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Neurobiology of Aging 30 (2009) 474–482

NEUROBIOLOGY OF AGING

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Identification and characterization of an insulin receptor substrate 4-interacting protein in rat brain: Implications for longevity

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Received 12 March 2007; received in revised form 14 June 2007; accepted 17 July 2007
Available online 27 August 2007

Abstract

The hypothalamus is organized as a collection of distinct, autonomously active nuclei that regulate discrete functions, such as feeding activity and metabolism. We used suppression subtractive hybridization (SSH) to identify genes that are enriched in the hypothalamus of the rat brain. We screened a subtractive library of 160 clones, and 4 genes that were predominantly expressed in the hypothalamus, compared to other brain regions. The mRNA for a member of the WD-repeat family of proteins, WDR6, was abundantly expressed in the hypothalamus, and we found that WDR6 interacted with insulin receptor substrate 4 (IRS-4) in the rat brain. Interestingly, WDR6 gene expression in the hypothalamic arcuate nucleus was decreased by caloric restriction, and in growth hormone (GH)-antisense transgenic rats, both of which are associated with an increased life span. Insulin-like growth factor (IGF)-I and insulin treatment increased WDR6 gene expression in mouse hypothalamus-derived GT1-7 cells. Our results might suggest that WDR6 participates in insulin/IGF-I signaling and the regulation of feeding behavior and longevity in the brain.

Keywords: Insulin/IGF-I signal; Calorie restriction; SSH; WD-repeat protein

1. Introduction

The hypothalamus is a key component of the homeostatic mechanism of energy balance, which also involves coordination between the brain and peripheral tissues. Electrical ablation studies have implicated several hypothalamic nuclei as central regulatory sites for the major homeostatic systems governing feeding behavior, stress responses, metabolism, and reproduction (Shepherd, 1994). Within the central nervous system (CNS), specific hypothalamic nuclei, such as the arcuate and paraventricular nuclei, have been identified as pivotal functional sites for the integration of central and peripheral signals that govern these processes (Schwartz et al., 2000). Therefore, the hypothalamus has been the focus of intensive research, and the development of pharmaceutical-based strategies targeting hypothalamic neuronal pathways is currently one of the most active areas of research on obesity, diabetes, and the aging process.

A growing body of evidence suggests a key role for the CNS in the regulation of both body fat content and glucose metabolism (Schwartz and Porte, 2005). In response to hormonal and nutrient-related input signals, the body adapts in order to maintain energy homeostasis and normal levels of blood glucose. Defects in this control system are linked to

Abbreviations: SSH, suppression subtractive hybridization; WDR6, WD-repeat protein 6.

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0197-4580/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2007.07.008

the development of metabolic disorders, such as obesity and diabetes, through excessive calorie intake. Caloric restriction (CR), on the other hand, increases life span and retards the development of various age-related disorders in many organisms (Weindruch and Wolford, 1988). It is believed that CR regulates the aging processes in part through its effects on endocrine and/or neural regulatory systems (Masoro, 1988). The underlying molecular mechanism of regulation of the neuroendocrine system by CR has not yet been clearly elucidated, however, insulin/IGF-I and leptin, a fat-derived adipocytokine, have been proposed as potential molecular mediators of the adaptive response to CR (Barzilai and Gupta, 1999; Chiba et al., 2002; Katic and Kahn, 2005; Shimokawa and Higami, 1999).

Recent studies have identified several regulatory molecules that are involved in energy homeostasis. Nevertheless, there are still large gaps in our understanding of the mechanisms of regulation and integration of the various hypothalamic neuronal pathways involved in feeding behavior and/or metabolism. Because existing techniques for isolating candidate regulatory molecules are limited, it is likely that critical regulatory molecules have not yet been identified. In an attempt to bridge the gaps in our knowledge of these systems, we tried to isolate regulatory factors in the hypothalamus that are involved in the regulation of feeding and/or metabolism by analyzing hypothalamic-specific gene transcription. Techniques for analyzing and isolating novel gene transcripts in specific brain regions, or food deprivation-induced genes in the hypothalamus, include differential display libraries (Qu et al., 1996), directional tag PCR subtraction (Gautvik et al., 1996), and oligonucleotide microarrays (Li et al., 2002). To date, however, there have been a limited number of studies applying SSH to the isolation of genes that are expressed in different regions of the brain. Thus we performed SSH analysis to identify genes that are enriched in the hypothalamus.

In the current study, we identified four genes that are abundantly expressed in the rat hypothalamus. One of the hypothalamus-enriched transcripts encoded WDR6, and we demonstrated that WDR6 interacted with IRS-4, an important insulin receptor substrate in the brain. Moreover, WDR6 gene expression was decreased by CR and in rats in which the GH/IGF-I axis was suppressed. These results might suggest that WDR6 is involved in the regulation of insulin/IGF-I signaling in the hypothalamus.

2. Materials and methods

2.1. Animals

Animal care and experimental procedures were performed in accordance with the Guidelines for Animal Experimentation of Nagasaki University, with the approval of the Institutional Animal Care and Use Committee. Male 4-week-old specific pathogen-free (SPF) Wistar rats (Jcl: Wistar) were purchased from Charles River Laboratory Japan (Yokohama, Japan). Transgenic dwarf rats (mini, tg/tg) were described previously (Matsumoto et al., 1993; Shimokawa et al., 2002). These rats are pituitary-specific GH-antisense transgenic rats. These rats were maintained under SPF conditions in our barrier facility (temperature: 22–25 °C; light/dark cycle: 12 h). To generate (tg/−) rats, F1 heterozygous progeny were generated by crossing female Wistar (−/−) rats, which have the same genetic background as (tg/tg) rats, with male (tg/tg) rats. Wistar (−/−), (tg/−), and (tg/tg) rats were fed ad libitum (AL) with a CR-LPF diet (Oriental Yeast Co., Ltd., Tsukuba, Japan). To construct the subtraction library, Wistar (−/−) rats were fasted for 48 h, then sacrificed by decapitation. The brain was removed from each rat, weighed, and dissected into slices according to the technique of Palkovits and Brownstein, with some modifications as described previously (Komatsu et al., 2006; Palkovits and Brownstein, 1983). Male rats were used in all experiments in this study.

2.2. Caloric restriction

From 6 weeks of age, Wistar (−/−), Wistar (tg/−), were divided into two groups: one fed AL (AL rats), and one subjected to CR (CR rats, 70% energy intake). CR rats were fed every other day, as described previously (Shimokawa et al., 2003). Their 2-day food allotment was equal to 140% of the mean daily intake of age-matched AL rats. This regimen yielded a 2-day cycle of food intake in the CR groups. The four consecutive 12 h light/dark phases after feeding were designated Dark 1, Light 1, Dark 2 and Light 2. We subdivided each CR group into CR1 and CR2, representing CR rats sacrificed within the first 24-h period (Dark 1 and Light 1) and the second 24-h period (Dark 2 and Light 2), respectively. Wistar (−/−) and (tg/−) rats were sacrificed at 6–7 months of age (after 4–5 months of CR), and brain tissues were isolated as described above. Insulin and IGF-I concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) kit as described previously (Shimokawa et al., 2003) and are listed in supplementary Table 1 along with body weight data.

2.3. RNA isolation and construction of the cDNA subtraction libraries

To construct subtraction libraries, we used five 2-month-old Wistar rats. The following regions were isolated from five different rats: hypothalamus, hippocampus, cerebrum cortex, and cerebellum as described previously (Komatsu et al., 2006). To perform real-time PCR, Wistar (−/−) and (tg/−) rats were sacrificed at 6–7 months of age (after 4–5 months of CR) and the arcuate nucleus was isolated as described previously (Komatsu et al., 2006). Total RNA was then isolated from each region using the RNeasy Mini Kit (Qiagen, CA). Poly(A) + RNA was purified and cDNA synthesis were performed as described previously (Chiba et al., 2007). SSH