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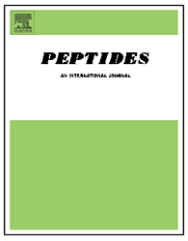

The relative impact of chronic food restriction and acute food deprivation on plasma hormone levels and hypothalamic neuropeptide expression

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ABSTRACT

Our understanding of the central regulation of food intake and body weight has increased tremendously through implication of a high number of neuropeptides. However, lack of all-embracing studies have made comparison difficult in the past. The objective of this study was to demonstrate the relative importance of the different neuropeptides in terms of involvement in appetite regulatory mechanisms. We quantified expression levels of 21 hypothalamic neuropeptides and circulating levels of leptin, insulin, corticosterone, adrenocorticotropic hormone, ghrelin and adiponectin in rats after acute food deprivation and chronic food restriction using validated quantitative real-time PCR and hormone measurements. Body weight, insulin and leptin were reduced whereas corticosterone was increased by both acute food deprivation and chronic food restriction. Our results confirmed the relative importance in body weight homeostasis of neuropeptide Y and proopiomelanocortin, which were increased and decreased as predicted. The expression of other neuropeptides previously attributed central roles in body weight homeostasis, e.g. melanin-concentrating hormone and orexin, appeared to be less affected by the treatments. Moreover, the expression of dynorphin, galanin-like peptide and neuropeptide B was dramatically reduced after both treatments. This suggests that the latter neuropeptides – although previously known to be involved in body weight homeostasis – may be of unexpected importance in states of negative energy balance.

1. Introduction

Hypothalamic neuropeptides are today believed to play a major role in body weight homeostasis. A homeostatic model has emerged, according to which adiposity is regulated by negative feedback by means of lipostatic signals that in some way correlate to adipose mass. These signals are perceived by first order neurons located in the arcuate nucleus (ARC) and are then conveyed to second order neurons in regions such as the lateral hypothalamic area (LHA) and paraventricular nucleus (PVN), that in turn exert regulatory effects on food intake and energy expenditure. Central integration of the

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lipostatic signal is mediated by orexigenic neuropeptides such as neuropeptide Y (NPY), agouti-related protein (AgRP) and melanin-concentrating hormone (MCH) or anorexigenic neuropeptides such as α-melanocyte-stimulating hormone (α-MSH), cocaine- and amphetamine regulated transcript (CART) and corticotropin releasing hormone (CRH). Different aspects of this model are presented in several recent reviews [2,6,24,50,57,61]. Body weight is maintained by comparing the incoming lipostatic signals to a set-point, followed by adjustment of energy intake and expenditure. In the lipostatic model, obesity is explained by genetic defects in any of the contributing components, individual differences in set-point or resistance to any of the lipostatic signals [49].

Some components of the lipostatic model are believed to be more important than others, as results generated from several approaches point to the same direction. One example of this is the melanocortin system originating in the ARC [46]. However, for several components of the model, data from different experimental approaches are less consistent. For example, whereas pharmacological and biochemical studies indicate that NPY is a powerful orexigenic neuropeptide, conventional inactivation of the NPY gene has no dramatic impact on feeding pattern or body weight, at least when the animal is fed with standard chow [35]. Other inconsistencies concern acute vs. chronic effects of various neuropeptides. Whereas galanin (GAL) acutely stimulates fat consumption, chronic administration does not cause hyperphagia or obesity [48]. Thus, the lipostatic model is incomplete, and the relative importance of the individual components of the model is still unknown.

The aim of the present study was to use a biochemical approach in order to estimate the roles of individual neuropeptides in terms of their involvement in food intake control. We reasoned that the relative importance of neuropeptides in response to manipulation of food availability would be reflected by the magnitude of change in gene transcription levels, and that the direction of the change would reflect the neuropeptide's orexigenic or anorexigenic properties. We used a validated real-time quantitative PCR (qPCR) protocol in order to quantify expression levels of 21 hypothalamic neuropeptides in male rats after acute food deprivation (AFD) or chronic food restriction (CFR). In addition, we quantified fasting plasma glucose and six circulating hormones.

2. Methods

2.1. Treatment protocol and tissue sampling

Twenty-four outbred male Sprague–Dawley rats (Alab, Sollentuna, Sweden) with an initial body weight of 223 ± 1.5 g were randomized into three groups, control, food restricted and food deprived animals, and kept in pairs in air-conditioned rooms (12 h dark/light cycle) at 22–23 °C and a humidity of 55%. All animals had free access to water. Animals in the control (CTRL) group had free access to R36 food pellets (Labfor, Lactamin, Vadstena, Sweden). The caloric content of the food was 1260 kJ/100 g. The food access of the CFR group was 45 ± 1% of the amount consumed by the control animals. The micronutrient intake was estimated to exceed the minimal amount for maintenance of bodily functions. For this group, food was delivered daily 3 h into the light period and was consumed within 2 h. The AFD group had free access to food until the last 48 h of the experiment, when the rats were completely deprived of food. Food consumption was monitored daily and body weight every 4th day. The experiment was maintained for 12 days. 12 h prior to the termination of the experiment, food was removed from all cages. The animals were killed by decapitation between 3 and 6 h into the light period and the brains rapidly removed. At decapitation, blood was collected in EDTA-prepared tubes, centrifuged for the preparation of plasma, which was stored at −20 °C until used for hormonal and nutritional measurements. The entire hypothalamus was isolated using a brain matrix (Activational System) and a brain atlas as guide [40]. The rostral border was at the crossing of the anterior commissure (bregma ca. −0.4 mm) and the caudal border at the end of the mammillary recess of the 3rd ventricle (bregma ca. −4.5 mm). The dissected tissue bordered dorsally to the bed nucleus of the stria terminalis (rostral part) and zona incerta (caudal part) and laterally to the substantia innominata (rostral part) and the optic tract and internal capsule (caudal part). Individual tissue samples were rapidly frozen on dry ice, immersed in RNAlater solution (Ambion) and kept in room temperature for approximately 1 h in order to allow the solution to infiltrate the tissue, then stored at −80 °C until further processed. RNA was extracted from the tissue samples and cDNA was synthesized as described previously [36]. Tissue samples from the same set of animals were used for analysis of gene expression in two previous studies [17,37].

2.2. Radioimmunoassays and glucose detection

Radioimmunoassays (RIA) were used to quantify fasting levels of plasma leptin, insulin, corticosterone, adiponectin and ghrelin. We used a rat leptin RIA kit (Linco Research) with a sensitivity of 0.5 ng/ml, a rat insulin RIA kit (Linco Research) with a sensitivity of 0.1 ng/ml, a rat and mouse corticosterone RIA kit (MP Biomedicals) with a sensitivity of 7.7 ng/ml, a ghrelin RIA kit specific for the active, octanoylated form (Linco Research) with a sensitivity of 7.8 pg/ml, a mouse adiponectin RIA kit (Linco Research) with a sensitivity of 1 ng/ml and a human ACTH kit (Eurodiagnostica) with a sensitivity of 1.0 pg/ml. All RIAs were performed according to the instructions from the manufacturer. Plasma glucose levels were assessed using a Glucose & Lactate Analyzer YSI 2300 STAT PLUS and a protocol provided by the manufacturer (VWR).

2.3. Primer design

All primers were designed using the Beacon Designer 4.02 software (PREMIER Biosoft Int.) and based on sequences downloaded for individual rat mRNAs. Primers were 18–21 nucleotides in length and formed products in the range of 70–100 bp. Primer efficiencies were calculated using serial dilutions of hypothalamic cDNA, and were all in the range of 80–100%. Primer specificity was confirmed using melting point curves and comparing with the predicted amplicon melting point. Forward and reverse primers for house-keeping genes (HKGs) and the genes of interest (GOIs) are presented in