial protein, apoptosis inducing factor (AIF), indicated that this protein was localized exclusively in the mitochondrial fraction of control and PQ-treated animals. Our previous study [60] indicated that DNA-bound enzyme (ADP-ribose) polymerase-1 (PARP-1) was localized exclusively in the nuclear fraction and has never been detected by us in mitochondria or in cytosol using a similar procedure for the isolation of subcellular fractions. In the aged striatum, the level of PARP-1 was significantly lower, which may suggest specific sensitivity of this brain part to age-related processes [60].

Electrophoresis and immunoblotting

The electrotransfer of proteins to PVDF membranes was performed for 1.5 hours at 100 V and then the membrane was blocked for 1 h at room temperature in 5% non-fat milk in TBS-T for GSK-3β or in 0.5% BSA/TBS-T for GSK-3β (pY216). After washing in TBS-T, the membranes were immunostained according to standard methods provided by the manufacturer (BD Transduction Laboratories, San Diego, CA, USA). They were probed with a primary mouse monoclonal anti-GSK-3β antibody (1 : 2,500) in 5% non-fat milk/TBS-T or anti (pY216)-GSK-3β IgG (1 : 1000) in 0.1% BSA/TBS-T (BD Transduction Laboratories, San Diego, CA, USA), were incubated overnight (4°C), washed three times in TBS-T and were further incubated with the secondary anti-mouse antibody conjugated with horseradish peroxidase (HRP) (1 : 4,000) (Amersham) in 2% non-fat milk/TBS-T. After stripping, membranes were probed with mouse monoclonal anti-beta-actin antibody (1 : 400) in 0.1% BSA/TBS-T to normalize protein levels (MP Biomedicals, Inc, Irvine, CA, USA). The blots were developed using the enhanced chemiluminescence (ECL) detection system (Amersham International, Aylesbury, U.K.) according to the manufacturer's protocol. After exposure, photographs were taken with a Kodak DC 290 zoom digital camera and were analysed using the Kodak EDAS 290/Kodak 1D 3.5 system.

Statistical analysis

We used Student's *t*-test for Western blotting; $p \leq 0.05$ was considered significant. The presented data are means ± SEM.

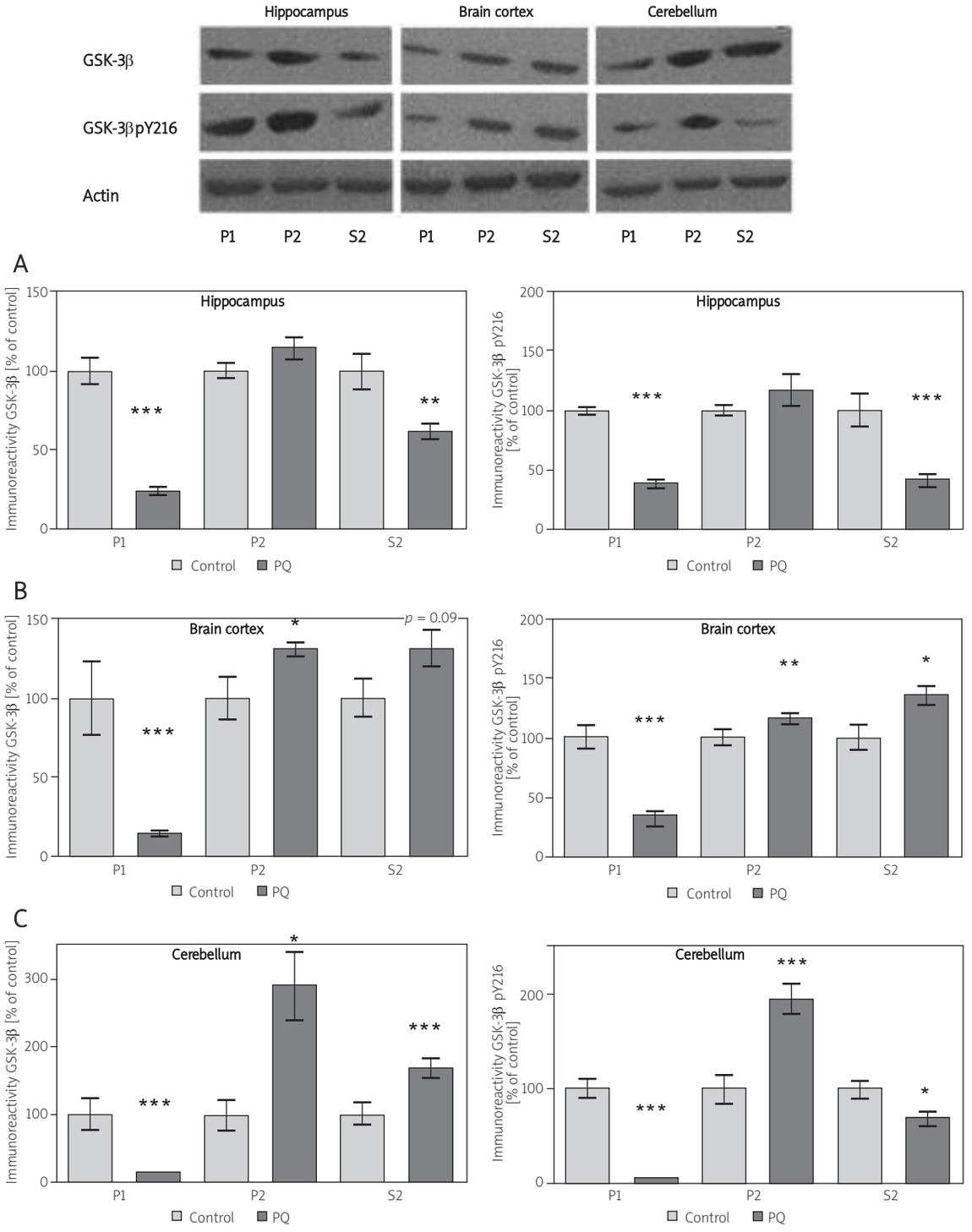

Fig. 1. Immunoreactivity of GSK-3β and GSK-3β (pY216) proteins in nuclear (P1), mitochondrial (P2) and cytosolic (S2) fractions in the hippocampus, brain cortex and cerebellum. Results are normalized to β-actin as loading control and are the means ± SEM. Representative blots of both total and phosphorylated forms of GSK-3β, as well as corresponding β-actin, are shown under bars. The number of animals per group n = 4-6. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Results

The influence of the long-term paraquat administration on the levels of GSK-3β and GSK-3β (pY216) in nuclear, mitochondrial and cytosolic fractions in the hippocampus, brain cortex and cerebellum.

Our data confirmed the presence of both total and active (pY216) form of GSK-3β in nuclear, mitochondrial and cytosolic fractions. The results presented in Fig. 1 demonstrate that paraquat administered in a dose of 10 mg/kg i.p. for 37 weeks influenced levels of that enzyme in the brain structures examined.

Hippocampus

PQ significantly decreased the total level of GSK-3β immunoreactivity in isolated nuclear (P1) and cytosolic fractions (S2) of the hippocampus. The level of GSK-3β (pY216) was also significantly lowered in the same fractions of this region (Fig. 1A).

Brain cortex

In the brain cortex, both total and phosphorylated (pY216) GSK-3β immunoreactivity levels were decreased in the nuclear fraction in rats treated with PQ (Fig. 1B). In contrast, the level of both forms of GSK-3β was increased significantly in the mitochondrial fraction and that of GSK-3β (pY216) in the cytosolic fraction. Moreover, a similar trend (but insignificant; $p = 0.09$) in the cytosolic fraction was observed for the total form of GSK-3β (Fig. 1B).

Cerebellum

In the cerebellum, the immunoreactivity levels of both total and active form of GSK-3β were significantly decreased in the nuclear fraction, whereas they were increased in the mitochondrial fraction after PQ (Fig. 1C). PQ additionally increased the level of total GSK-3β in this structure in the cytosolic fraction, but decreased slightly but significantly the phosphorylated form of this enzyme (Fig. 1C).

Discussion

This study showed that the long-term administration of PQ in rats diversely influenced the levels of GSK-3β in different brain structures. We found that PQ lowered levels of both total and active (pY216) forms of this kinase in the nuclear and cytosolic fractions in the hippocampus. In contrast, in the brain cortex and cerebellum this pesticide decreased levels of GSK-3β and GSK-3β (pY216) in the nuclear fraction, but increased them in the mitochondrial fractions and in some cases also in the cytosol, which suggested translocation of this kinase from the nucleus to other cellular compartments.

In our previous paper [58], we demonstrated that the same 37-week regime of PQ administration as used in the present study increased the level of GSK-3β and its active (pY216) form in all cellular compartments in the midbrain and pons, and induced an opposite effect in the striatum. All these results showed that the long-term exposure to PQ not only influenced variably the level of GSK-3β but also affected its activation in different brain structures. These alterations may have significant implications for cell functions.

Our previous papers have shown that long-term 24-37-week treatment with PQ moderately decreased the number of dopaminergic neurons in the substantia nigra pars compacta and ventral tegmental area, as well as noradrenergic cells in the locus coeruleus, and weakly lowered the level of dopamine, or its metabolism in the striatum in rats [43-45,58]. These results were in good agreement with several others which showed that PQ induced loss of dopaminergic neurons and disturbances of dopaminergic transmission [6,25,28,37,57]. Our last paper also suggested that increased levels of the total and activated (pY216) form of GSK-3β in the midbrain and pons may be involved in the PQ-induced degeneration processes progressing in catecholaminergic neurons [58]. Such a role of GSK-3β may be supposed because this kinase contributes to the production of reactive oxygen species [62] and its overexpression promotes cell death caused by the p53-dependent mitochondrial intrinsic apoptotic pathway [3,31], which are the main mechanisms responsible for the PQ-induced toxicity [66]. Moreover, recent studies suggested that microglia-dependent inflammatory processes (including activation of pro-inflammatory cytokines and nuclear factor [NF]-κB) contribute to PQ-induced toxicity towards dopaminergic neurons [35,48]. GSK-3β is known as a regulator of inflammation via its influence on NF-κB [24,63] and therefore the activation of this kinase in the midbrain and pons [58] is likely to be involved in neuroinflammation induced by PQ in these regions. Further support for a crucial role of GSK-3β for de-

generative processes in catecholaminergic neurons comes from studies of other authors who showed that activation of this kinase (via increased phosphorylation on tyrosine 216 or decreased phosphorylation of serine 9) contributed to the promotion of cell death caused by other PD-related toxic agents, such as 6-hydroxydopamine (6-OHDA), rotenone and MPTP [8,27,64] and was involved in proteasomal inhibition [1].

Selective toxic influence of PQ on dopaminergic neurons in mice after its systemic administration was postulated by the earliest studies [37]. However, levels of this pesticide are elevated in parallel with increasing number of injections [47]. This may lead to its deleterious action on non-catecholaminergic neurons after prolonged (37-week) administration, as well. In agreement with this view, repeated injections of PQ alone or in combination with the fungicide maneb in normal and/or α-synuclein mutant mice were found to induce histopathological alterations (e.g. changes in neuronal arrangement, presence of pyknotic nuclei, disorganization of mitochondrial membrane and others) in neurons of several brain structures including hippocampus and cerebellum [9,40]. Our present study showing an influence of PQ on levels of GSK-3β in the hippocampus, cerebral cortex and cerebellum also indicates a rather non-selective, widely spread action of PQ throughout the brain. However, the mechanisms underlying these alterations are not understood and at this stage of study it is difficult to judge what kind of consequences they could cause.

PQ is known to destroy the cytoskeleton of different cells, including neurons, and has been found to induce microtubule aggregation and bundling, microfilament redistribution, as well as axonopathy, measured by a decrease in the number of neurofilaments [30,39,53,54,56]. All these alterations may disturb axonal transport. Our previous study [45,58] showed that PQ destroyed the sources of catecholaminergic hippocampal innervations: the noradrenergic locus coeruleus [36] and the dopaminergic ventral tegmental area [61]. Therefore, it seems possible that PQ also destroyed transportation of GSK-3β and GSK-3β (pY216) from catecholaminergic cell bodies in the above regions to their terminals in the hippocampus, which resulted in the lower levels of this kinase in nuclear and cytosolic fractions of the latter structure. Similar processes related to degeneration of the dopaminergic nigrostriatal pathway could be responsible for the decrease in levels of GSK-3β and its active form in the striatum reported in our previous paper [58]. However, in the light of the aforementioned signs of neuropathology in the hippocampus induced by PQ administration [9,40], it is highly probable that alterations of GSK-3β in this structure could be an adaptive, protective reaction activated concomitantly with degenerative processes triggered by this pesticide. The appearance of such processes in response to several neuronal toxins is a well-known phenomenon and with regard to PQ recent studies have shown activation of both pro- and antiapoptotic processes in cell lines after low doses of this pesticide [51]. Since some earlier studies have demonstrated changes in neurotransmissions in the hippocampus induced by PQ which were dependent on IFN-γ, it is likely that reduction of GSK-3β levels and its activation could oppose the development of inflammation in this structure [33].

However, in other brain structures, such as the brain cortex and cerebellum, 37-week PQ administration changed the subcellular localization of GSK-3β and GSK-3β (pY216), which were translocated from the nucleus to the mitochondria. Since GSK-3β is a multifunctional kinase [3], the consequences of these alterations are unclear at present. Although GSK-3β is located predominantly in the cytosol, activities of this enzyme in the nucleus and mitochondria are much greater than in the cytosolic fraction [5] and every redistribution of this enzyme between the subcellular organelles may be of special importance for its function. The translocation of GSK-3β from the cytosol to the nucleus or mitochondria is known to appear during apoptotic conditions [3]. GSK-3β influences a large number of transcription factors that control gene expression of apoptotic and anti-apoptotic proteins, as well as proteins involved in cellular growth and survival, neuroinflammation, or oxidative stress [3,13,22]. Therefore the reverse process, a decrease in the level of GSK-3β in the nucleus (which was observed in all examined brain structures except the midbrain and pons after PQ), may diminish its transcription activity and in this way may affect both degenerative and protective compensatory processes developing in response to this herbicide [22]. On the other hand, PQ is a toxin which disrupts mitochondrial structure and several of its functions [12,38,40,50,66]. Therefore, increased levels of GSK-3β and GSK-3β (pY216)

in this subcellular compartment in brain cortex and cerebellum may facilitate these processes [3]. Finally, it seems that the fate of neurons in the above-mentioned brain structures after PQ administration may depend on the result of alterations of all processes which involve GSK-3β.

Summing up, the present paper suggests that long-term PQ administration influences levels and activation of GSK-3β in different brain structures, which may contribute to its toxicity, but on the other hand may suggest development of adaptive, protective processes.

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References

1. Avraham E, Szargel R, Eyal A, Rott R, Engelender S. Glycogen synthase kinase-3β modulates synphilin-1 ubiquitylation and cellular inclusion formation by SIAH; implications for proteasomal function and Lewy body formation. J Biol Chem 2005; 280: 42877-42886.
2. Beaulieu JM, Zhang X, Rodriguiz RM, Sotnikova TD, Cools MJ, Wetsel WC, Gainetdinov RR, Caron MG. Role of GSK3 beta in behavioral abnormalities induced by serotonin deficiency. Proc Natl Acad Sci USA 2008; 105: 1333-1338.
3. Beurel E, Jope RS. The paradoxical pro- and anti-apoptotic actions of GSK3 in the intrinsic and extrinsic apoptosis signaling pathways. Progr Neurobiol 2006; 79: 173-189.
4. Bhat RV, Shanley J, Correll MP, Fieles WE, Keith RA, Scott CW, Lee CM. Regulation and localization of tyrosine 216 phosphorylation of glycogen synthase kinase-3β in cellular and animal models of neuronal degeneration. PNAS 2000; 97: 11074-11079.
5. Bijur G, Jope RS. Glycogen synthase kinase-3β is highly activated in nuclei and mitochondria. Neuroreport 2003; 14:2415-2418.
6. Brooks AI, Chadwick CA, Gelbard HA, Cory-Slechta DA, Federoff HJ. Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. Brain Res 1999; 823: 1-10.
7. Castello G, Drechsel DA, Patel M. Mitochondria are a major source of paraquat-induced reactive oxygen species production in the brain. J Biol Chem 2007; 282: 14186-14193.
8. Chen G, Bower KA, Ma C, Fang S, Thiele CJ, Luo JIA. Glycogen synthase kinase 3β mediates 6-hydroxydopamine-induced neuronal death. FASEB J 2004; 18: 1162-1164.
9. Chen Q, Niu Y, Zhang R, Guo H, Gao Y, Li Y, Liu R. The toxic influence of paraquat on hippocampus of mice: involvement of oxidative stress. Neurotoxicology 2010; 31: 310-316.
10. Cho J, Rameshwar P, Sadoshima J. Distinct roles of glycogen synthase kinase (GSK)-3alpha and GSK-3beta in mediating cardiomyocyte differentiation in murine bone marrow-derived mesenchymal stem cells. J Biol Chem 2009; 284: 36647-36658.
11. Clodfelder-Miller B, Sarno P, Zmijewska AA, Song L, Jope RS. Physiological and pathological changes in glucose regulated brain Akt and glycogen synthase kinase-3. J Biol Chem 2005; 280: 39723-39731.
12. Cochemé HM, Murphy MP. Complex I is the major site of mitochondrial superoxide production by paraquat. J Biol Chem 2008; 283: 1786-1798.
13. Doble BW, Woodgett JR. GSK-3: tricks of the trade for a multitasking kinase. J Cell Sci 2003; 116: 1175-1186.
14. Duarte AI, Santos P, Oliveira CR, Santos MS, Rego AC. Insulin neuroprotection against oxidative stress is mediated by Akt and GSK-3β signaling pathways and changes in protein expression. Bioch Bioph Acta 2008; 1783: 994-1002.
15. Embi N, Rylatt DB, Cohen P. Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMPdependent protein kinase and phosphorylase kinase. Eur J Biochem 1980; 107: 519-527.
16. Fukushima T, Yamada K, Hojo N, Isobe A, Shiwaku K, Yamane Y. Mechanism of cytotoxicity of paraquat. III. The effect of acute paraquat exposure on the electron transport system in rat mitochondria. Exp Toxicol Pathol 1994; 46: 437-441.
17. Hardt SE, Sadoshima J. Glycogen Synthase Kinase-3β: A Novel Regulator of Cardiac Hypertrophy and Development. Circ Res 2002; 90: 1055-1063.
18. Hertzman C, Wiens M, Bowering D, Snow B, Calne D. Parkinson's disease: a case-control study of occupational and environmental risk factors. Am J Ind Med 1990; 17: 349-355.
19. Hoeflich KP, Luo J, Rubie EA, Tsao MS, Jin O, Woodgett JR. Requirement for GSK3beta in cell surival and NFκB activation. Nature 2000; 406: 86-90.
20. Hughes K, Nikolakaki E, Plyte SE, Totty NF, Woodgett JR. Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. EMBO J 1993; 12: 803-808.
21. Iqbal K, Wang I-Z, Grudke-Iqbal I. Kinases and phosphatases and tau sites involved in Alzheimer neurofibrillary degeneration. Eur J Neurosience 2007; 55: 59-68.
22. Jain AK, Jaiswal AK. GSK-3β acts upstream of Fyn kinase in regulation of nuclear export and degradation of NF-E2-related factor 2. J Biol Chemistry 2007; 282: 16502-16510.
23. Jope RS, Yuskaitis CJ, Beurel E. Glycogen synthase kinase-3 (GSK3): Inflamation, Diseases and Therapeutics. Neurochem Res 2007; 32: 577-595.
24. Kai JI, Huang WC, Tsai CC, Chang WT, Chen CL, Lin CF. Glycogen synthase kinase-3β indirectly facilitates interferon-κ-induced nuclear factor – κB activation and nitric oxide biosynthesis. J Cell Biochem 2010; 111: 1522-1530.
25. Kang MJ, Gil SJ, Koh HC. Paraquat induces alteration of the dopamine catabolic pathways and glutathione levels in the substantia nigra of mice. Toxicol Lett 2009; 188: 148-152.
26. Kimura T, Yamashita S, Nakao S, Park JM, Murayama M, Mizoroki T, Yoshiike Y, Sahara N, Takashima A. GSK-3beta is required for memory reconsolidation in adult brain. PLoS ONE 2008; 3: e3540.
27. King TD, Jope RS. Inhibition of glycogen synthase kinase-3 protects cells from intrinsic but not extrinsic oxidative stress. Neuro report 2005; 16: 597-601.
28. Kuter K, Śmiałowska M, Wierońska J, Zięba B, Wardas J, Pietraszek M, Nowak P, Biedka I, Roczniak W, Konieczny J, Wolfarth S, Ossowska K. Toxic influence of subchronic paraquat

administration on dopaminergic neurons in rats. Brain Res 2007; 1155: 196-207.
29. Kwok JBJ, Hallupp M, Loy CT, Chan DKY, Woo J, Mellick GD, Buchanan DD, Silburn PA, Halliday GM, Schofield PR. GSK3β polymorphisms alter transcription and splicing in Parkinson's disease. Ann Neurol 2005; 58: 829-839.
30. Li WD, Zhao YZ, Chou IN. Paraquat-induced cytoskeletal injury in cultured cells. Toxicol Appl Pharmacol 1987; 91: 96-106.
31. Linseman DA, Butts BD, Precht TA, Phelps RA, Le SS, Laessig TA, Bouchard RJ, Florez-McClure ML, Heidenreich KA. Glycogen synthase kinase-3beta phosphorylates Bax and promotes its mitochondrial localization during neuronal apoptosis. J Neurosci 2004; 24: 9993-10002.
32. Liou HH, Tsai MC, Chen CJ, Jeng JS, Chang YC, Chen SY, Chen RC. Environmental risk factors and Parkinson's disease: a case-control study in Taiwan. Neurology 1997; 48: 1583-1588.
33. Littlejohn D, Mangano E, Shukla N, Hayley S. Interferon-γ deficiency modifies the motor and co-morbid behavioral pathology and neruochemical changes provoked by the pesticide paraquat. Neuroscience 2009; 164: 1894-1906.
34. Liu KJ, Arron JR, Stankunas K, Crabtree GR, Longaker MT. Chemical rescue of cleft palate and midline defects in conditional GSK3b mice. Nature 2007; 446: 79-82.
35. Mangano EN, Litteljohn D, So R, Nelson E, Peters S, Bethune C, Bobyn J, Hayley S. Interferon-γ plays a role in paraquat-induced neurodegeneration involving oxidative and proinflammatory pathways. Neurobiol Aging 2011; doi: 10.1016/j/neurobiolaging.2011.02.016.
36. Mason ST, Fibiger HC. Regional topography within noradrenergic locus coeruleus as revealed by retrograde transport of horseradish peroxidase. J Comp Neurol 1979; 187: 703-724.
37. McCormack AL, Thiruchelvam M, Manning-Bog AB, Thiffault C, Langston JA, Cory-Slechta DA, Di Monte DA. Environmental risk factors and Parkinson's disease: selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat. Neurobiol Dis 2002; 10: 119-127.
38. Miller GW. Paraquat: the red herring of Parkinson's disease research. Toxicol Sci 2007; 100: 1-2.
39. Milzani A, Dalledonne I, Vailati G, Colombo R. Paraquat induces actin assembly in depolymerizing conditions. FASEB 1997; 11: 261-270.
40. Norris EH, Uryu K, Leight S, Giasson BI, Trojanowski JQ, Lee VM Y. Pesticide exposure exacerbates α-synucleinopathy in an A53T transgenic mouse model. Am J Pathol 2007; 170: 658-666.
41. O'Brien WT, Harper AD, Jove F, Woodgett JR, Maretto S, Piccolo S, Klein PS. Glycogen synthase kinase-3beta haploinsufficiency mimics the behavioral and molecular effects of lithium. J Neurosci 2004; 24: 6791-6798.
42. Ohori K, Miura T, Tanno M, Miki T, Sato T, Ishikawa S, Horio Y, Shimamoto K. Ser9 phosphorylation of mitochondrial GSK-3beta is a primary mechanism of cardiomyocyte protection by erythropoietin against oxidant-induced apoptosis. Am J Physiol Heart Circ Physiol 2008; 295: H2079-2086.
43. Ossowska K, Wardas J, Kuter K, Nowak P, Dąbrowska J, Bortel A, Labus L, Kwieciński A, Krygowska-Wajs A, Wolfarth S. Influence of paraquat on dopaminergic transporter in the rat brain. Pharmacol Rep 2005; 57: 330-335.
44. Ossowska K, Wardas J, Śmiałowska M, Kuter K, Lenda T, Wierońska JM, Zięba B, Nowak P, Dąbrowska J, Bortel A, Kwieciński A, Wolfarth S. A slowly developing dysfunction of dopaminergic nigrostriatal neurons induced by long-term paraquat administration in rats: an animal model of preclinical stages. Eur J Neurosci 2005; 22: 1294-1304.
45. Ossowska K, Śmiałowska M, Kuter K, Wierońska J, Zięba B, Wardas J, Nowak P, Dąbrowska J, Bortel A, Biedka I, Schulze G, Rommelspacher H. Degeneration of dopaminergic mesocortical neurons and activation of compensatory processes induced by a long-term paraquat administration in rats: implications for Parkinson's disease. Neuroscience 2006; 141: 2155-2165.
46. Pajak B, Songin M, Strosznajder JB, Gajkowska B. Alzheimer's disease genetic mutation evokes ultrastructural alterations: correlation to an intracellular Abeta deposition and the level of GSK-3beta-P(Y216) phosphorylated form. Neurotoxicology 2009; 30: 581-588.
47. Prasad K, Winnik B, Thiruchelvam MJ, Buckley B, Mirochnitchenko O, Richfield EK. Prolonged toxicokinetics and toxicodynamics of paraquat in mouse brain. Environ Health Perspect 2007; 115: 1448-1453.
48. Purisai MG, McCormack AL, Cumine S, Li J, Isla MZ, DiMonte DA. Microglial activation as a priming event leading to paraquat-induced dopaminergci cell degeneration. Neurobiol Dis 2007; 25: 392-400.
49. Ragano-Caracciola M, Berlin WK, Miller MW, Hanover JA. Nuclear glycogen and glycogen synthase kinase 3. Biochem Biophys Res Commun 1998; 249: 422-427.
50. Richardson JR, Quan Y, Sherer TB, Greenamyre JT, Miller GW. Paraquat neurotoxicity is distinct from that of MPTP and rotenone. Toxicol Sci 2005; 88: 193-201.
51. Santano MN, Morán JM, Garcia-Rubio L, Gómez-Martin A, González-Polo, Soler G, Fuentes JM. Low concentrations of paraquat induces early activation of extracellular signal-regulated kinase 1/2, protein kinase B, c-Jun N-terminal linase 1/2 pathway: role of c-June N-terminal kinase in paraquat-induced cell death. Toxicol Sci 2006; 92: 507-515.
52. Sarno P, Bijur GN, Zmijewska AA, Li X, Jope RS. In vivo regulation of GSK3 phosphorylation by cholinergic and NMDA receptors. Neurobiol Aging 2006; 27: 413-422.
53. Schmuck G, Schlüter G. An in vitro model for toxicological investigations of environmental neurotoxins in primary neuronal cell cultures. Toxicol Ind Health 1996; 12: 683-696.
54. Schmuck G, Ahr HJ, Schlüter G. Rat cortical neurons cultures: an in vitro model for differentiating mechanisms of chemically induced neurotoxicity. In Vitr Mol Toxicol 2000; 13: 37-50.
55. Schmuck G, Rőhrdanz E, Tran-Thi Q-H, Kahl R, Schlüter G. Oxidative stress in rat cortical neurons and astrocytes induced by paraquat in vitro. Neurotox Res 2002; 4: 1-13.
56. Schmuck G, Kahl R. The use of Fluro-Jade in primary neuronal cell cultures. Arch Toxicol 2009; 83: 397-403.
57. Shimizu K, Matsubara K, Ohtaki K, Fujimaru S, Saito O, Shiono H. Paraquat induces long-lasting dopamine overflow through the excitotoxic pathway in the striatum of freely moving rats. Brain Res 2003; 976: 243-252.

58. Songin M, Strosznajder JB, Fitał M, Kuter K, Kolasiewicz W, Nowak P, Ossowska K. Glycogen synthase kinase 3β and its phosphorylated form (Y216) in the paraquat-induced model of parkinsonism. Neurotox Res 2011; 19: 162-171.
59. Soutar MP, Kim WY, Williamson R, Peggie M, Hastie CJ, McLauchlan H, Snider WD, Gordon-Weeks PR, Sutherland C. Evidence that glycogen synthase kinase-3 isoforms have distinct substrate preference in the brain. J Neurochem 2010; 115: 974-983.
60. Strosznajder RP, Jesko H, Adamczyk A. Effect of aging and oxidative/genotoxic stress on poly(ADP-ribose) polymerase-1 activity in rat brain. Acta Biochim Pol 2005; 52: 909-914.
61. Swanson LW. The projection of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull 1982; 9: 321-353.
62. Valerio A, Bertolotti P, Delbarba A, perego C, Dossena M, Ragni M, Spano P, Carruba MO, De Simoni MG, Nisoli E. Glycogen synthase kinase-3 inhibition reduces ischemic cerebral damage, restores impaired mitochondrial biogenesis and prevents ROS production. J Neurochem 2011; 116: 1148-1159.
63. Wang MJ, Huang HY, Chen WF, Chang HF, Kuo JS. Glycogen synthase kinase-3β inactivation inhibits tumor necrosis factor-β production in microglia by modulating nuclear factor βB and MLK/JNK signaling cascades. J Neuroinflammation 2010; 7: 99.
64. Wang W, Yang Y, Ying C, Li W, Ruan H, Zhu X, You Y, Han Y, Chen R, Wang Y, Li M. Inhibition of glycogen synthase kinase-3β protects dopaminergic neurons from MPTP toxicity. Neuropharmacology 2007; 52: 1678-1684.
65. Winterbourne CC. Production of hydroxyl radicals from paraquat radicals and H2O2. FEBS Lett 1981; 128: 339-342.
66. Yang W, Tiffany-Castiglioni E. Paraquat-induced apoptosis in human neuroblastoma SH-SY5Y cells: involvement of p53 and mitochondria. J Toxicol Environ Health 2008; 71: 289-299.
67. Yang W, Tiffany-Castiglioni E, Koh HC, Son IH. Paraquat activates the IRE1/ASK1/JNK cascade associated with apoptosis in human neuroblastoma SH-SY5Y cells. Toxicol Lett 2009; 191: 203-210.