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Cafeteria diet differentially alters the expression of feeding-related genes through DNA methylation mechanisms in individual hypothalamic nuclei

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ABSTRACT

We evaluated the effect of cafeteria diet (CAF) on the mRNA levels and DNA methylation state of feeding-related neuropeptides, and neurosteroidogenic enzymes in discrete hypothalamic nuclei. Besides, the expression of steroid hormone receptors was analyzed. Female rats fed with CAF from weaning increased their energy intake, body weight, and fat depots, but did not develop metabolic syndrome. The increase in energy intake was related to an orexigenic signal of paraventricular (PVN) and ventromedial (VMN) nuclei, given principally by upregulation of AgRP and NPY. This was mildly counteracted by the arcuate nucleus, with decreased AgRP expression and increased POMC and kisspeptin expression. CAF altered the transcription of neurosteroidogenic enzymes in PVN and VMN, and epigenetic mechanisms associated with differential promoter methylation were involved. The changes observed in the hypothalamic nuclei studied could add information about their differential role in food intake control and how their action is disrupted in obesity.

1. Introduction

Obesity has become one of the most serious public health problems in the developed world. Although it is a multifactorial metabolic condition, its main cause is presumably the combination of overeating and inactivity. Cafeteria diet (CAF) is an experimental rodent diet model that accurately reflects the variety of highly palatable and energy dense foods that are prevalent in the Western society and that are associated with the current obesity pandemic (Sampey et al., 2011). The interplay of various hypothalamic nuclei with peripheral hormones, neuropeptides, and nuclear receptors represents a critical aspect of the hypothalamic regulation of energy metabolism (Frank et al., 2014). The body weight is regulated by a complex inter-organ circuit connecting the periphery and the brain (Caminos et al., 2008). The hypothalamus is a major hub that integrates nutritionally relevant information originated from all peripheral organs, mediated through circulating metabolites and hormones such as glucose, insulin, ghrelin, and leptin (Lenard and Berthoud, 2008). The central control of energy homeostasis is highly dependent on the activity of peptidergic neuronal circuits located in the hypothalamus (Torri et al., 2002). The arcuate nucleus (ARC) plays a central role in the integration of signals regulating nutritional status and energy homeostasis (Coupe et al., 2010). The ARC contains orexigenic neurons that coexpress neuropeptide Y (NPY) and agouti-related protein (AgRP) and anorexigenic neurons that coexpress proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) (Coupe et al., 2010). All these neuropeptide circuits project to downstream hypothalamic areas, including the paraventricular (PVN) and the ventromedial (VMN) nuclei, modulating the release of further anorectic or

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Abbreviations

17β-HSD	17β-hydroxysteroid dehydrogenase
3β-HSD	3β-hydroxysteroid dehydrogenase
3α-HSD	3α-hydroxysteroid dehydrogenase
5αR-1	5α-reductase-1
AgRP	Agouti-related protein
ARC	Arcuate nucleus
ARO	p450-aromatase
AUC	Area under curve
CAF	Cafeteria diet
CART	Cocaine- and amphetamine-regulated transcript
E_2	Estradiol
ERα	Estrogen receptor alpha
HOMA	Homeostasis model assessment
ipGTT	Intraperitoneal glucose tolerance test
KISS	Kisspeptin
nGRE	negative glucocorticoid-responsive element
NPY	Neuropeptide Y
Ob-Rb	Long form of leptin receptor
P450-17α	Cytochrome P450 17α-hydroxylase
P450scc	Cytochrome P450 side-chain cleavage
PND	Postnatal day
POMC	Proopiomelanocortin
PVN	Paraventricular nucleus
StAR	Steroidogenic acute regulatory protein
VMN	Ventromedial nucleus

orexigenic peptides that adjust energy intake and expenditure to maintain a stable body weight (Torri et al., 2002). Kisspeptin (KISS) is a neuropeptide synthesized by neurons in the ARC. Although its major role is in reproduction, KISS also has a role in the control of energy homeostasis, as it has the ability to directly excite POMC neurons and indirectly inhibit neurons that express NPY (Fu and van den Pol, 2010).

The brain, like the adrenal glands, gonads, and placenta, is a steroidogenic organ (Mellon and Griffin, 2002). Neurosteroids are endogenous modulators of neuronal function responsible for a broad spectrum of biological and pathophysiological effects (Do Rego et al., 2009). Although it has been recently reported that several neurosteroidogenic enzymes are expressed in the hypothalamus (Munetomo et al., 2015) the relationship between their expression and the metabolic or dietary status of the animals remains unknown. Numerous experiments have shown that the diet and/or nutritional status of the animals affect/s steroidogenesis in different organs including ovary (Newell-Fugate et al., 2015) and adrenal gland (Swierczynska et al., 2015), and in brain regions such as the hippocampus (Ohashi et al., 2015). Our group has recently reported that obese phytoestrogen-deprived rats show alterations in the hypothalamic expression of different neurosteroidogenic enzymes (Andreoli et al., 2016). Numerous experiments have established that steroids may affect food intake. Chronic administration of progesterone by subcutaneous patches increases energy intake in adult female rats (Grueso et al., 2001). Besides, it has been reported that rats fed with standard chow *ad libitum* and treated with allopregnanolone show increased food intake and weight gain (Nakhate et al., 2013). Otherwise, female rats treated with estradiol (E_2), administered either centrally or by subcutaneous implants, show decreased food intake and body weight (Dagnault and Richard, 1997; Puerta et al., 1990). Moreover, estrogen signaling potentiates leptin sensitivity, possibly by increasing the expression of the leptin receptor (Ob-Rb) in the hypothalamus (Frank et al., 2014), which is another mechanism through which E_2 exerts its anorectic action. We have previously reported that, in phytoestrogen-deprived rats, an alternative mechanism for the induction of obesity is a reduction of circulating E_2 levels, which affects hypothalamic estrogen receptor alpha (ERα) signaling (Andreoli et al., 2015).

DNA methylation is a major epigenetic modification that controls gene expression in physiologic and pathologic states. Different metabolic disorders, including obesity, are associated with alterations in gene expression that are caused by genetic and environmental factors, which could modify the DNA methylation pattern in somatic tissues (Barres and Zierath, 2011). DNA methylation at CpG dinucleotides alters gene expression by affecting transcription factor binding activity (Marco et al., 2013). It has been reported that different environmental exposures, including a high caloric environment, could affect DNA epigenetic patterns in hypothalamic feeding control centers resulting in altered gene expression and obesity (Marco et al., 2013). Thus, DNA methylation provides a mechanism by which diet can modify genetic predisposition to disease.

Our hypothesis was that the endogenous synthesis of molecules involved in the homeostatic regulation of food intake may be altered in obese animals fed with CAF, implicating variations in the gene expression of neuropeptides, and receptors involved in food intake control. The expression of neurosteroidogenic enzymes in the rat hippocampus has been widely reported, but more information in discrete hypothalamic nuclei is needed. Thus, the aim of the present study was to evaluate the effect of CAF diet on the mRNA levels of peptides, steroid hormone receptors, and neurosteroidogenic enzymes in individual hypothalamic nuclei obtained using a micropunch dissection technique. Besides, we propose that epigenetic changes, such as altered DNA methylation of genes involved in the regulation of body weight and metabolism, could be involved.

2. Materials and methods

2.1. Ethics statement

All procedures were approved by the Ethical Committee of the School of Biochemistry and Biological Sciences (Universidad Nacional del Litoral, UNL, Santa Fe, Argentina) and were performed in accordance with the principles and procedures outlined in the Guide for the Care and Use of Laboratory Animals issued by the U.S. National Academy of Sciences (Commission on Life Sciences, National Research Council, Institute of Laboratory Animal Resources, 1996).

2.2. Animals, diets, and dietary treatment

Thirty-two female Wistar rats were obtained at the Department of Human Physiology of the School of Biochemistry and Biological Sciences (UNL), weaned at 21 days of age, and randomly divided into two weight-matched groups. Rats were housed two per cage and maintained in controlled conditions (22 ± 2 °C and 12-h light–dark cycle). Animals were fed with either standard chow or a CAF diet (n = 16 per group) from weaning and for 20 weeks. Water was available throughout the experiment. The standard