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Sgk1, a cell survival response in neurodegenerative diseases

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Serum and glucocorticoid-regulated kinase 1 (sgk1) belongs to a family of serine/threonine kinases that is under acute transcriptional control by serum and glucocorticoids. An expanding set of receptors and cellular stress pathways has been shown to enhance sgk1 expression, which is implicated in the regulation of ion channel conductance, cell volume, cell cycle progression, and apoptosis. Recent evidence for the involvement of sgk1 in the early pathogenesis of MPTP-induced Parkinson's disease (PD) prompted us to investigate in more detail its expression and role in animal models of different neurodegenerative diseases.

Here, we show that transcription of sgk1 is increased in several animal models of PD and a transgenic model of amyotrophic lateral sclerosis (ALS). The upregulation of sgk1 strongly correlates with the occurrence of cell death. Furthermore, we provide evidence that the Forkhead transcription factor FKHRL1 and some of the voltage-gated potassium channels are physiological substrates of sgk1 in vivo. Using a small interfering RNA approach to silence sgk1 transcripts in vitro, we give evidence that sgk1 exerts a protective role in oxidative stress situations.

These findings underline a key role for sgk1 in the molecular pathway of cell death, in which sgk1 seems to exert a protective role.

Introduction

Parkinson's disease (PD) is a slow progressive disorder of the nervous system. Cardinal symptoms include resting tremor, rigidity, bradykinesia, and postural instability. The primary cause of the symptoms is the death of dopamine (DA)-producing neurons in the substantia nigra (SN) and the resultant depletion of DA in the striatum (ST).

The search for the molecular basis of dopaminergic cell death has been the recent focus in PD research. Studies using human postmortem brain tissue, animal models and primary mesencephalic neurons elucidated that relevant processes inducing neurodegeneration include (i) abnormal protein dynamics with defective protein degradation and aggregation, many of which are related to the ubiquitin–proteasomal system, (ii) oxidative stress and free radical formation, (iii) impaired bioenergetics and mitochondrial dysfunctions, and (iv) neuroinflammatory processes. Many of these processes are triggered by changes in gene transcription. Understanding this genetic cascade may provide the basis for the development of neuroprotective therapies for PD.

In a recent study, we analyzed the gene expression pattern after MPTP intoxication in the mouse ventral midbrain, i.e., in the phase in which dopaminergic cell degeneration occurs. Applying an automated, PCR-based expression profiling system with high sensitivity and reliability (digital expression pattern display, DEPD; Maelicke and Lübbert, 2002), we detected several gene expression changes accompanying the degenerative process. We identified the serum and glucocorticoid-dependent kinase 1 (sgk1) as one of the most strongly upregulated genes.

Sgk1, a serine/threonine-specific protein kinase, was originally cloned as a glucocorticoid (Webster et al., 1993a,b)- and cell-volume-sensitive gene (Waldegger et al., 1997) that regulates sodium absorption by the amiloride-sensitive sodium channel in kidney principal cells (Chen et al., 1999). An expanding set of cell surface receptors, nuclear receptors, and cellular stress pathways has been shown to target sgk1 (for reviews, see Lang and Cohen, 2001; Firestone et al., 2003; Lang et al., 2003). There is increasing evidence that sgk1 expression is regulated during both discrete developmental stages and pathological conditions such as hyper-

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; ASA, acetylsalicylic acid; C, cytoplasmic fraction; DA, dopamine; DAT, dopamine transporter; DEPD, digital expression pattern display; DEX, dexamethasone; DIC, days in culture; DIG, digoxigenin; FKHRL1, forkhead transcription factor, Forkhead box protein O3A (FOXO3A); hm, human; IHC, immunohistochemistry; ISH, in situ hybridization; LM, littermate; M, membrane fraction; Ma, maneb; MPP+, 1-methyl-4-phenylpyridinium ion; MPTP, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; N, nuclear fraction; OD, optical density; PA, paraquat; PD, Parkinson's disease; PFA, paraformaldehyde; sgk1, serum and glucocorticoid-regulated kinase 1; SDS, sodium dodecyl sulfate; siRNA, small interfering RNA; SN, substantia nigra; SOD1, superoxide dismutase 1; SPA, sodium potassium ATPase; sqPCR, semiquantitative PCR; SSC, saline sodium citrate; ST, striatum; TBP, TATA-binding protein; TG, transgene; TH, tyrosine hydroxylase.

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tension, diabetic neuropathy (Lang and Cohen, 2001), ischemia (Nishida et al., 2004b), trauma (Imaizumi et al., 1994), and neurodegenerative diseases (Hollister et al., 1997; Rangone et al., 2004; Iwata et al., 2004; Stichel et al., 2005). Several observations point to a physiological role of sgk1 in cell cycle progression (Buse et al., 1999) and cell survival pathways. For instance, activated sgk1 regulates the voltage-gated K+ channel Kv1.3 (Gamper et al., 2002a,b), expected to play a role in neuroexcitability, and inactivates the proapoptotic enzyme glycogen synthase kinase 3 (GSK3) (Kaytor and Orr, 2002; Jope and Johnson, 2004) as well as the proapoptotic forkhead transcription factor family member FKHRL1 (FOXO3a) (Brunet et al., 2001; You et al., 2004), which stimulates the expression of FasL and GADD45.

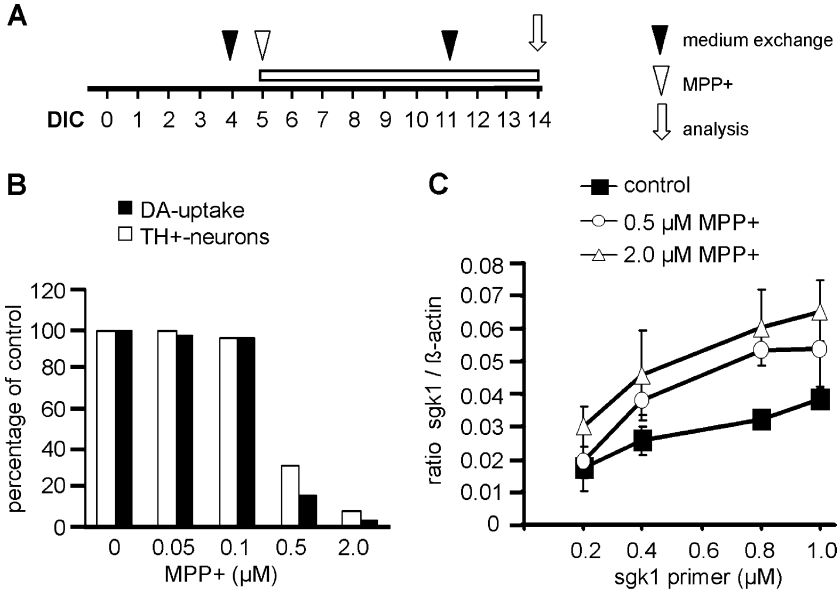
These data prompted us to investigate the role of sgk1 in animal models of neurodegenerative diseases. Hence, in this study, we examined

(i) the differential expression of sgk1 transcript in human PD tissue as well as in additional chemical and genetic animal models to determine whether sgk1 regulation plays a general role in PD pathogenesis;
(ii) the expression pattern of sgk1-protein in PD, to determine whether the altered gene regulation is also reflected by enhanced protein expression;
(iii) the expression profile of proteins regulated by sgk1, in order to elucidate molecular processes induced by sgk1;
(iv) the influence of pharmacological interventions on sgk1 expression in PD;
(v) the involvement of sgk1 in other neurodegenerative diseases; and
(vi) the effect of RNAi-mediated downregulation of sgk1 during cell stress.

Results

Sgk1-mRNA is upregulated in various neurotoxic animal models of Parkinson's disease

Using gene expression profiling, we have previously shown that sgk1 is strongly upregulated in the brains of mice after subacute or acute MPTP treatment (Stichel et al., 2005). In this



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primer (µM) conc.	ratio sgk / ß-actin								
	saline			0.5 µM MPP+			2.0 µM MPP+		
	exp 1	exp 2	mean	exp 1	exp 2	mean	exp 1	exp 2	mean
0.2	0.014	0.019	0.017	0.025	0.013	0.019	0.025	0.034	0.030
0.4	0.022	0.028	0.025	0.041	0.034	0.038	0.036	0.055	0.045
0.8	0.031	0.032	0.032	0.053	0.052	0.053	0.052	0.068	0.060
1.0	0.036	0.04	0.038	0.045	0.061	0.053	0.058	0.071	0.065

Fig. 1. MPP+ induces sgk1 upregulation in primary mesencephalic cultures. (A) Experimental protocol of chronic MPP^+ treatment. (B) Effect of different MPP^+ concentrations on dopamine (DA) uptake and the number of tyrosine hydroxylase (TH)-immunopositive neurons. Exposure to concentrations higher than 0.1 µM MPP+ induces a strong reduction of DA uptake and cell death of dopaminergic neurons at DIC14. Data represent the percentage of control of 2 to 7 independent experiments with 4 wells for DA uptake and 3 coverslips for TH-immunocytochemistry each. (C, D) Semiquantitative PCR analysis of sgk1 expression, showing upregulation of sgk1 transcripts in mesencephalic neurons treated with 0.5 or 2.0 µM MPP^+, presented as raw data (D) and in a histogram (C). A competitive RT-PCR approach has been used, in which increasing concentrations of sgk1 primers compete with a constant concentration of β-actin primers in the same PCR reaction. The ratio between the amounts of two PCR products provides a robust mRNA quantification. Data are the means ± SD of two independent experiments, each with $n = 25–29$ embryos.