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Leptin and Insulin Induce Mutual Resistance for Nitric Oxide Synthase III Activation in Adipocytes

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ABSTRACT

Obesity-induced hyperleptinemia is frequently associated with insulin resistance suggesting a crosstalk between leptin and insulin signaling pathways. Our aim was to determine whether insulin and leptin together interfere on NOS activation in adipocytes. We examined insulin and leptin-induced nitric oxide synthase (NOS) activity, protein amount and NOS III phosphorylation at Ser^{1179} in isolated epididymal adipocytes from rat, in the presence or not of inhibitors of kinases implicated in insulin or leptin signaling pathways. Insulin or leptin induced NOS III phosphorylation at Ser^{1179} leading to increased NO production in rat adipocytes, in agreement with our previous observations. When insulin and leptin at a concentration found in obese rats (10 ng/ml) were combined, NOS activity was not increased, suggesting a negative crosstalk between insulin and leptin signaling mechanisms. Chemical inhibitors of kinases implicated in signaling pathways of insulin, such as PI-3 kinase, or of leptin, such as JAK-2, did not prevent this negative interaction. When leptin signaling was blocked by PKA inhibitors, insulin-induced NOS activity and NOS III phosphorylation at Ser^{1179} was observed. In the presence of leptin and insulin, (i) IRS-1 was phosphorylated on Ser^{307} and this effect was prevented by PKA inhibitors, (ii) JAK-2 was dephosphorylated, an effect prevented by SHP-1 inhibitor. A mutual resistance occurs with leptin and insulin. Leptin phosphorylates IRS-1 to induce insulin resistance while insulin dephosphorylates JAK-2 to favor leptin resistance. This interference between insulin and leptin signaling could play a crucial role in insulin- and leptin-resistance correlated with obesity. J. Cell. Biochem. 108: 982–988, 2009.

KEY WORDS: LEPTIN; INSULIN; NITRIC OXIDE; ADIPOSE TISSUE

Obesity is a major cause of insulin resistance and contributes to the development of type-2 diabetes. The causes of peripheral insulin resistance are multiple, linked to nonspecific or regulated degradation of elements in the insulin signaling. There are evidences showing that fatty acids, adipose-derived cytokines like TNF-α and interleukins or adipokines such as leptin are relevant for impaired insulin action [Ceddia et al., 2002; Pirola et al., 2004]. Leptin, synthesized and secreted by adipocytes, plays an important role in the central regulation of body weight [Turban et al., 2002]. Leptin has also important functions as a metabolic and neuroendocrine hormone [Robertson et al., 2008]. Leptin acts predominantly in the central nervous system, mainly in the hypothalamus, bringing about effects on appetite and in neuroendocrine pathways, as well as on autonomic nerves, which are transmitted to periphery [Irani et al., 2008]. It is now well known that leptin acts also on peripheral tissues like adipose tissue [Frühbeck et al., 1997; Wang et al., 1999; Kim et al., 2000; William et al., 2002]. In agreement with that, leptin receptors (Ob-R) are expressed in such tissues. In confluent and differentiated preadipocytes, leptin receptors—Ob-Ra and Ob-Rb—were identified and exposure to leptin induced STAT3 phosphorylation in these cells [Machinal-Quélin et al., 2002]. Leptin regulates many intracellular signaling pathways mediated by insulin [Sweeney, 2002]. Thus there is clearly potential crosstalk between intracellular signaling in response to these hormones. Complex interactions between leptin and insulin signaling pathways are cell- and tissue specific [Szanto and Kahn, 2000]. Positive or negative crosstalks between leptin and insulin can be observed [Muller et al., 1997; Carvalheira et al., 2001, 2003; Kellerer et al., 2001; Vecchione

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et al., 2003; Perez et al., 2004]. The leptin effects observed in vivo tend to support that leptin has an insulin-sensitizing effect, which may depend on central mechanisms whereas in vitro observations suggest that leptin plays an inhibitory role such as on metabolic actions of insulin in adipocytes [Muller et al., 1997; Perez et al., 2004]. In obesity and metabolic diseases, alterations in Ob-R signaling leading to evidence for cellular leptin resistance is often associated with a state of insulin resistance with attenuation of insulin signaling [Perseghin et al., 2003; Myers et al., 2008]. A negative crosstalk between insulin and leptin signaling chain seems a possible regulatory mechanism leading to these states.

We have previously shown that rat adipose tissue expresses two isoforms of nitric oxide synthases (NOS), the endothelial isoform (NOS III) and the inductible isoform (NOS II) [Ribiere et al., 1996]. We and others have described the important role played by nitric oxide (NO) in regulation of fatty acid metabolism in adipose tissue [Gaudiot et al., 2000; Jobgen et al., 2006]. NOS III activity is regulated by reversible phosphorylation through multiple protein kinases and phosphatases [Fleming and Busse, 2003]. We showed that insulin or leptin increased rapidly NOS activity in isolated adipocytes, an effect linked to NOS III phosphorylation on Ser^{1179} [Ribiere et al., 2002; Mehebik et al., 2005]. However the signaling pathways were specific for each hormone. Insulin induced this phosphorylation through PI3-kinase/Akt activation whereas leptin effect was Jak-2/PKA dependent. Furthermore MAP-kinase activation was indispensable for both hormone-induced NOS III phosphorylation. The aim of the present study is to investigate whether an interference of insulin and leptin signaling occurs in isolated adipocytes and to identify potential molecular mechanisms.

MATERIALS AND METHODS

MATERIALS

Recombinant rat leptin was from Peprotech (Rocky Hill, NJ). L-[2,3,4,5-^{3}H] arginine (58 Ci/mmol) and ECL detection kit were products of Amersham Biosciences (Little Chalfont, UK). AG Dowex 50W-X8, Bradford protein dye reagent, and electrophoretic chemicals were from Bio-Rad Laboratories (Hercules, CA). Anti-dual-phospho Thr^{183} and Tyr^{185} p42/p44. MAP-kinases (V8031) antibodies were obtained from Promega (Madison, WI). Anti-phospho Ser^{1179} NOS III antibodies were from Cell Signaling Technology (Beverley, MA). Polyclonal anti-NOS III and anti-ERK (pan ERK) antibodies were obtained from Transduction Laboratories (San Diego, CA). AG490 and protein tyrosine phosphatase inhibitor (PTP inhibitor I) were from Calbiochem (San Diego, CA). KT5720 was from Biomol International (Plymouth Meeting, PA). Anti-phospho Ser^{307} IRS-1 antibodies were from Upstate (Lake Placid, NY) and anti-phospho $Tyr^{1007/1008}$ JAK-2 antibodies from Santa Cruz Biotechnology (Santa Cruz, CA). Insulin, H89, wortmannin and other reagents were obtained from Sigma (St. Louis, MO).

ANIMALS

Male Sprague–Dawley rats (180–200 g) obtained from Centre d'Elevage de Rats Janvier (Le Genest St Isle, France) at 8 weeks of age, were maintained at constant room temperature (24°C) on a 12 h light/dark cycle. Fed rats were killed by decapitation and white adipose tissue from epididymal fat depots were carefully removed and rapidly used for adipocyte preparation. All experimental protocols were approved by the University Animal Use and Care Committee.

ADIPOCYTE INCUBATION AND NOS ACTIVITY

Isolated epididymal adipocytes were prepared as previously described [Ribiere et al., 1996]. NOS activity was measured following L-[^{3}H] arginine conversion into L-[^{3}H] citrulline by intact adipocytes [Mehebik et al., 2005]. Briefly, adipocytes ($3–5 \times 10^5$ cells/ml) were incubated at 37°C in KRB (pH 7.4) containing 2% (w/v) BSA, 5 mM glucose, 1.5 μCi/ml L-[^{3}H] arginine and 50 mM valine (to inhibit arginase), in the absence or presence of insulin (10^{-9} M) and/or various concentrations of leptin. Each incubation was performed without and with a specific NOS inhibitor, diphenyl-iodonium (DPI). Incubations were stopped by adding 250 μl ethanol followed by 5 ml of 1:1 (v/v) H_2O/Dowex 50W-X8 (Na^+ form) resin to retain arginine and left to settle for 10 min at 4°C. Supernatant was removed and the main product detected by HPLC was citrulline. The citrulline production was linear between 10 and 60 min. One milliliter of supernatant was added to liquid scintillation cocktail for counting. Values obtained in the presence of DPI were subtracted from each sample. DMSO which was used as vehicle for the tested inhibitors was added to controls and separate experiments revealed no effect of this compound on the adipocyte NOS activity.

CELL LYSATES

Adipocytes ($3–5 \times 10^5$ cells/ml) were incubated during 20 min at 37°C in KRB (pH 7.4) containing 5 mM glucose in the absence or in the presence of the effectors to be tested. Then adipocytes were harvested, and disrupted in buffer containing 20 mM Tris, pH 7.5, 5 mM EDTA, 10 mM $Na_4P_2O_7$, 100 mM NaF, 2 mM Na_3VO_4, 1% NP-40, and protease inhibitors [Kim et al., 2000]. Cell lysates were solubilized by continuous stirring for 30 min at 4°C and centrifuged for 10 min at 14,000*g*. Supernatants were used for protein determination according to Bradford [1976] and, after Laemmli buffer addition, for electrophoresis and immunoblotting studies.

PROTEIN DETECTION

Cell lysates (10–20 μg protein/lane) were subjected to SDS–PAGE and then blotted onto polyvinylidine difluoride membranes. Blots were incubated with the primary antibody at 4°C overnight and incubated with the secondary antibody linked to peroxidase. Immunoreactive proteins were visualized on X-ray film by an enhanced chemiluminescence ECL reagents. Immunoblotting was performed using specific, anti-NOS III, anti-phospho NOS III (Ser^{1179}), anti-active MAP kinases (phospho Thr^{183} and phospho Tyr^{185}) and anti-ERK (pan ERK), anti-phospho IRS-1 (Ser^{307}), anti-phospho JAK-2 ($Tyr^{1007/1008}$) antibodies.

STATISTICS

Data are presented as means ± SEM. Statistical analyses were performed using unpaired Student's *t*-test.

RESULTS

NEGATIVE LEPTIN–INSULIN CROSSTALK ON NOS ACTIVITY

As previously shown [Ribiere et al., 2002; Mehebik et al., 2005], insulin or leptin alone increased NOS activity. When leptin (10 ng/ml) and insulin (1 nM) where tested in combination, the NOS activity was not increased but was similar to control value (Fig. 1) suggesting an interaction between leptin and insulin signaling networks. To test this negative crosstalk between the two hormones, we inhibited selectively the PI-3K transduction pathway implicated in insulin effect, using wortmannin. Wortmannin blocked NOS activity induced by insulin but was without action on leptin-induced NOS activity and did not prevent the negative crosstalk induced by insulin on leptin signaling (Fig. 2A). This result suggested that insulin interfered with leptin transduction pathways by a signal upstream the PI3-K activation. Leptin can activate PKA in adipocytes and increase NOS III activation [Ribiere et al., 2002; Mehebik et al., 2005] and lipolysis [Kawaji et al., 2001]. Additionally, leptin induces fatty acid oxidation in endothelial cells through PKA activation [Yamagishi et al., 2001]. To discriminate between signaling pathways, we alternatively inhibited the leptin pathways implicated in NOS activation, either JAK-2 using AG-490, or PKA using H89 and KT5720. As shown in Figure 2B, AG-490 prevented leptin effect and was without effect on insulin activation of NOS but did not reverse the inhibitory crosstalk induced by leptin on insulin. H89 or KT5720, two inhibitors of PKA known to prevent leptin-induced NOS activity [Mehebik et al., 2005], were without effect on insulin activation but prevented negative crosstalk induced by leptin on insulin signaling responsiveness of NOS activation (Fig. 3). Thus, leptin interferes with insulin signaling pathways independently of JAK-2 activation but involves PKA activation.

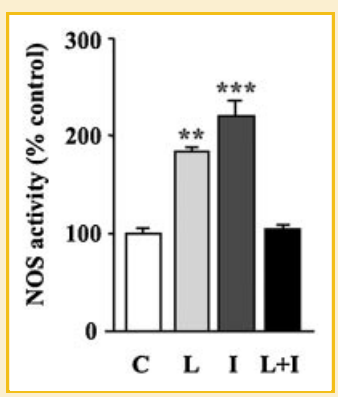

Fig. 1. NOS activity in rat adipocytes in the presence of insulin and leptin. Isolated adipocytes were incubated with insulin (I: 1 nM) and/or leptin (L: 10 ng/ml) for 20 min. NOS activity was measured by the conversion of L-[^{3}H] arginine into L-[^{3}H] citrulline as described under "Materials and Methods Section." Results are means ± SEM of independent experiments performed in duplicate with 5 separate adipocyte preparations and are expressed as the percentage of NOS activity in control adipocytes (C). $^{**}P < 0.01$; $^{***}P < 0.001$ versus control.

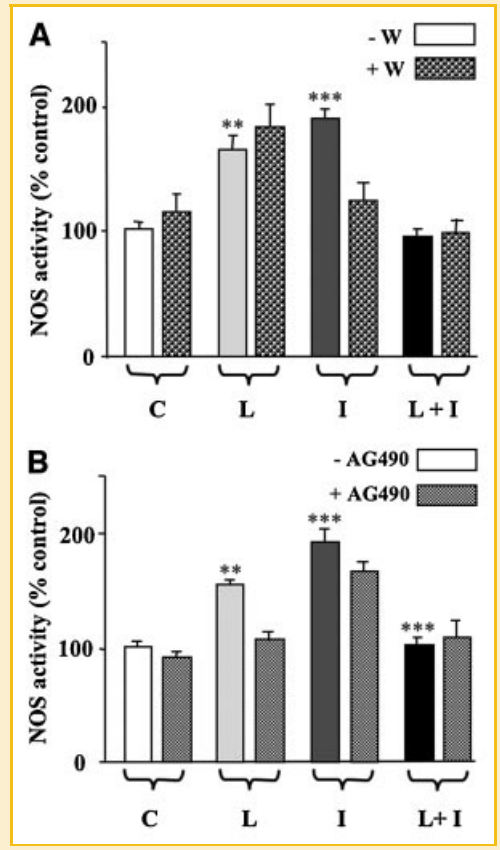

Fig. 2. A PI-3 kinase inhibitor (A) or a JAK-2 inhibitor (B) does not prevent insulin interaction on leptin-induced NOS activity. Isolated adipocytes were pretreated with vehicle alone or PI3-kinase inhibitor, 1 μM wortmannin (W), or JAK-2 inhibitor, 10 μM AG490, for 15 min, then exposed to vehicle or insulin (I: 1 nM) and/or leptin (L: 10 ng/ml) for another 20 min. NOS activity was measured by the conversion of L-[^{3}H] arginine into L-[^{3}H] citrulline as described under "Materials and Methods Section." Results are means ± SEM of independent experiments performed in duplicate with five separate adipocyte preparations and are expressed as the percentage of NOS activity in control adipocytes (C). $^{**}P < 0.01$; $^{***}P < 0.001$ versus control.

INHIBITORY EFFECT OF PKA ON INSULIN TRANSDUCTION PATHWAYS FOR NOS ACTIVATION

The leptin or insulin-induced NOS activity was linked to NOS III phosphorylation at Ser^{1179} [Mehebik et al., 2005]. If leptin and insulin were tested in combination, Ser^{1179} phosphorylation was blunted (Fig. 4). When adipocytes were pretreated with H89 and incubated with leptin plus insulin, NOS III was phosphorylated at Ser^{1179}. This phosphorylation observed in the presence of PKA inhibitors, when leptin and insulin are in combination was due to insulin since leptin-induced NOS III phosphorylation was PKA dependent [Mehebik et al., 2005]. As MAP-kinase activation was shown necessary to Ser^{1179} phosphorylation on NOS III by insulin or leptin [Ribiere et al., 2002; Mehebik et al., 2005], we studied this activation in the presence of both hormones. As shown in Figure 5, leptin or insulin activated MAP-kinase as previously observed [Ribiere et al., 2002; Mehebik et al., 2005]. However, such activation was not detected when both hormones were used in combination but was maintained in H89-pretreated adipocytes. These results show

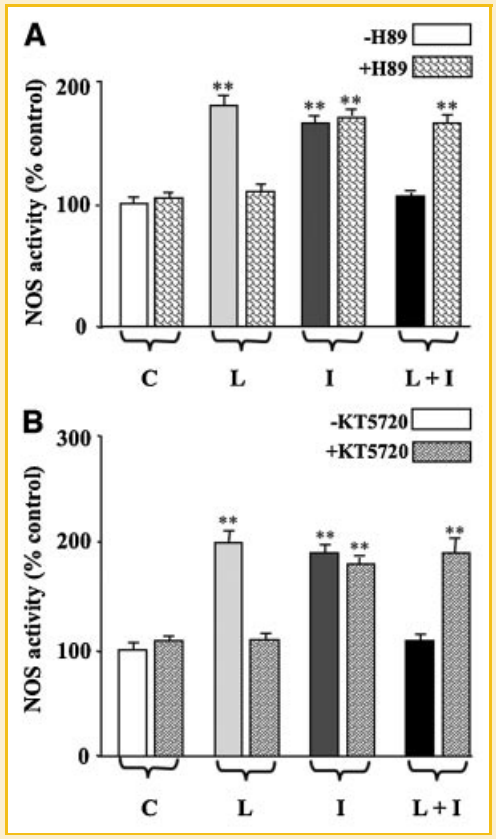

Fig. 3. PKA inhibitors prevent leptin interaction on insulin-induced NOS activity. Isolated adipocytes were pretreated with vehicle alone or A: H89 (10 μM) or B: KT5720 (1 μM) for 15 min, and then exposed to vehicle or insulin (I: 1 nM) and/or leptin (L: 10 ng/ml) for another 20 min. NOS activity was measured by the conversion of L-[^{3}H] arginine into L-[^{3}H] citrulline as described under "Materials and Methods Section." Results are means ± SEM of independent experiments performed in duplicate with five separate adipocyte preparations and are expressed as the percentage of NOS activity in control adipocytes (C). $^{**}P < 0.01$ versus control.

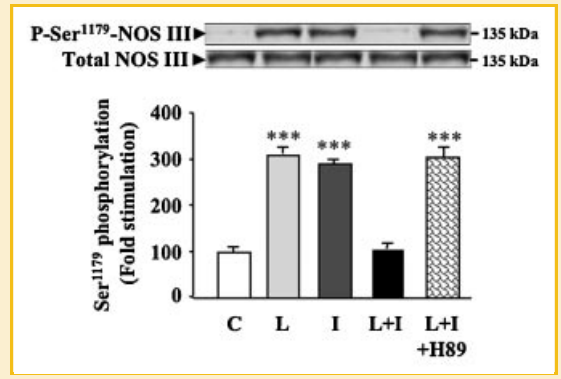

Fig. 4. Phosphorylation of NOS III at Ser^{1179} is blunted in the presence of insulin plus leptin, an effect reversed by PKA inhibitor. Isolated adipocytes were pretreated with vehicle alone or H89 (10 μM) for 15 min, and then exposed to vehicle or insulin (I: 1 nM) and/or leptin (L: 10 ng/ml) for another 20 min. Cell lysates, prepared as described under "Materials and Methods Section," were separated on SDS–7% polyacrylamide gels, then transferred to nitrocellulose membranes and analyzed with antibody specific for phosphorylated form of NOS III at Ser^{1179} and with polyclonal anti-NOS III antibody to ensure equal loading of the samples. Densitometry was performed to quantify phosphorylated bands. Data represent means ± SEM of independent experiments with five separate adipocyte preparations. $^{***}P < 0.001$ versus control.

that leptin interferes with insulin transduction mechanisms via PKA. Once activated, insulin receptor phosphorylates a number of important proximal substrates on tyrosine including members of the insulin receptor-substrate family (IRS-1/2/3/4). Serine/threonine kinases could phosphorylate IRSs on serine residues which are ubiquitinated and finally degraded in proteasome [Potashnik et al., 2003]. We hypothesized that leptin could phosphorylate IRS-1 by PKA on serine thus blocking insulin transduction. To address this issue, we used phospho-specific IRS-1 antibodies which recognize Ser^{307} residue on IRS-1. Insulin or leptin alone did not phosphorylate Ser^{307} on IRS-1 but in the presence of both hormones this residue was phosphorylated and this effect was prevented by H89 pretreatment (Fig. 6). Thus leptin-induced PKA activation alters insulin transduction pathways at IRS-1 level.

INSULIN ALTERS LEPTIN RECEPTOR SIGNALING AT THE LEVEL OF JANUS KINASE-2 (JAK-2)

Leptin signaling is mediated by ligand-induced conformational changes of Ob-Rb which activates the intracellular signal transducing protein JAK-2. JAK-2 has intrinsic tyrosine kinase activity causing autophosphorylation and subsequently phosphorylation of Ob-Rb on different tyrosine residues [Kloek et al., 2002]. It was previously shown that insulin interfered with JAK-2 and that SHP-1 was, at least in part, involved in the negative effect of insulin on JAK-2 [Kellerer et al., 2001]. We, therefore, studied JAK-2 phosphorylation induced by leptin in the presence and in the

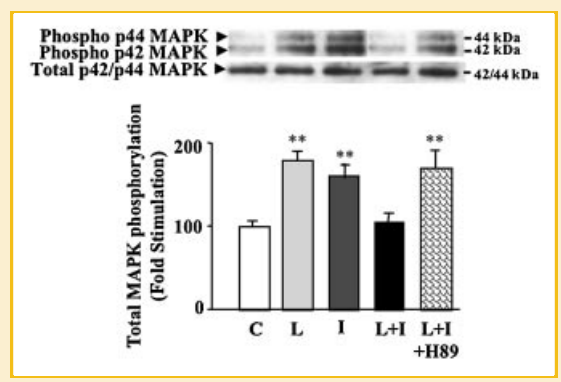

Fig. 5. Phosphorylation of p42/p44 MAP kinases is blunted in the presence of insulin plus leptin, an effect reversed by PKA inhibitor. Isolated adipocytes were pretreated with vehicle alone or H89 (10 μM) for 15 min, and then exposed to vehicle or insulin (I: 1 nM) and/or leptin (L: 10 ng/ml) for another 20 min. Cell lysates, prepared as described under "Materials and Methods Section," were separated on SDS–12% polyacrylamide gels, then transferred to nitrocellulose membranes and analyzed with antibody specific for dual phosphorylated form of p42/p44 MAP kinases or with anti-total p42/p44 MAP kinases (pan ERK) antibody to ensure equal loading of the samples. Densitometry was performed to quantify phosphorylated bands. Data represent means ± SEM of independent experiments with five separate adipocyte preparations. $^{**}P < 0.01$ versus control.

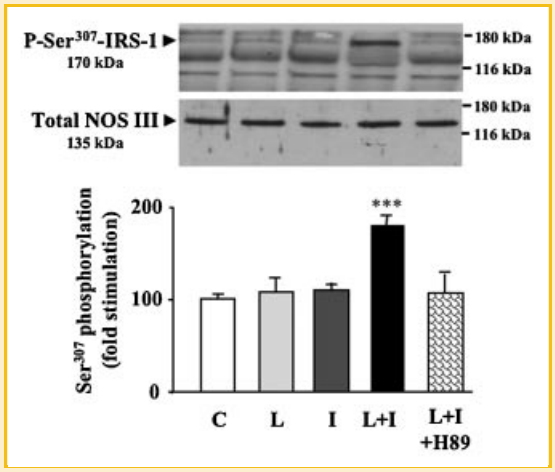

Fig. 6. Phosphorylation of IRS-1 at Ser^{307} in the presence of insulin plus leptin is prevented by PKA inhibitor. Isolated adipocytes were pretreated with vehicle alone or H89 (10 μM) for 15 min, and then exposed to vehicle or insulin (I: 1 nM) and/or leptin (L: 10 ng/ml) for another 20 min. Cell lysates, prepared as described under "Materials and Methods Section," were separated on SDS–7% polyacrylamide gels, then transferred to nitrocellulose membranes and analyzed with antibody specific for phosphorylated form of IRS-1 at Ser^{307} and with polyclonal anti-NOS III antibody to ensure equal loading of the samples. Densitometry was performed to quantify phosphorylated bands. Data represent means ± SEM of independent experiments with four separate adipocyte preparations. $^{***}P < 0.001$ versus control.

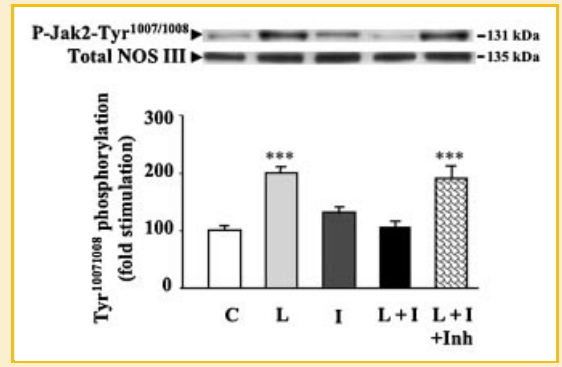

Fig. 7. Phosphorylation of JAK-2 is blunted in the presence of insulin plus leptin, an effect reversed by a protein tyrosine phosphatase inhibitor. Isolated adipocytes were pretreated with vehicle alone or protein tyrosine phosphatase inhibitor I [4-hydroxyphenacyl bromide (Inh)] (50 μM) for 15 min, then exposed to vehicle or insulin (I: 1 nM) and/or leptin (L: 10 ng/ml) for another 20 min. Cell lysates, prepared as described under "Materials and Methods Section," were separated on SDS–7% polyacrylamide gels, then transferred to nitrocellulose membranes and analyzed with antibody specific for phosphorylated form of JAK-2 at $Tyr^{1007/1008}$ and with polyclonal anti-NOS III antibody to ensure equal loading of the samples. Densitometry was performed to quantify phosphorylated bands. Data represent means ± SEM of independent experiments with four separate adipocyte preparations. $^{***}P < 0.001$ versus control.

absence of insulin using phospho-specific JAK-2 antibodies which recognize $Tyr^{1007/1008}$ on JAK-2. Leptin alone induced JAK-2 phosphorylation but in the presence of insulin this phosphorylation did not occur (Fig. 7). A potent, cell-permeable, and covalent protein tyrosine phosphatase inhibitor I [4-hydroxyphenacyl bromide (Inh)] which is able to inhibit SHP-1, restored JAK-2 phosphorylation in the simultaneous presence of leptin and insulin.

DISCUSSION

Our results reveal a negative crosstalk in insulin and leptin signaling pathways implicated in NOS III activation. This effect occurs at leptin level close to its plasma concentration observed during obesity in cafeteria-fed rats [Coatmellec-Taglioni et al., 2002]. The NOS III activation observed in the presence of insulin or leptin results in Ser^{1179} phosphorylation on NOS III [Ribiere et al., 2002; Mehebik et al., 2005]. When leptin and insulin are in combination, Ser^{1179} phosphorylation disappears completely suggesting that leptin induces an insulin resistance state while insulin induces leptin resistance. If indeed activation of MAP-kinases by leptin or insulin is required for NOS III activation produced by these two hormones, it appears that signal transductions related to NOS III phosphorylation are different, that is, PI-3K/Akt for insulin [Ribiere et al., 2002] and JAK-2/PKA for leptin [Mehebik et al., 2005]. Since the PI-3K inhibitor, wortmannin, is unable to prevent leptin interference on insulin signaling, we have tested an upstream target for this activation, IRS-1. In fact, phosphorylation of Ser^{307} on IRS-1 inhibits insulin-stimulated tyrosine phosphorylation and IRS-1-associated PI-3 kinase activity, potentially through disruption of the protein-protein interaction between IRS-1 and the insulin receptor [Aguirre et al., 2002; Gual et al., 2005; He et al., 2006]. In the absence of insulin, IRS-1 is located in the cytoplasm but under insulin stimulation, IRS-1 is translocated to the plasma membrane where it binds to the insulin receptor [Jacobs et al., 2001]. Since it was reported that leptin stimulated PKA, we should have expected a stimulation in Ser^{307}–IRS-1 phosphorylation by leptin [Mehebik et al., 2005]. We did not observe this stimulation under our experimental conditions, that is, using membrane extracts, probably because in the absence of insulin, IRS-1 remained in the cytoplasm [Jacobs et al., 2001]. However, we show clearly that in the presence of insulin and leptin, IRS-1 is phosphorylated on Ser^{307}, an effect which is prevented by a PKA inhibitor (H89). Hence, leptin, through PKA activation, blocks insulin signaling implicated at least in NOS III regulation since H89 re-establishes MAP-kinase activation and NOS III phosphorylation induced by insulin in the presence of leptin (Fig. 8). It appears then that IRS-1 is a target for leptin, mediating inhibitory signal on the insulin-signaling cascade in adipocytes as previously observed in muscle tissue [Hennige et al., 2006].

While the hypothalamus was the first target organ described for leptin, several evidences at the level of mRNA and cellular function suggested that adipose tissue also expressed Ob-Rb and was responsive to leptin effects [Siegrist-Kaiser et al., 1997; Machinal-Quélin et al., 2002]. Several reports concerning leptin signaling demonstrated that only the long isoform of the leptin receptor (Ob-Rb) mediated leptin-dependent tyrosine phosphorylation of JAK-2 [Ghilardi and Skoda, 1997; Bahrenberg et al., 2002; Kloek et al., 2002]. In addition, no lipolytic effect of leptin was observed in adipocytes isolated from fa/fa [Wang et al., 1999; Frühbeck and

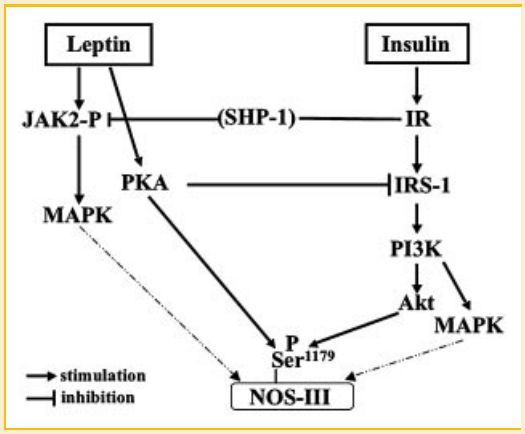

Fig. 8. Schematic diagram of insulin and leptin interaction on signaling pathways involved in the rapid activation of NOS III. Insulin and leptin alone acutely stimulate NOS activity by phosphorylation of NOS III at serine ^{1179}. In the presence of both hormones, insulin interferes with JAK-2 phosphorylation induced by leptin whereas leptin blocks insulin signaling by IRS-1 phosphorylation leading to suppression of NOS III phosphorylation. In adipocytes, phosphorylation of MAPK by insulin, but not leptin, depends on PI3K, while MAPK activation in response to leptin depends on JAK2 activation [Ribiere et al., 2002].

Gomez-Ambrosi, 2001] or db/db rodents [Frühbeck et al., 1998], both known to harbor a partially inactivating mutation in the Ob-Rb gene. A few studies reported that leptin was inefficient on adipocyte metabolism [Mick et al., 1998; Ranganathan et al., 1998; Eliman et al., 2002]. However, this lack of effect could result from the use of mouse recombinant leptin on rat adipose tissue since species-related specificities have been reported, including from our own group (Mehebik, Jaubert and Ribière, unpublished results). The report from Eliman et al. [2002] is puzzling because leptin of human origin had no effect on human adipocytes. However, in this report leptin action on lipolysis was monitored in the presence of insulin and we show here that insulin counteracts leptin action through a PKA-dependent mechanism.

Our results suggest that leptin-induced PKA activation is independent of JAK-2 and could explain why AG490 does not prevent leptin-induced alterations in insulin signaling linked to NOS III activation (Fig. 8). JAK-2 phosphorylation induced by leptin is observed in the presence of insulin when adipocytes are pretreated with a tyrosine phosphatase inhibitor. This effect could be linked to insulin-activation of SHP-1, as previously reported [Kellerer et al., 2001]. On the one hand, insulin induces phosphorylation and activation of SHP-1, presumably by a direct association between SHP-1 and the insulin receptor [Uchida et al., 1994]. Besides direct association of SHP-1 with JAK-2 results in activation of the phosphatase and subsequent dephosphorylation of JAK-2 [Jiao et al., 1996; Li and Friedman, 1999]. In the presence of leptin, SHP-1 could modulate JAK-2 phosphorylation [Li and Friedman, 1999] and insulin activation of SHP-1 could strengthen this modulation, thus preventing JAK-2 phosphorylation. These results clearly show that increased leptin as a result of obesity impairs insulin action in adipocytes, in agreement with what has been reported previously [Muller et al., 1997; Perez et al., 2004]. In mirror of this, insulin accelerates the release and secretion of leptin from a preformed intracellular pool in rat adipose tissue [Lee and Fried, 2006]. We demonstrate here that insulin blocks leptin action observed at concentrations corresponding to obesity at least on NOS activity. This effect is in sharp contrast with that observed in the vessels where insulin and leptin cooperate in the modulation of vascular tone through enhancement of endothelial NO release [Vecchione et al., 2003]. In this tissue, leptin phosphorylates NOS III at Ser^{1179} by Akt activation. Such results underline the tissue- and signaling specificity for crosstalk of hormones.

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