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Obesity/hyperleptinemic phenotype adversely affects hippocampal plasticity: Effects of dietary restriction

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ABSTRACT

Epidemiological studies estimate that greater than 60% of the adult US population may be categorized as either overweight or obese and there is a growing appreciation that obesity affects the functional integrity of the central nervous system (CNS). We recently developed a lentivirus (LV) vector that produces an insulin receptor (IR) antisense RNA sequence (IRAS) that when injected into the hypothalamus selectively decreases IR signaling in hypothalamus, resulting in increased body weight, peripheral adiposity and plasma leptin levels. To test the hypothesis that this obesity/hyperleptinemic phenotype would impair hippocampal synaptic transmission, we examined short term potentiation (STP) and long term potentiation (LTP) in the hippocampus of rats that received the LV-IRAS construct or the LV-Control construct in the hypothalamus (hypo-IRAS and hypo-Con, respectively). Stimulation of the Schaffer collaterals elicits STP that develops into LTP in the CA1 region of hypo-Con rats; conversely, hypo-IRAS rats exhibit STP that fails to develop into LTP. To more closely examine the potential role of hyperleptinemia in these electrophysiological deficits, hypo-IRAS were subjected to mild food restriction paradigms that would either: 1) prevent the development of the obesity phenotype; or 2) reverse an established obesity phenotype in hypo-IRAS rats. Both of these paradigms restored LTP in the CA1 region and reversed the decreases in the phosphorylated/total ratio of GluA1 Ser845 AMPA receptor subunit expression observed in the hippocampus of hypo-IRAS rats. Collectively, these data support the hypothesis that obesity impairs hippocampal synaptic transmission and support the hypothesis that these deficits are mediated through the impairment of hippocampal leptin activity.

1. Introduction

Ongoing epidemiological studies by the Centers for Disease Control estimate that greater than 60% of the adult US population may be categorized as either overweight or obese [1]. In addition to peripheral complications, there is a growing appreciation that the complications of obesity extend to the central nervous system (CNS). While the vast majority of these studies have focused upon the hypothalamus, more recent studies suggest that the complications of obesity may also affect the functional integrity of the hippocampus. For example, hippocampal-dependent behaviors are impaired in experimental models of obesity [2–14] and obesity/increased body mass index (BMI) is associated with decreased cognitive function in humans [15–18]. Unfortunately, the underlying molecular and cellular mechanisms responsible for obesity-induced cognitive deficits remain unclear. One potential mediator of hippocampal plasticity deficits in obesity may be hyperleptinemia.

Leptin is synthesized and secreted by adipocytes and is transported across the blood-brain barrier (BBB) via a saturable transport system [19]. In the hypothalamus, leptin is recognized as an important integration factor for the coordination of peripheral and central signals to regulate food intake, metabolism, body weight and body composition (for review see [20]). In addition, there is a growing literature to support a role for leptin in the facilitation of hippocampal function [21]. For example, leptin regulates hippocampal plasticity by converting short term potentiation (STP) into long term potentiation (LTP) [22], a functional enhancement that may contribute to leptin's ability to improve hippocampal-dependent behavioral performance [23,24]. In this regard, intrahippocampal [23], as well as peripheral administration of leptin [24], enhances behavioral performance in a dose-dependent manner. However, leptin transport across the blood-brain barrier is impaired in obesity phenotypes [19,25,26], which led to the hypothesis that decreases in hippocampal leptin signaling and/or leptin transport are associated with decreases in synaptic plasticity.

We recently developed a model of obesity using a lentiviral vector that produces an antisense RNA selective for the insulin receptor (IRAS) [27]. When injected into the third ventricle to target IRs expressed in the arcuate nucleus, hypo-IRAS rats exhibit significant decreases in IR

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expression and signaling when compared to rats treated with the control virus (hypo-Con). Additionally, hypo-IRAS rats exhibit increases in body weight, body adiposity and plasma leptin levels in the absence of other metabolic and neuroendocrine abnormalities or deficits in hippocampal IR expression and signaling. As such, the hypo-IRAS rat provides a unique model system to more selectively examine the mechanisms through which impaired hippocampal leptin activity contributes to or is responsible for hippocampal plasticity deficits in obesity/hyperleptinemic phenotypes. Accordingly, the aim of the current studies was to examine hippocampal synaptic transmission in hypo-IRAS rats, as well as examine the ability of food restriction paradigms to reverse this obesity/hyperleptinemic phenotype and thereby restore hippocampal synaptic plasticity.

2. Materials and methods

2.1. Animal protocols

Adult male Sprague Dawley rats (CD strain, Charles River) weighing 200–250 g were housed in groups of three with *ad libitum* access to food and water, in accordance with all guidelines and regulations of The University of South Carolina Animal Care and Use Committee. Prior to administration of lentiviral vectors, tail bleeds were performed for analysis of baseline plasma leptin levels. Hypothalamic IRAS administration was performed as described in our previous studies [27]; rats received either the LV-Control construct (hypo-Con) or the LV-IRAS construct (hypo-IRAS). After surgery, hypo-IRAS and hypo-Con rats were housed individually in a BSL2 facility for at least four weeks. Body weight was monitored daily. In experiment 1, hypo-IRAS and hypo-Con rats were sacrificed and used for electrophysiological analyses as described later. Prior to electrophysiology, the hypothalamus was dissected and membrane homogenates were prepared for the analysis of IR expression. Additionally, trunk blood was collected and the bodies were saved and frozen at −20 °C for subsequent body composition analysis. In experiment 2, hypo-IRAS rats were divided into three experimental groups: 1) rats provided *ad libitum* access to food, 2) rats subjected to food restriction at day 5 (Prevention group), and 3) rats subjected to food restriction at day 21 (Reversal group). The hypo-Con group was treated as described earlier. The rationale of the day 5 time point was that hypo-IRAS rats have recovered from surgery but not yet developed the obesity/hyperleptinemia phenotype and food restriction would 'prevent' the development of the obesity/hyperleptinemic phenotype. In the Reversal group, the obesity/hyperleptinemic phenotype was allowed to develop in the hypo-IRAS rats and the goal of the food restriction paradigm was to return body weight, body adiposity and plasma leptin levels to those observed in hypo-Con rats. In order to achieve these outcomes, the prevention and reversal groups were provided approximately 80% of their normal food intake; food intake was determined during the 5 days following lentivirus administration. Food restriction was maintained in the Prevention group and the Reversal group until the completion of the study.

2.2. Acute restraint stress test

Hypo-Con and hypo-IRAS rats were subjected to an acute restraint stress session as described in our previous studies [28]. Briefly, rats were subjected to restraint stress in wire mesh restrainers secured at the head and tail ends with clips. Immediately after the rat was secured in the restrainer, a tail bleed was performed to determine baseline corticosterone (CORT) values. Thirty minutes after the initiation of stress, the tail was gently massaged to recover blood for peak stress-mediated increases in plasma CORT. Upon completion of this tail bleed, rats were released from the restrainers and returned to their home cage. One hour later, the tail was gently messaged to recover blood for post-stress measurement of plasma CORT levels. Plasma CORT levels were determined by enzyme-linked immunosorbent assay.

2.3. Enzyme-linked immunosorbent assay (ELISA)

ELISA analysis was performed for leptin (Linco Research, Billerica, MA) and CORT (Assay Designs, Ann Arbor, Michigan) in plasma isolated from hypo-Con and hypo-IRAS rats [27]. ELISA plates were analyzed according to the manufacturer's instructions using a Tecan SPECTRAFluor plate reader (Tecan U.S., Inc., Durham, NC). Statistical analysis was performed using a one-way ANOVA followed by a Student-Newman-Keuls post hoc test with $P<0.05$ as the criterion for statistical significance.

2.4. Immunoblot analysis

Immunoblot analysis was performed as described in our previous studies [5]. Briefly, 20 μg of total membrane fractions was separated by SDS/PAGE (10%), transferred to nitrocellulose (NC) membranes and blocked in TBS plus 10% non fat dry milk for 60 min. NC membranes were incubated with primary antisera in TBS/5% non fat dry milk. After an overnight incubation at 4 °C, blots were washed with TBS plus 0.05% Tween 20 (TBST) and incubated with peroxidase-labeled species-specific secondary antibodies. NC membranes were then washed with TBST and developed using enhanced chemiluminescence reagents (ECL, Amersham) as described by the manufacturer. Normalization for protein loading was performed using a mouse monoclonal primary antibody selective for actin (Sigma Chemical Company).

2.5. In vitro phosphorylation assays

In vitro phosphorylation of the insulin receptor was performed as described in our previous study [27] based upon protocols developed by Alkon and co-workers [29,30]. Briefly, 50 μg of hypothalamic total membrane fractions was incubated with a reaction buffer (50 mM Tris–HCl, pH 7.4; 1 mM $MgCl_2$; 2 mM EGTA; 1x protease inhibitor cocktail [Sigma Chemical Company]; 1x phosphatase inhibitor cocktail [Sigma Chemical Company]). In vitro phosphorylation was stimulated by an addition of 1 μM insulin and 5 mM ATP. Following the addition of insulin/ATP, samples were incubated for 3 min at 37 °C. SDS/PAGE sample buffer was quickly added, the samples were boiled for 10 min and added to a precast 4–20% SDS/PAGE gel (Bio-Rad Laboratories).

2.6. Hippocampal slice electrophysiology

Hippocampal slices were prepared from isoflurane anesthetized rats and maintained as previously described [31,32]. Briefly, brain slices were prepared in cold (4 °C), oxygenated (95% O_2/% CO_2) sucrose-based 'cutting' artificial cerebrospinal fluid (aCSF) that contained (in mM): 248 sucrose, 2.7 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose, 0.5 $CaCl_2$ and 7 $MgSO_4$ (350 mOsm). Slices were incubated for approximately one hour at room temperature in oxygenated (95% O_2/5% CO_2) aCSF containing (in mM): 125 NaCl, 2.7 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 10 glucose, 0.5 $CaCl_2$ and 7 $MgSO_4$, 0.02 D-APV and 1 kynurenic acid (pH 7.4; 305–312 mOsm). For field potential recording, slices were transferred to a recording chamber maintained at 32–34 °C and continuously perfused (2–3 ml/min) with oxygenated recording aCSF containing (in mM): 125 NaCl, 2.7 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 10 glucose, 2.0 $CaCl_2$ and 1.0 $MgSO_4$ (pH 7.4; 305–312 mOsm). Stimuli were 0.1 ms, monophasic, cathodal, rectangular, constant current pulses (10–200 μA) delivered to CA3 axons in stratum radiatum of CA1c through a monopolar platinum–iridium electrode. For recording, a glass pipette (resistance 2–3 MΩ) filled with recording aCSF was placed in stratum radiatum of CA1b. Responses were recorded using an AxoProbe