Behavioural Brain Research 196 (2009) 279–285

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

ICV vs. VMH injection of leptin: Comparative effects on hypothalamic gene expression

Suresh Ambati[a], Jiuhua Duan[a,1], Yang-Ho Choi[a,2], Diane L. Hartzell[a], Mary Anne Della-Fera[a], Clifton A. Baile[a,b,*]

[a] Department of Animal and Dairy Science, University of Georgia, Athens, GA 30602, USA
[b] Department of Foods and Nutrition, University of Georgia, Athens, GA 30602, USA

ARTICLE INFO

Article history:
Received 6 August 2008
Received in revised form
15 September 2008
Accepted 17 September 2008
Available online 24 September 2008

Keywords:
Feeding
Hypothalamus
Neuropeptide
mRNA
Serum
Fat pad

ABSTRACT

Leptin regulates feeding behavior and body weight by binding to its receptors localized in specific areas of the hypothalamus. Leptin injected twice daily for 4 days either into the right ventromedial hypothalamus (VMH) or into the right lateral cerebral ventricle (ICV) and using Real-Time Taqman™ RT-PCR, mRNA expression levels of selected genes in the arcuate nucleus-median eminence (ARC-ME) complex were quantitatively measured. Expression of selected genes from the ipsi- vs. contralateral VMH areas in rats injected with leptin into the VMH was also compared. VMH injections of leptin increased ARC-ME mRNAs of proopiomelanocortin (POMC), 27.3% ($p < 0.05$); gamma-aminobutyric acid A receptor (GABRD), 89.3% ($p < 0.01$); and thyrotropin-releasing hormone (TRH), 57.7% ($p < 0.01$); and decreased janus kinase 2 (JAK2), 44.4% ($p < 0.001$); suppressor of cytokine signaling 3 (SOCS3), 86.6% ($p < 0.001$); signal transducer and activator of transcription 3 (STAT3), 46.8% ($p < 0.01$); tyrosine hydroxylase (TH), 51.1% ($p < 0.001$); prostaglandin E synthase (PTGES), 96.5% ($p < 0.001$); tumor necrosis factor-α (TNF-α), 47% ($p < 0.01$); and secretin, 55.4% ($p < 0.001$). Only GABRD, 76.6% ($p < 0.01$) and SCT, 64.9% ($p < 0.01$) were up-regulated in the hypothalamic ARC-ME of rats with ICV leptin injections.VMH injections of leptin induced identical reductions in expression levels of CART, SOCS3, PTGES, and TNF-α in both VMH areas; except TH mRNA, whose expression was lowered ipsilaterally. Food intake, body and fat pad weights and serum insulin and leptin were also decreased in rats given leptin through VMH. This study suggests that leptin either unilateral exposure through VMH or bilateral exposure through ICV injections induces divergent ARC-ME gene profiles.

1. Introduction

Leptin, a protein hormone of adipocytes and a product of the obese (ob) gene, is an indicator of the body's nutritional status. Effects of circulating leptin on energy homeostasis and body weight are primarily mediated through its influence on hypothalamic nuclei that control hunger, satiety, body temperature and energy expenditure [10]. Leptin is also suggested to regulate several central and neuroendocrine functions [44] and the hypothalamic–pituitary–adrenal (HPA) axis [56].

Leptin receptors are localized in specific areas of the hypothalamus, including the ARC and VMH [51]. Leptin reportedly phosphorylates STAT3 which in turn induces nuclear translocation of STAT3, representing a critical step in the leptin-dependent regulation of gene expression [28]. Studies involving intra-hypothalamic injections of leptin or site-specific leptin gene therapy were carried out to demonstrate the areas involved in leptin's effects on food intake, body weight and metabolism. Satoh et al. showed that injections of leptin ranging from 0.125 to 0.5 μg administered in the VMH, ARC or LH areas of the brain effectively decreased food intake and body weight gain in a dose-dependent manner [49]. In another study, injections of leptin given directly into the VMH of rats for 3 days (0.05 μg/day) reduced food intake and body weight, whereas similar doses when administered into either the dorsal raphe or intracerebroventricularly (ICV) had no effect [30].

Although many studies have provided data on leptin action focused on the ARC, leptin injections directly into the VMH provides further insight into its role in leptin-mediated suppression of food

* Corresponding author at: 444 Animal Science Complex, University of Georgia, Athens, GA 30602-2771, USA. Tel.: +1 706 542 4094; fax: +1 706 542 7925.
E-mail address: cbaile@uga.edu (C.A. Baile).
[1] Current address: Department of Nutritional Sciences, University of Toronto, Toronto, ON M5S 3E2, Canada.
[2] Current address: Department of Animal Science and Institute of Agriculture & Life Sciences, Gyeongsang National University, Jinju 660-701, South Korea.

0166-4328/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2008.09.014

intake and stimulation of the sympathetic nervous system. Recent studies have shown that leptin can directly activate SF1 neurons in the VMH, which has a direct bearing on body weight homeostasis [14]. We also hypothesized that the unilateral leptin exposure with VMH injections and bilateral leptin exposure with ICV injections might result in divergent physiological effects and gene expression profiles. In this study, we investigated the effects of VMH and ICV injections of leptin on gene expression profiles in the hypothalamic ARC-ME complex. The median eminence is an important circumventricular region with its rich vascular network extending to various subcellular regions of the arcuate nucleus [45] and its higher interactive capabilities with peptide hormones [58], and it has a crucial role in the control of homeostatic systems. We also determined whether unilateral VMH injection of leptin resulted in a gene expression profile in the non-injected VMH similar to that of the injected nucleus.

2. Materials and methods

Male Sprague-Dawley rats (250–274 g) from Harlan, Inc. (Indianapolis, IN) were housed singly in a light (12 h on/12 h off) and temperature-controlled environment (22 ± 1 °C). Rats were implanted with either right side lateral cerebroventricular (ICV) cannulas or unilateral right side VMH cannulas as described previously [9,24]. All experimental and surgical procedures in this study were approved by the Animal Care and Use Committee at The University of Georgia.

Following recovery from surgery, both ICV and VMH rats were randomly assigned to treatments of vehicle control (*a*CSF, 0.5 μl/injection) or 0.05 μg leptin/injection ($N = 8$). Treatments were administered twice daily for 4 successive days. Recombinant rat leptin (R&D Systems, Minneapolis, MN) was dissolved in an artificial cerebrospinal fluid (*a*CSF), which was constituted as described previously [16]. The body weights and food intakes were monitored daily. The experiment was terminated approximately 24 h after the last treatment. On day 5 rats were killed by CO_2 asphyxiation and decapitation.

Trunk blood was collected for serum preparation; serum was isolated by centrifugation and stored at −80 °C until assayed by RIA for insulin and leptin concentrations. Epididymal (EPI), retroperitoneal (RP) white adipose tissues and interscapular brown adipose tissue (iBAT) were collected, weighed and stored at −80 °C. The brains were removed rapidly and carefully frozen by placing them on trays over crushed ice. Frozen brains were stored in plastic cassettes at −80 °C until micro-dissection. Hypothalamic ARC-ME from ICV and VMH-cannulated rats were punched out using micro-dissection needles. Both ipsi- and contralateral VMH areas were dissected out separately for comparing leptin treatment effects among VMH-cannulated rats.

2.1. Micro-dissection of hypothalamic nuclei

Coronal sections of 40 μm thick were cut from frozen rat brains at −9 °C in a cryostat (Leica cryostat, Model CM3050, Vashaw Scientific, Norcross, GA), and thaw-mounted on to glass slides. Sections were carefully examined under the microscope for accuracy of hypothalamic injections, with reference to the rat brain atlas [41]. Using the micro-dissection technique described by Palkovits and Brownstein [40], the ARC-ME complex and bilateral VMH areas from respective treatment groups were carefully micropunched with a blunted stainless steel needle of 750 μm internal diameter (Fig. 1).

2.2. Extraction of total RNA, reverse transcription (RT) and real-time PCR

Total RNA was extracted from each of the punched-out hypothalamic tissue samples using Trizol Reagent (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's protocol. The integrity of the total RNA obtained from all the samples was verified using the RNA 6000 Nano Assay and the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The RT reaction was performed on 100 ng of total RNA per sample in a 20 μl reaction mixture using the *c*DNA Archive Kit with MultiScribe™ Reverse Transcriptase (ABI; Applied Biosystems, Inc., Foster City, CA; Part #4322171) according to the manufacturer's instructions. Reactions were incubated initially at 25 °C for 10 min and subsequently at 37 °C for 120 min. Quantitative PCR (Taqman™) assays chosen for the transcripts to be evaluated were from Assays-On-Demand™ (ABI), a pre-validated library of qPCR assays, and were incorporated into 384-well MicroFluidic™ cards. All of the oligonucleotide primer and fluorogenic probe sets for Taqman™ real time PCR were from ABI (Table 1). Two μl of the complementary DNA (*c*DNA) along with 50 μl of 2 × PCR master mixes were loaded into respective channels on the microfluidic card followed by a brief centrifugation (3000 rpm for 3 min). The card was then sealed and real-time PCR and relative quantification were carried out using the ABI PRISM 7900 Sequence Detection System. The cycle conditions were: 94.5 °C for 15 min, followed by 40 cycles of 97 °C for 30 s, 59.7 °C for 1 min. Data were analyzed using Sequence Detection Systems software (ABI) and the relative quantification (RQ) method, which represents the fold difference of mRNA level in rats treated with leptin relative to vehicle control (*a*CSF-treated) rats. Expression levels of mRNAs were normalized with 18S as an endogenous control to correct the differences in the amount of total RNA added to each reaction. The ΔC_T values were calculated initially using C_T for a specific gene mRNA minus C_T for 18 S mRNA in the sample. The mean mRNA expressions from the rats treated with leptin (ICV or VMH) were compared with those from the *a*CSF-treated rats using the formula: relative quantification (RQ) = $2^{-\Delta\Delta CT}$ ($\Delta\Delta C_T$ is the average *a*CSF control group ΔC_T values minus the average experimental (leptin-treated) group ΔC_T values, and $\Delta\Delta C_T$ of 1 equates to a two-fold difference in starting amount of *c*DNA). The relative quantification values from each gene were used to compare the hypothalamic gene expression levels between the two groups.

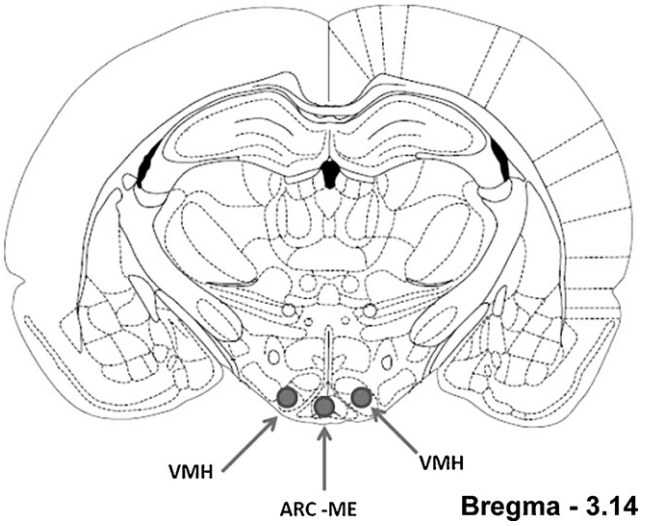

Fig. 1. Diagram showing locations of micropunches for the ARC-ME and lateral sides of VMH brain regions. Diagram is taken from the Paxinos and Watson rat brain atlas (1998), which was used to identify different regions of the brain [42].

2.3. Statistical analysis

Data are means ± S.E.M. and are expressed as % of control for relative quantification (RQ) values from the Sequence Detection System (ABI) files for all the genes tested. Statistical significance was assessed by general linear model analysis of variance (ANOVA) for multiple comparisons between the means for the different treatment groups within VMH or ICV cannulated animals. A p-value less than 0.05 was considered to be statistically significant.

Table 1
Probes used for real time PCR.

Gene	ABI assay ID	Function
18S	4342379-18S	House-keeping gene
CART	Rn00567382_m1	Anxiety [2,8] and bone formation [18]
CALM2	Rn00820919_g1	Signaling [15]
CREB1	Rn00578829_g1	Anxiety [7,17]
GABRD	Rn00568740_m1	Feeding [15]
GHRH	Rn00580832_m1	Growth [29]
GnRH	Rn00562754_m1	Reproduction, growth [13]
HCRT	Rn00565995_m1	Feeding, behavior [52]
JAK2	Rn00580452_m1	Signal transduction [47]
MAPK1	Rn00587719_m1	Learning [54], circadian rhythm [11]
NPY	Rn00561681_m1	Feeding [32]
POMC	Rn00595020_m1	Feeding [52]
PGES	Rn00572047_m1	Inflammation [15]
SECRETIN	Rn00575360_g1	Stress and autism [39,59]
SOCS3	Rn00585674_s1	Signal transduction [3]
STAT3	Rn00562562_m1	Signal transduction [3]
TH	Rn00562500_m1	Anxiety and depression [48]
TNF	Rn00562055_m1	Depression, inflammation, sleep [1,20,31]
TRH	Rn00564880_m1	Signaling [15]
UCN3	Rn00591306_s1	Stress responses, mood disorders [33]