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GLYCOGEN SYNTHASE KINASE-3 REDUCES ACETYLCHOLINE LEVEL IN STRIATUM VIA DISTURBING CELLULAR DISTRIBUTION OF CHOLINE ACETYLTRANSFERASE IN CHOLINERGIC INTERNEURONS IN RATS

L. ZHAO,[b] C.-B. CHU,[c] J.-F. LI,[b] Y.-T. YANG,[b] S.-Q. NIU,[a] W. QIN,[a] Y.-G. HAO,[a] Q. DONG,[a] R. GUAN,[d] W.-L. HU[a]* AND Y. WANG[a]*

[a] *Department of Neurology, Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China*

[b] *Department of Neurobiology and Beijing Institute for Brain Disorders, Capital Medical University, Beijing, China*

[c] *Department of Neurology, Xuan Wu Hospital of the Capital Medical University, Beijing, China*

[d] *Department of Neurology, University of Chicago, Chicago, USA*

Abstract—Cholinergic interneurons, which provide the main source of acetylcholine (ACh) in the striatum, control the striatal local circuits and deeply involve in the pathogenesis of neurodegenerative diseases. Glycogen synthase kinase-3 (GSK-3) is a crucial kinase with diverse fundamental functions and accepted that deregulation of GSK-3 activity also plays important roles in diverse neurodegenerative diseases. However, up to now, there is no direct proof indicating whether GSK-3 activation is responsible for cholinergic dysfunction. In the present study, with combined intracerebroventricular injection of Wortmannin and GF-109203X, we activated GSK-3 and demonstrated the increased phosphorylation level of microtubule-associated protein tau and neurofilaments (NFs) in the rat striatum. The activated GSK-3 consequently decreased ACh level in the striatum as a result of the reduction of choline acetyltransferase (ChAT) activity. The alteration of ChAT activity was due to impaired ChAT distribution rather than its expression. Furthermore, we proved that cellular ChAT distribution was dependent on low phosphorylation level of NFs. Nevertheless, the cholinergic dysfunction in the striatum failed to induce significant neuronal number reduction. In summary, our data demonstrates the link between GSK-3 activation and cholinergic dysfunction in the striatum and provided beneficial evidence for the pathogenesis study of relevant neurodegenerative diseases.

Key words: glycogen synthase kinase-3, acetylcholine, choline acetyltransferase, neurofilaments.

INTRODUCTION

The striatum is a subcortical structure that integrates multiple inputs from the cortex, thalamus, and midbrain (Bolam et al., 2000; Zhou et al., 2002). It relays information to the output domains of the basal ganglia and involves in motor control, reinforcement learning, motivational processes and drug addiction (Haber, 2003; Aosaki et al., 2010). The striatum contains two types of neurons: projection neurons and interneurons (Bolam et al., 2000; Tepper and Bolam, 2004). The projection neurons, responsible for output signal of the striatum, are medium-sized spiny cells (Koos and Tepper, 2002) and account for 96% of the striatal cells in rodents (Yelnik et al., 1991), but this percentage decreases to 74% while the number of local circuit neurons increases significantly in humans (Roberts et al., 1996). The local circuits are controlled by striatal giant aspiny cholinergic interneurons, which have widespread connections throughout the striatum and provide the main source of acetylcholine (ACh) to the striatum (Woolf and Butcher, 1981). Although few in number, the cholinergic interneurons have extremely dense axonal arbors and help regulate the duration, strength and spatial pattern of striatal medium-sized neuron output (Graveland et al., 1985; Zhou et al., 2002; Oldenburg and Ding, 2011), thus they play a crucial role in modulating striatal function including dopamine release through the activation of ACh receptor subtypes (Smolders et al., 1997; Tepper and Bolam, 2004; Apicella, 2007). While the harmonious ACh–dopamine interactions determine the production of fluid voluntary movements (Lester et al., 2010), dysfunction of cholinergic signaling is primarily associated with pathophysiological changes in movement disorders such as Huntington's disease (HD) and Parkinson's disease (PD) (Ding et al., 2006; Pisani et al., 2007). Moreover, the striatal cholinergic interneurons are also involved in the processing of

*Corresponding authors. Address: Department of Neurology, Beijing Chao-Yang Hospital, Capital Medical University, 8 Gongren Tiyuchang Nanlu, Chaoyang District, Beijing 100020, China. Tel: +86-10-85231398 (W.-L. Hu), +86-10-85231548 (Y. Wang).
E-mail addresses: huwenli@sina.com (W.-L. Hu), wyjoe2000@163.com (Y. Wang).

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; aCSF, artificial cerebrospinal fluid; AD, Alzheimer's disease; ChAT, choline acetyltransferase; EGTA, ethylene glycol tetraacetic acid; GFX, GF-109203X; GSK-3, glycogen synthase kinase-3; HD, Huntington's disease; HPLC, High-performance liquid chromatography; NFs, neurofilaments; PD, Parkinson's disease; pS214, phosphorylated tau at Ser 214 epitope; pS396, phosphorylated tau at Ser 396 epitope; pS9-GSK-3β, phosphorylated GSK-3β at Ser 9 epitope; pT231, phosphorylated tau at Thr 231 epitope; SMI31, phosphorylated NFs; SMI33, nonphosphorylated NFs; WT, wortmannin.

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cognition (Stocco et al., 2010; Ashby and Crossley, 2011), synaptic plasticity (Wang et al., 2006) and learning (Aosaki et al., 1994), and their number is decreased in certain neurodegenerative diseases, such as Alzheimer's disease (AD) (Selden et al., 1994). But the mechanism underlying the cholinergic interneuron impairment is still unclear.

Glycogen synthase kinase-3 (GSK-3) is a highly conserved, constitutively active serine/threonine kinase which is initially discovered involving in the regulation of glucose metabolism and later as a participant in Wnt/wingless signaling (McManus et al., 2005; Rao et al., 2007). Moreover, GSK-3 has been identified as a crucial kinase with diverse fundamental functions in cell cycle, gene transcription, cytoskeletal integrity, and apoptosis, as a result of its ability to phosphorylate key proteins that modulate these processes (Frame and Cohen, 2001). GSK-3 is ubiquitously expressed, including abundant expression in the brain (Woodgett, 1990). It has been widely accepted that deregulation of GSK-3 activity (activated or inhibited) has been postulated to play important roles in diverse neurodegenerative diseases. In cell model of HD, lithium reduced polyglutamine toxicity and rescued cell death through GSK-3 inhibition (Carmichael et al., 2002). Similarly, lithium also showed antiapoptotic neuroprotective effect in the striatum of animal model of HD (Senatorov et al., 2004; Chuang, 2005). Tau is a phosphoprotein containing normally 1–3 mol of phosphate per mole of tau protein. In the pathological condition, the phosphorylation level of tau is three- to fourfold higher than that of tau isolated from age-matched control brains. GSK-3 was found robustly activated in the postmortem of PD patients (Duka et al., 2009) resulting in high level of phosphorylated tau specifically in the striatum, but not in the inferior frontal gyrus (Haggerty et al., 2011). Blockage of GSK-3β activity protected dopaminergic neurons from apoptosis in PD model, restored the depletion of dopamine and ameliorated the PD-like behavioral impairments (Wang et al., 2007). GSK-3 has also been identified as a crucial kinase that can actively phosphorylate tau at most of the abnormal sites seen in the AD brains (Ishiguro et al., 1993; Plattner et al., 2006) and the activity of GSK-3 is significantly associated with spatial memory parameters in an AD animal model (Wang et al., 2008).

All the above data suggest that striatal cholinergic interneurons and GSK-3 are both deeply involved in neurodegenerative disease, including HD, PD and AD. However, the relationship between them, in more detail, whether and how GSK-3 activation regulates cholinergic function of striatal interneurons is elusive. In our earlier research we found simultaneous inhibition of phosphoinositol-3 kinase and protein kinase C by wortmannin (WT) and GF-109203X (GFX) activated GSK-3 and decreased the level of hippocampal ACh remarkably (Wang et al., 2008). By using this rat model, we presently explored the effects of GSK-3 on cholinergic function in the rat striatum. We found that activation of GSK-3 decreased ACh level and inhibited choline acetyltransferase (ChAT) activity via altering the cellular distribution, with invariable acetylcholinesterase (AChE) activity.

EXPERIMENTAL PROCEDURES

Ethics statement

The experimental procedures were carried out in accordance with the Chinese regulations involving animal protection and approved by the animal ethics committee of the China Capital Medical University. Male Wistar rats (weight 200–250 g) were housed in groups of two per cage with free access to food (standard rodent) and water. The room was maintained on 12-h light:12-h dark cycle (lights on at 18:00 pm, and off at 06:00 am) and stable temperature (23–25 °C) and humidity.

Antibodies and chemicals

The detailed information for the antibodies used in this work is listed in Table 1. WT, GFX and LiCl (inhibitor of the GSK-3 enzyme) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Intracerebroventricular injection

Intracerebroventricular injection was performed as described before (Jiang et al., 2011). Briefly, the rats were first anesthetized with chloral hydrate (30 mg/kg, intraperitoneally) and placed on a stereotaxic instrument with the incisor bar set 2 mm below the ear bars (i.e. flat skull). A 10 μl syringe (Hamilton) was placed into the left ventricle at the co-ordinates for bregma and dura of AP-0.9, L-1.5 and V-4 (in mm) after the scalp was incised and retracted. The drugs were dissolved in

Table 1. Antibodies employed in the study

Antibody	Specific	Type	WB	IHC or IF	Source
Ser9-phospho-GSK-3β	Phosphorylated GSK-3β at Ser9	pAb	1:1000		Cell Signaling, MA, USA
GSK-3β	Total GSK-3β	mAb	1:1000		Cell Signaling, MA, USA
pS396	Phosphorylated tau at Ser396	pAb	1:1000	1:500	Invitrogen, NY, USA
pT231	Phosphorylated tau at Thr231	pAb	1:1000		Invitrogen, NY, USA
pS214	Phosphorylated tau at Ser214	pAb	1:1000		Invitrogen, NY, USA
SMI31	Phosphorylated neurofilaments	mAb	1:1000	1:500	Covance, North Yorkshire, UK
SMI33	Non-phosphorylated neurofilaments	mAb	1:1000	1:500	Covance, North Yorkshire, UK
ChAT	Choline Acetyltransferase	pAb	1:1000	1:200	Chemicon, CA, USA
β-actin	β-actin	mAb	1:1000		Abcam, MA, USA

WB – Western blot, IHC – immunohistochemistry, IF – immunofluorescence, mAb – monoclonal antibody, pAb – polyclonal antibody.