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ACUTE UP-REGULATION OF THE RAT BRAIN SOMATOSTATIN RECEPTOR-EFFECTOR SYSTEM BY LEPTIN IS RELATED TO ACTIVATION OF INSULIN SIGNALING AND MAY COUNTERACT CENTRAL LEPTIN ACTIONS

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Abstract—Leptin and somatostatin (SRIF) have opposite effects on food seeking and ingestive behaviors, functions partially regulated by the frontoparietal cortex and hippocampus. Although it is known that the acute suppression of food intake mediated by leptin decreases with time, the counter-regulatory mechanisms remain unclear. Our aims were to analyze the effect of acute central leptin infusion on the SRIF receptor-effector system in these areas and the implication of related intracellular signaling mechanisms in this response. We studied 20 adult male Wister rats including controls and those treated intracerebroventricularly with a single dose of 5 μg of leptin and sacrificed 1 or 6 h later. Density of SRIF receptors was unchanged at 1 h, whereas leptin increased the density of SRIF receptors at 6 h, which was correlated with an elevated capacity of SRIF to inhibit forskolin-stimulated adenylyl cyclase activity in both areas. The functional capacity of SRIF receptors was unaltered as cell membrane levels of αi1 and αi2 subunits of G inhibitory proteins were unaffected in both brain areas. The increased density of SRIF receptors was due to enhanced SRIF receptor subtype 2 (sst2) protein levels that correlated with higher mRNA levels for this receptor. These changes in sst2 mRNA levels were concomitant with increased activation of the insulin signaling, c-Jun and cyclic AMP response element-binding protein (CREB); however, activation of signal transducer and activator of transcription 3 was reduced in the cortex and unchanged in the hippocampus and suppressor of cytokine signaling 3 remained unchanged in these areas. In addition, the leptin antagonist L39A/D40A/F41A blocked the leptin-induced changes in SRIF receptors, leptin signaling and CREB activation. In conclusion, increased activation of insulin signaling after leptin infusion is related to acute up-regulation of the SRIF receptor-effector system that may antagonize short-term leptin actions in the rat brain. **© 2013 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: adenylyl cyclase, frontoparietal cortex, hippocampus, insulin signaling, leptin, somatostatin receptors.

INTRODUCTION

Leptin is a hormone produced mainly in adipose tissue (Zhang et al., 1994), but also in other tissues including the brain, and acts in the central nervous system controlling energy balance through inhibition of feeding behavior and stimulation of energy expenditure (Morash et al., 1999). Leptin acts preferentially in the hypothalamus, after binding to the long-form of its

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Abbreviations: AC, adenylyl cyclase; BSA, bovine serum albumin; CRE, cyclic AMP response element; CREB, CRE binding protein; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; FK, forskolin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; icv, intracerebroventricular; IRS1, insulin receptor substrate 1; JAK2, Janus kinase 2; JNK, c-Jun N-terminal kinase; MBIA, multiplexed bead immunoassay; NDS, normal donkey serum; Ob-Rb, long-form of leptin receptor; p, phosphorylated; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; SOCS3, suppressor of cytokine signaling 3; SRIF, somatostatin; sst, SRIF receptor subtype; STAT3, signal transducer and activator of transcription 3; TBS, Tris-buffered saline; TTBS, Tris–Tween-buffered saline.

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receptor (Ob-Rb) and activation of the subsequent intracellular signaling (Vaisse et al., 1996). This hormone suppresses food intake and reduces body weight by modulating the synthesis and secretion of both orexigenic and anorexigenic peptides produced mainly by neurons of the arcuate nucleus (Schwartz et al., 1996, 1997).

The presence of the Ob-Rb in other regions of the brain not classically associated with energy homeostasis such as the frontoparietal cortex and hippocampus (Burgos-Ramos et al., 2010; Caron et al., 2010), suggests a possible role for leptin in the regulation of food intake related behaviors. Indeed, leptin reduces postprandial locomotor activity (Ruffin and Nicolaidis, 2000) and hippocampal leptin resistance is linked to cognitive alterations (Fadel et al., 2013). Moreover, activation of the cortex in response to high-calorie food images has been reported (Killgore et al., 2013), as well as the negative effects of high-fat diets on hippocampal cognitive processes related to energy homeostasis and the association of hippocampal damage with augmented appetite (Davidson et al., 2010).

Although acute leptin-mediated suppression of food intake is reported to decline with time (Passos et al., 2004), the counter-regulatory mechanisms remain uncertain. The mechanism by which leptin exerts its actions in metabolic control involves the binding of leptin to the long-form of its receptor, Ob-Rb (Tartaglia et al., 1995) and the subsequent intracellular signaling, initiated by the autophosphorylation of Janus kinase 2 (JAK2) and activation of signal transducer and activator of transcription 3 (STAT3). Phosphorylation of STAT3 on Tyr705 is a prerequisite for its dimerization and nuclear translocation, whereas Ser727 phosphorylation is needed for activation of transcription. After the translocation of STAT3 to the nucleus, suppressor of cytokine signaling 3 (SOCS3) is synthesized, exerting feedback inhibition on leptin signaling (Bjorbaek et al., 1999). Several mechanisms modulate acute leptin actions on energy balance, one of which is the improvement of glucose homeostasis in obese animals through increased hypothalamic insulin signaling (Koch et al., 2010).

Various neurotransmitters and hormones inhibit leptin signaling, including somatostatin (SRIF), an orexigenic neuropeptide widely distributed in the CNS that acts as a neuromodulator (Blake et al., 2004). In this regard, when SRIF receptor agonists are delivered in combination with leptin, nuclear STAT3 translocation is reduced compared to leptin infusion alone (Stepanyan et al., 2007). The actions of SRIF are mediated via five transmembrane receptors located in several brain areas, including the frontoparietal cortex and hippocampus (Aguado-Llera et al., 2011). These receptors are coupled via G proteins to inhibition of adenylyl cyclase (AC), among other signal transduction pathways. Orexigenic peptides increase SRIF mRNA content and experimental activation of brain SRIF receptors rapidly stimulates ingestive behavior, increasing body weight (Stengel et al., 2010). Insulin is also involved in the central modulation of this system, thus; pituitary SRIF receptor mRNAs are reduced in diabetic rats and insulin therapy partially restores SRIF receptor density (Berelowitz et al., 1995).

These findings indicate that the frontoparietal cortex and hippocampus may be involved in modulation of food intake and energy homeostasis, with insulin signaling and the SRIFergic system counter-acting acute leptin actions. In light of the above, we first assessed the effect of acute leptin infusion on the binding of ^{125}I–Tyr^{11}–SRIF to its specific receptors, as well as SRIF receptor subtype (sst) 1–sst4 protein levels in frontoparietal cortical and hippocampal membranes. In addition, we evaluated basal AC activity, SRIF-mediated inhibition of basal and FK-stimulated AC activity and Giα1–2 protein levels. In addition, the changes in activation of the insulin signaling cascade and its relationship with modulation of SRIF receptor subtypes, as well as possible association of SRIFergic tone with changes in leptin signaling were also examined. Finally, in order to further address the specificity of the effect of leptin signaling on cortical and hippocampal SRIF receptors, the leptin antagonist L39A/D40A/F41A was used in combination with acute leptin treatment.

EXPERIMENTAL PROCEDURES

Animals and leptin infusion

This study was approved by the Ethics Committee of the Universidad de Alcalá de Henares (SAF 2010-22277, Ministerio de Ciencia y Tecnología) and complied with Royal Decree 1201/2005 (Boletín Oficial del Estado, BOE No. 252) pertaining to the protection of experimental animals and with the European Communities Council Directive (86/609/EEC). All efforts were made to minimize suffering and to reduce the number of animals used here.

Twenty male Wistar rats weighing 250 ± 10 g were anesthetized (200 μl ketamine/kg wt and 400 μl xylazine/kg wt) and a cannula attached to a catheter was implanted in the left lateral cerebral ventricle (−0.3 mm anteroposterior, 1.1 mm lateral from Bregma). Two days after surgery, the rats were fasted for a period of 12 h before infusing 5 μg of leptin (Preprotech, Rocky Hill, NJ, USA) at 9.00 a.m. (dissolved in 10 μl of saline) intracerebroventricularly (icv), as previously described (Lecklin et al., 2000). Control rats received equivalent volume of the vehicle by the same route. The rats were sacrificed by decapitation 1 or 6 h after infusion ($n = 5$ per group). The brain was quickly removed and the frontoparietal cortex and the hippocampus were dissected on ice according to the method of Glowinski and Iversen (1966).

In addition, 20 male Wistar rats were used to analyze whether leptin receptor antagonism modifies the density of the SRIF receptors, mRNA and protein levels of sst2 and leptin-related signaling, as well as to determine whether the response to exogenous leptin on the SRIF system is mediated through the leptin receptor. To this end, rats were treated twice (9 a.m. and 9 p.m.) the day before acute leptin treatment with a PEG-SRLA, L39A/

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