Insulin modulates leptin-induced STAT3 activation in rat hypothalamus

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Abstract Insulin and leptin have overlapping effects in the control of energy homeostasis, but the molecular basis of this synergism is unknown. Insulin signals through a receptor tyrosine kinase that phosphorylates and activates the docking proteins IRSs (insulin receptor substrates), whereas the leptin receptor and its associated protein tyrosine kinase JAK2 (Janus kinase 2) mediate phosphorylation and activation of the transcription factor STAT3 (signal transducer and activator of transcription). Here, we present evidence for the integration of leptin and insulin signals in the hypothalamus. Insulin induced JAK2 tyrosine phosphorylation, leptin receptor phosphorylation which, in the presence of leptin, augmented the interaction between STAT3 and this receptor. Insulin also increased the leptin-induced phosphorylation of STAT3 and its activation. These results indicate that insulin modulates the leptin signal transduction pathway, and may provide a molecular basis for the coordinated effects of insulin and leptin in feeding behavior and weight control.

Key words: Leptin; Insulin; Tyrosine phosphorylation; Janus kinase 2; Signal transducer and activator of transcription; Long form of the leptin receptor

1. Introduction

The circulating peptide leptin, which is the product of the ob gene [1], is secreted predominantly by white adipose tissue and provides feedback information on the extent of the body's fat stores to central OB receptors (long form of the leptin receptor (OBR)) [2] that control food intake and body weight homeostasis [3,4]. The hypothalamus is thought to be the major target for leptin, which acts through receptors that bear strong sequence homology to the class I cytokine receptor family [2]. The leptin receptor exists as multiple splice variants. OBR is expressed most abundantly in paraventricular, arcuate and ventromedial nuclei of the hypothalamus, where it mediates most of the neural signaling of leptin [5–7]. Ligand binding to the OBR results in the activation of Janus kinase 2 (JAK2) by transphosphorylation and the subsequent phosphorylation of tyrosine residues on the intracellular portion of OBR [8–11]. In general, tyrosine phosphorylation of cytokine and growth factor receptors activates intracellular signals by recruiting specific signaling proteins with specialized phosphotyrosine-binding domains called src homology 2 (SH2) domains [12,13]. Tyrosine phosphorylation of OBR recruits the tyrosine phosphatase, phosphotyrosine phosphatase 2 (SHP-2) [14,15] and the signal transducer and activator of transcription (STAT3) transcription factor [14–16]. The use of these SH2 domain-containing proteins by OBR in the hypothalamus implies that they may mediate physiologically important signals [17,18]. In addition to the direct binding of SHP-2 and STAT3, OBR also mediates a number of downstream actions in a variety of cells and tissues [8].

Insulin acts in the same hypothalamic areas as leptin to suppress feeding [19]. In the past decade, many of the proteins involved in insulin action have been defined at a molecular level. The insulin receptor (IR) is a protein tyrosine kinase which, when activated by insulin binding, undergoes rapid autophosphorylation and phosphorylates intracellular protein substrates, including insulin receptor substrates (IRSs) [20–22], Shc [23] and Tub [24]. Following tyrosine phosphorylation, the IRSs act as docking proteins for several SH2 domain-containing proteins, including phosphatidylinositol 3-kinase (PI 3-kinase), Grb2, SHP2, Nck and Fyn [25–29]. A potential mitogen-activated protein kinase-independent mechanism for the regulation of gene expression is the JAK–STAT pathway. The role of insulin in the activation of the JAK/STAT pathway is controversial [30,31]. While this activation was originally thought to occur through cytokine receptors, recent work has shown that the key activating event, STAT tyrosine phosphorylation, is regulated by receptor protein tyrosine kinases [32]. Furthermore, STAT5b activation by insulin has also been reported [31]. However, there is no tyrosine phosphorylation of STAT3 after stimulation by insulin [33].

Obesity in humans and rodents is usually associated with high circulating levels of insulin and leptin as well as insulin and leptin resistance [34–38]. The interaction between insulin and leptin signaling pathways is therefore fundamental for an integrated response of the signal inputs to the hypothalamus. In this study, we examined the cross-talk between the signaling pathways of insulin and leptin leading to STAT3 activation.

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Abbreviations: IR, insulin receptor; IRS, insulin receptor substrate; OBR, long form of the leptin receptor; JAK2, Janus kinase 2; STAT3, signal transducer and activator of transcription; SH2, src homology 2; SHP-2, phosphotyrosine phosphatase 2; PI 3-kinase, phosphatidylinositol 3-kinase; EMSA, electrophoretic mobility shift assay

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2. Materials and methods

2.1. Animals and surgical procedures

Adult male Wistar rats (250–300 g) were used in all the experiments in accordance with the guidelines of the Brazilian College for Animal Experimentation (COBEA). After an overnight fast, the rats were anesthetized with ketamine hydrochloride (100 mg/kg, i.p.) and positioned on a Stoelting stereotaxic apparatus using head and chin holders. An appropriate side arm holding the guide cannula was attached. The vertical coordinate of the side arm was set at 0° in the lateral plane and at 90° in the horizontal plane. The scalp was removed in the midline to expose the saggital suture and the periosteum was then opened and reflected away from the surgical field. The lateral, anteroposterior and dorsoventral coordinates of the third ventricle were obtained from a standard atlas [39]. The bregma was used as the reference point and a hole was drilled in the parietal bone at the junction of the lateral and anteroposterior coordinates. Two smaller holes were drilled around this central hole, and stainless-steel screws were inserted half-way through the holes. The cannula was positioned over the central hole and lowered carefully until the necessary dorsoventral coordinate was reached. The cannula was fixed to the skull using cranioplastic cement after ensuring hemostasis. After the cement had set, the cannula was freed from the side arm and a dummy cannula was inserted to close the outer cannula. The rats were then allowed to recover from anesthesia and the surgical procedure for 7 days.

2.2. Intracerebroventricular infusions

After a 6-h fast, the rats were anesthetized with sodium amobarbital (15 mg/kg body weight, i.p.), and used as soon as anesthesia was assured by the loss of pedal and corneal reflexes. Insulin (1.9 μg/μl – human insulin from E. Lilly), leptin (5 μg/μl – rat leptin from NIH), saline (2 μl in the control animals) or an equimolar mixture of insulin and leptin (1.9 μg/μl insulin, 5 μg/μl leptin) were taken up into an internal fusion cannula connected to a polyethylene supply tube. This tubing was then connected to a 2-μl syringe primed with the infusate. The dummy cannula was removed and the infusion cannula was introduced into the outer guide cannula after which the solution was injected into the third ventricle. The cranium was opened and the hypothalamus excised. In preliminary experiments we determined blood glucose and serum insulin levels in animals that received i.c.v. insulin infusion. Insulinemia and glycemia were not altered by third ventricle insulin or saline microinjection. Under the experimental conditions, no retrograde insulin trafficking through the blood–brain barrier was observed.

2.3. Western blot analysis

The hypothalami were removed minced coarsely and homogenized immediately in the solubilization buffer containing 100 mM Tris (pH 7.6), 1% Triton X-100, 150 mM NaCl, 0.1 mg aprotinin, 35 mg PMSF/ml, 10 mM Na_3VO_4, 100 mM NaF, 10 mM $Na_4P_20_7$, and 4 mM EDTA, using a politron PTA 20S generator operated at maximum speed for 30 s and clarified by centrifugation. Equal amounts of protein were used for immunoprecipitation followed by Western blot analysis with the indicated antibodies and ^{125}I-Protein A. Quantitative analysis of the blots was done using Scion Image software. The antibodies to: OBR (SC-1835 and SC-8325), phosphotyrosine (SC-508), STAT3 (SC-483), JAK2 (SC-294-G) were obtained from Santa Cruz Biotechnology, and the STAT3 phosphotyrosine 705-specific antibody (9131) was from New England Biolabs.

2.4. IR kinase assay

IR tyrosine kinase activity was measured by autophosphorylation. Insulin was infused into the third ventricle to stimulate limited receptor activation and partial IR autophosphorylation. IR was then immunoprecipitated and allowed to autophosphorylate in vitro in the presence of exogenous ATP. Tyrosine autophosphorylation was measured by immunoblotting with anti-phosphotyrosine antibody.

2.5. Nuclear extracts

Tissues were dissected and dounce-homogenized 10 times in 100: 1 (v:v) of buffer A (10 mM KCI, 1.5 mM $MgCl_2$, 10 mM HEPES pH 7.9, 1 mM DTT, 1 mM $NaVO_4$, 1×complete TM protease inhibitor (Boehringer Mannheim). After centrifugation at 2000×*g* for 10 min at 4°C, the pellet was carefully resuspended in two volumes of buffer B (420 mM NaCl, 10 mM KCI, 20 mM HEPES pH 7.9, 20% glycerol, 1 mM DTT, 1 mM Na VO_4, 1×complete TM protease inhibitor) and extracted for 30 min at 4°C on a shaking rotor. After centrifugation at 16 000×*g*, the supernatant was diluted 10-fold in buffer C (10 mM KCl, 20 mM HEPES pH 7.9, 20% glycerol, 1 mM DTT, 1 mM $NaVO_4$, 1× complete TM protease inhibitor) and centrifuged for 10 min at 16 000×*g*. The supernatant was loaded onto a Microcon 50 ultrafiltration column (Amicon) and centrifuged for 15 min at 4000×*g* The protein concentration was determined by the Bradford assay (Bio-Rad). Samples were snap-frozen and kept at −80°C. The procedure was carried out at 4°C. DTT, $NaVO_4$ and protease inhibitors were added at the time of the experiment.

2.6. Electrophoretic mobility shift assay (EMSA)

EMSA was done as described [40]. Double-stranded oligonucleotide probes were synthesized with 5′-GATC protruding ends for fill-in labeling. The M67-SIE probe sequence was 5′-CATTTCCCG-TAAATCAT-3′. 2 μg of hypothalamic nuclear extract were incubated at room temperature for 15 min in the presence of 100 pg of labeled probe (2×10^4–10^5 dpm), 2 μg de poly dI-dC (Pharmacia), 40 mM KCI, 1 mM $MgCl_2$, 20 mM HEPES pH 7,9, 100 μM EGTA, 0.5 mM DTT and 4% Ficoll in a total volume of 12μl. Samples were run on 4% native polyacrylamide gels in 0.25×TBE at 4°C.

2.7. Statistical analysis

Where appropriate, the results were expressed as the mean ± S.E.M. accompanied by the indicated number of experiments. ANOVA factorial test was used in all statistical comparisons with $P < 0.05$ indicating significance.

3. Results and discussion

The aim of this study was to examine the cross-talk between the insulin and leptin signal transduction pathways in the hypothalamus of normal rats. The rats were studied 7 days after the implantation of a cannula in the third ventricle. Leptin, insulin or both hormones were administered i.c.v., after which the ability of leptin to activate STAT3 in the hypothalamus was assessed.

OBR has been detected in various hypothalamic regions, including the arcuate nucleus [2,41], where it completes a feedback loop that delivers information on peripheral energy stocks to the hypothalamus, thereby altering both food intake

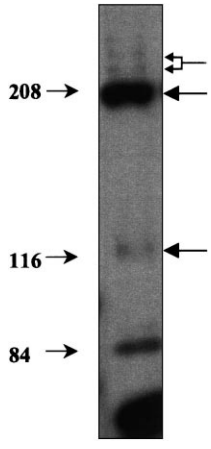

Fig. 1. Different isoforms of OBR are present in the hypothalamus. Hypothalamic lysates were run on 8% polyacrylamide gels and blotted with antibody raised against amino acids 32–51 of the extracellular domain of the OBR. Several bands representing the long and short isoforms were detected. OBR migrated as a major band just below the 208-kDa marker, whereas a band close to 116 kDa represented the short form. The bands migrating in the 60–85-kDa range may represent degradation products of the leptin receptor.

and metabolic rate. The presence of various isoforms of OBR in hypothalamic extracts was analyzed by Western blotting using a polyclonal antibody generated against amino acids 32–51 of the extracellular domain of the mouse OBR. The hypothalamus expressed the long and short isoforms of the OBR (Fig. 1). In agreement with data obtained using cells transfected with OBR-S or OBR-L [8,9], OBR migrated as a weak doublet band above the 208-kDa marker and as a major band just below this marker; the short form migrated as a minor band close to the 116-kDa marker. The bands at 60 kDa and 85 kDa were not characterized further and may represent the non-glycosylated short form of the OBR or degradation fragments as previously described in Fao cells [42].

The OBR is a member of the class I cytokine receptor family that causes a ligand-dependent increase in intracellular protein tyrosine phosphorylation which is essential for receptor function [43]. Receptors of this class lack intrinsic tyrosine kinase activity and act through receptor-associated kinases of the Janus family (JAKs). Activated JAKs can phosphorylate each other, the receptor and the recruited cellular substrates [44]. To examine leptin-induced tyrosine phosphorylation of JAK2, immunoprecipitation and Western blotting of hypothalamic extracts were done using anti-JAK2 and anti-phosphotyrosine antibodies, respectively. As shown in Fig. 2, there was an increase in the phosphorylation of JAK2 1 min after exposure to leptin. Maximal tyrosine phosphorylation of JAK2 occurred 15–30 min after leptin infusion (Fig. 2A) and then decreased dramatically (data not shown). This is the first demonstration that such signaling, previously described in cultured cells, occurs in the rat hypothalamus after the i.c.v. infusion of leptin. These results differ from those of McCowen et al. [45], who reported that leptin signaling was distinguishable from that of GH and other ligands in this class of receptors by its inability to stimulate JAK family proteins in the rat hypothalamus. This discrepancy may reflect the

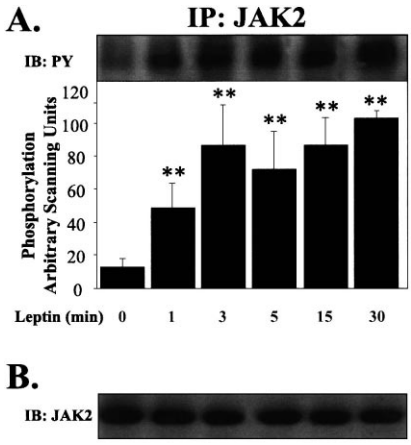

Fig. 2. Effect of leptin on JAK2 phosphorylation. Hypothalamic extracts of from rats treated with 10 μg of leptin for the indicated times, were prepared as described in Section 2. A: Tissue extracts were immunoprecipitated with anti-JAK2 (IP) and immunoblotted with anti-phosphotyrosine antibody (pY) (IB, immunoblotting). Quantitation of the phosphorylation of JAK2 protein is shown in the bar graph, and represents the mean ± S.E.M. of four independent experiments. B: The equal loading of proteins was verified by reblotting with anti-JAK2 antibodies. $^{**}P < 0.01$, compared to rats treated with saline (control) (factorial ANOVA).

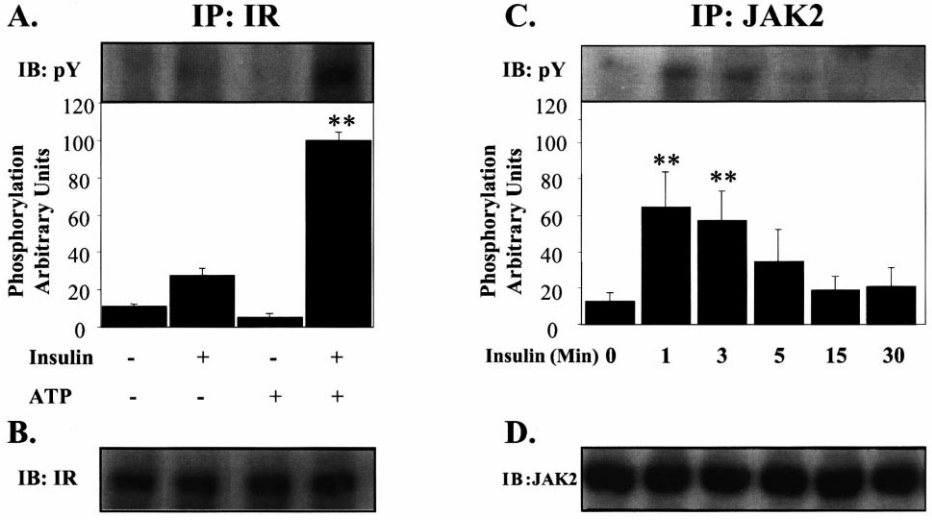

Fig. 3. Insulin induces IR tyrosine kinase activity and JAK2 phosphorylation in rat hypothalamus. A: IR tyrosine kinase activity measured by autophosphorylation. Insulin (10 ng) was injected into the third ventricle to partially stimulate IR autophosphorylation. The IR was then immunoprecipitated and allowed to autophosphorylate in vitro in the presence of exogenous ATP (lane 4). Tyrosine phosphorylation was measured by immunoblotting with an anti-phosphotyrosine antibody. Control conditions are shown in lanes 1–3. Lane 1: The hypothalamic extract was not exposed to insulin nor was exogenous ATP added to the in vitro autophosphorylation reaction. Lane 2: A low dose of insulin (10 ng) was infused into the third ventricle and the hypothalamus was then extracted, but no exogenous ATP was added during the autophosphorylation reaction in vitro. The faint signal seen in this lane probably represents IR autophosphorylation using endogenous ATP. Lane 3: The hypothalamus was not exposed to insulin, but ATP was added to the autophosphorylation reaction in vitro. B: The equal loading of proteins was verified by reblotting with anti-IR antibodies. Bar graphs are representative of five independent experiments (mean ± S.E.M.). $^{**}P < 0.01$ compared to rats treated with saline (control) (factorial ANOVA). In (C) and (D) extracts of bar graphs are representative of five independent experiments (mean ± S.E.M.). Hypothalami from rats treated with 10 μg leptin for the indicated times, were prepared as described in Section 2. C: Tissue extracts were immunoprecipitated with anti-JAK2 (IP) and immunoblotted with anti-phosphotyrosine antibody (pY) (IB, immunoblotting). Quantitation of the phosphorylation of JAK2 protein is shown in the bar graph, and represents the mean ± S.E.M. of four independent experiments. D: The equal loading of proteins was verified by reblotting with anti-JAK2 antibodies. $^{**}P < 0.01$ compared to rats treated with saline (control) (factorial ANOVA).

dephosphorylation of JAK2 by one of several phosphatases during the storage and manipulation of hypothalamic tissue.

As demonstrated in other tissues, insulin stimulated IR autophosphorylation and kinase activity in the rat hypothalamus (Fig. 3A,B). To investigate whether insulin could induce JAK2 tyrosine phosphorylation in the rat hypothalamus, as described in other tissues and cultured cells [30,46], we infused insulin i.c.v. followed by immunoprecipitation and Western blotting of hypothalamic extracts using anti-JAK2 and anti-phosphotyrosine antibodies, respectively. As shown in Fig. 3, maximal tyrosine phosphorylation of JAK2 occurred 1 min after insulin infusion and disappeared by 15 min (Fig. 3C,D).

Insulin acts in the same hypothalamic areas as leptin to suppress feeding [19]. We have performed immunohistochemical analysis of rat hypothalami using IR- and OBR-specific antibodies and the results showed high concentrations of both receptors in the arcuate nucleus and, to a lesser extent, in some periventricular neuronal bodies (data not shown). The presence of both receptors in the same hypothalamic areas may allow a cross-talk between these two hormones. To determine whether there was a direct interaction between the insulin and leptin signaling systems, insulin, leptin or insulin plus leptin were infused i.c.v. and OBR tyrosine phosphorylation was assessed. Immunoprecipitation and Western blotting of hypothalamic extracts were done using the anti-leptin receptor (SC 8325) and anti-phosphotyrosine antibodies, respectively. As shown in Fig. 4A (right side), the leptin receptor (the band just below 208 kDa) was strongly phosphorylated 15 and 30 min after an i.c.v. infusion of leptin. There was also an increase in OBR tyrosine phosphorylation as early as 5 min after exposure to insulin; maximal phosphorylation occurred at 15 min and then decreased by 30 min after insulin infusion (Fig. 4A, left side). Based on these results, we hypothesized that insulin could interfere with the leptin signaling system. When insulin was infused simultaneously with leptin, the levels of OBR phosphorylation increased by 1.7 ± 0.2-fold ($P < 0.01$) compared with leptin treatment alone. The mechanism by which insulin can induce leptin receptor phosphorylation is unknown, but may involve JAK2. In agreement with this hypothesis, the time-course of

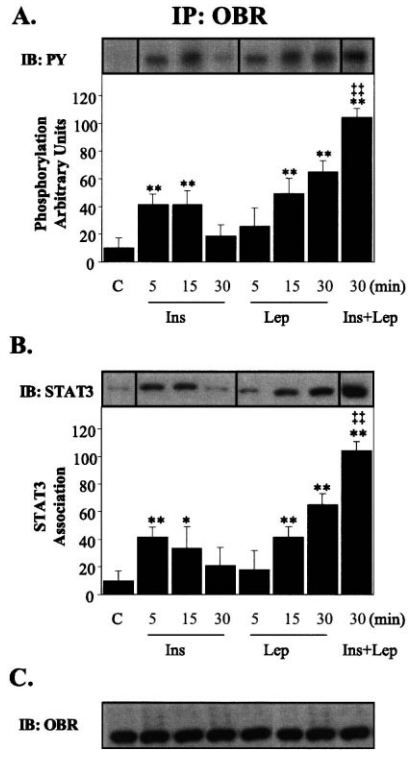

Fig. 4. Effect of insulin and leptin on OBR phosphorylation and STAT3 association in extracts of hypothalami from rats treated with leptin, insulin or a combination of the two for the indicated times. A: Western blots of anti-OBR immunoprecipitates (IP) with anti-phosphotyrosine antibody (pY) (IB, immunoblotting). The bar graph shows the quantitative phosphorylation of OBR proteins. B: The same membranes were reblotted with anti-STAT3 antibodies. The bar graph represents the quantitative association of STAT3 with OBR. The data in (A) and (B) are the means ± S.E.M. of four to six independent experiments. C: The equal loading of proteins was verified by reblotting with OBR antibodies. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control; $^{‡‡}P < 0.01$ vs. leptin-treated rats (factorial ANOVA).

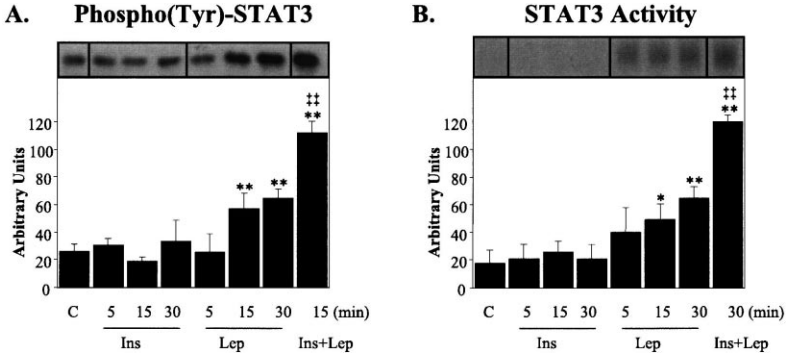

Fig. 5. Insulin (Ins) does not induce STAT3 activation but positively modulates leptin-induced activation of this protein. A: Hypothalami were stimulated with insulin, leptin (Lep), or a combination of the two for the indicated times. Hypothalami were lysed and the proteins were separated by SDS–PAGE on 8% gels and blotted with phosphotyrosine-specific STAT.3 antibodies. Leptin, but not insulin increased the phosphorylation of STAT3; combining the two hormones increased the phosphorylation compared to leptin alone. A representative Western blotting of each is shown. The bar graphs represent the means ± S.E.M. of three to four independent experiments. B: Insulin- and leptin-induced STAT3 DNA-binding activity in rat hypothalami. Rats were starved for 6 h and then received leptin, insulin or insulin plus leptin. The animals were sacrificed at the indicated times, and nuclear extractions were used for EMSA (see Section 2). Each lane represents four hypothalami. All experiments were done twice with similar results. A representative gel shift is shown for each treatment. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control; $^{‡‡}P < 0.01$ vs. leptin-treated rats (factorial ANOVA).

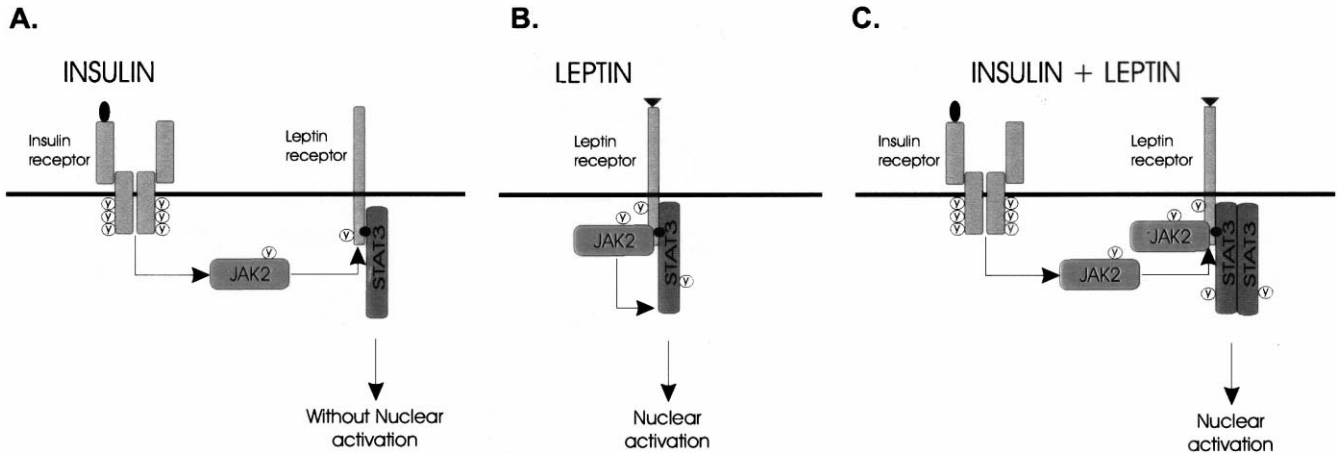

Fig. 6. Model for insulin/leptin cross-talk in rat hypothalamus. A: Insulin infusion. The phosphorylated IR binds and phosphorylates JAK2, which phosphorylates the leptin receptor. The leptin receptor binds STAT3, but there is no nuclear activation. B: Leptin infusion. The leptin receptor binds and activates JAK2, which phosphorylates the leptin receptor. The leptin receptor then binds and activates STAT3. C: Insulin+-leptin infusion. The simultaneous stimulation with insulin and leptin delivers an addictive signal, which leads to enhanced STAT3 association and nuclear activation.

insulin-induced JAK2 tyrosine phosphorylation preceded that of OBR phosphorylation.

Tyrosine phosphorylation of the OBR leads to the binding of STAT3 [47]. To detect the OBR/STAT3 association, nitrocellulose membranes containing samples previously immunoprecipitated with anti-OBR antibody were blotted with anti-STAT3 antibodies. There was an increase in OBR/STAT3 association after leptin stimulation, and this was concomitant with the increase in OBR tyrosine phosphorylation (Fig. 4B, right side). The same membrane was reprobed with anti-IR antibody, and no band was detected, demonstrating that there was not non-specific antibody interaction. Insulin was also able to recruit STAT3 to OBR (Fig. 4B, left side). This insulin-induced increase in the OBR recruitment of STAT3 indicated that insulin can modulate the OBR. The simultaneous stimulation with leptin and insulin dramatically increased this association compared to leptin treatment alone (Fig. 4B).

To determine the additive effect of insulin on the leptin signal transduction pathway, we evaluated the activity of STAT3 after the infusion of these hormones (Fig. 5A,B). Initially, we measured insulin-induced tyrosine phosphorylation of STAT3 by immunoblotting with specific phospho-STAT3 antibodies. As shown in Fig. 5A, insulin did not induce tyrosine phosphorylation of STAT3. Western blotting was also used to examine the tyrosyl phosphorylated STAT3 proteins before and after stimulation with leptin. Fig. 5A shows that there was a marked increase in leptin-stimulated STAT3 phosphorylation in rat hypothalami which was maximal at 15 min. Comparison of the bands from simultaneous stimulation revealed that the extent of STAT3 phosphorylation increased 2.1 ± 0.4-fold ($P < 0.05$) in rats receiving leptin plus insulin compared to the leptin-treated group. The results were confirmed by EMSA experiments. A time-course curve for STAT3 activation after i.c.v. leptin administration in normal rats showed an increase in STAT3 nuclear binding after stimulation with this hormone. The maximal response was obtained immediately after tyrosine phosphorylation, i.e. 15–30 min after 10 μg of leptin i.c.v. and was similar to the maximal response time observed after the iv injection of leptin [17]. In agreement with the results for STAT3 phosphorylation, there was no STAT3 activation after stimulation with insulin. However, the simultaneous administration of insulin and leptin increased the activation of STAT3 by 1.9 ± 0.5-fold compared to treatment with leptin alone. These findings indicate that insulin modulates leptin signal transduction by increasing the association of the leptin receptor with STAT3, leading to activation of the latter.

Based on these results we propose a model for the cross-talk between the insulin and leptin pathways in rat hypothalamus (Fig. 6). According to this model insulin alone induces IR tyrosine phosphorylation which is rapidly followed by JAK2 tyrosine phosphorylation. Activated JAK2 then induces the phosphorylation of OBR. This pathway leads to OBR/STAT3 association, but no activation of STAT3. Leptin on its turn, phosphorylates and activates JAK2, which leads to OBR tyrosine phosphorylation and STAT3 recruitment accompanied by its activation. Co-stimulation with insulin and leptin provides an additive signal which leads to enhanced STAT3 association and nuclear activation.

Integrated responses to insulin and leptin in the hypothalamus participate in the physiological control of food ingestion and body weight. The clinical outcomes observed in some animal models and in humans may reflect a disruption of the normal events in either of these signaling pathways. Obesity occurs in mice lacking the IR in the central nervous system (NIRKO) [19], despite elevated circulating leptin concentrations. This suggests that organ-specific insulin resistance in the central nervous system leads to central leptin resistance. On the other hand, the lack of leptin in ob/ob mice leads to obesity accompanied by hyperinsulinemia, which by itself is not sufficient to reverse the obese phenotype. Finally, in humans with type 1 diabetes, a hypoinsulinemic and a hypoleptinemic status may contribute to the ensuing hyperphagia. Thus insulin resistance, as well as a complete absence of insulin, may impair some of the leptin responses. Our results indicate a mechanism for modulation of the leptin/STAT3 signal transduction pathway by insulin. Insulin rapidly increased the phosphorylation of OBR, and improved the stimulation of leptin-mediated STAT3 phosphorylation, with a consequent gain in STAT3 signaling to the nucleus. This

mechanism may normally function to potentiate the activity of both of these pathways, and to increase stimulation in physiological processes such as the regulation of body weight and food intake, that are under the combined control of insulin and leptin. The potential for this interaction as a site for new therapeutic approaches in treating leptin resistance, and for increasing our understanding of leptin signaling and tyrosine kinase-mediated pathways, deserves further exploration.

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