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Impact of lipid phosphatases SHIP2 and PTEN on the time- and Akt-isoform-specific amelioration of TNF-α-induced insulin resistance in 3T3-L1 adipocytes

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Ikubo M, Wada T, Fukui K, Ishiki M, Ishihara H, Asano T, Tsuneki H, Sasaoka T. Impact of lipid phosphatases SHIP2 and PTEN on the time- and Akt-isoform-specific amelioration of TNF-α-induced insulin resistance in 3T3-L1 adipocytes. *Am J Physiol Endocrinol Metab* 296: E157–E164, 2009. First published November 11, 2008; doi:10.1152/ajpendo.90581.2008.—TNF-α is a major contributor to the pathogenesis of insulin resistance associated with obesity and inflammation by serine phosphorylating and degrading insulin receptor substrate-1. Presently, we further found that pretreatment with TNF-α inhibited insulin-induced phosphorylation of Akt2 greater than Akt1. Since lipid phosphatases SH2-containing inositol 5′-phoshatase 2 (SHIP2) and phosphatase and tensin homologs deleted on chromosome 10 (PTEN) are negative regulators of insulin's metabolic signaling at the step downstream of phosphatidylinositol 3-kinase, we investigated the Akt isoform-specific properties of these phosphatases in the negative regulation after short- and long-term insulin treatment and examined the influence of inhibition on the amelioration of insulin resistance caused by TNF-α in 3T3-L1 adipocytes. Adenovirus-mediated overexpression of WT-SHIP2 decreased the phosphorylation of Akt2 greater than Akt1 after insulin stimulation up to 15 min. Expression of a dominant-negative ΔIP-SHIP2 enhanced the phosphorylation of Akt2 up to 120 min. On the other hand, overexpression of WT-PTEN inhibited the phosphorylation of both Akt1 and Akt2 after short- but not long-term insulin treatment. The expression of ΔIP-PTEN enhanced the phosphorylation of Akt1 at 120 min and that of Akt2 at 2 min. Interestingly, the expression of ΔIP-SHIP2, but not ΔIP-PTEN, protected against the TNF-α inhibition of insulin-induced phosphorylation of Akt2, GSK3, and AS160, whereas both improved the TNF-α inhibition of insulin-induced 2-deoxyglucose uptake. The results indicate that these lipid phosphatases possess different characteristics according to the time and preference of Akt isoform-dependent signaling in the negative regulation of the metabolic actions of insulin, whereas both inhibitions are effective in the amelioration of insulin resistance caused by TNF-α.

insulin signaling; SH2-containing inositol 5′-phoshatase 2; phosphatase and tensin homologs deleted on chromosome 10

THE ACTIVATED INSULIN RECEPTOR phosphorylates insulin receptor substrates (IRS) at tyrosine residues (17, 26, 28). The tyrosine phosphorylated IRS binds to the regulatory subunit of phosphatidylinositol 3-kinase (PI3-kinase), which in turn activates the p110 catalytic subunit (3, 32, 36). The activation of PI3-kinase is known to be important for the various metabolic actions of insulin (3, 33, 37). PI3-kinase functions as a lipid kinase to produce $PI(3,4,5)P_3$ from $PI(4,5)P_2$ in vivo (32). $PI(3,4,5)P_3$ acts as a key lipid second messenger in insulin signaling to further downstream molecules, including Akt (30, 38). Lipid phosphatases were identified to hydrolyze $PI(3,4,5)P_3$ in the negative regulation of insulin signaling (12, 20, 25). SH2-containing inositol 5′-phoshatase 2 (SHIP2) functions as a lipid phosphatase possessing 5′-phosphatase activity to hydrolyze $PI(3,4,5)P_3$ to $PI(3,4)P_2$ (38). Phosphatase and tensin homologs deleted on chromosome 10 (PTEN) act as a 3′-lipid phosphatase hydrolyzing $PI(3,4,5)P_3$ to $PI(4,5)P_2$ (20). Targeted disruption of the SHIP2 gene in mice resulted in increased insulin sensitivity and conferred protection against obesity induced by a high-fat diet (4, 33). Since homozygous disruption of the PTEN gene in mice results in embryonic lethality, the tissue-specific role of PTEN was investigated (7). Liver, skeletal muscle, or adipose tissue-specific disruption of PTEN ameliorated glucose metabolism in the animal model of diabetes (18, 34, 39); therefore, lipid phosphatases, both SHIP2 and PTEN, appear to be implicated in glucose metabolism.

Adipocytes are important target tissues of insulin, and 3T3-L1 cells are well-characterized adipocytes (22, 23, 38). Overexpression of SHIP2 and PTEN is reported to inhibit insulin-induced phosphorylation of Akt and glucose uptake (22, 23, 38); however, the effect of SHIP2 and PTEN expression has been examined only after short-term insulin treatment with controversial results (23, 35). In addition, the role of these lipid phosphatases in the regulation of metabolic signaling after long-term insulin treatment is unknown; therefore, it would be of particular importance to clarify the possible differences in characteristics and properties among these lipid phosphatases to further understand the molecular mechanism of the negative regulation of insulin signaling.

Akt is one of the downstream target molecules of PI3-kinase important for glucose metabolism (5, 6, 38). Akt1 and Akt2 are the isoforms mainly expressed in adipocytes (31, 38). Studies (5, 6) with Akt1 and Akt2 knockout mice revealed that Akt2 is preferentially implicated in glucose metabolism, whereas Akt1 is mainly involved in cell growth. The comparative effect of SHIP2 and PTEN on short- and long-term insulin-induced phosphorylation of Akt1 and Akt2 is uncertain in 3T3-L1 adipocytes. In addition, TNF-α is an important cytokine implicated in the development of insulin resistance in type 2 diabetes (14, 27, 39); therefore, investigation of the ameliorative effect by inhibition of these endogenous lipid phosphatases

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on TNF-α-induced insulin resistance is important to clarify the therapeutic value in type 2 diabetes.

In the present study, we directly compared the role of lipid phosphatases SHIP2 and PTEN in short- and long-term insulin-induced phosphorylation of Akt1 and Akt2 in 3T3-L1 adipocytes. Furthermore, we investigated whether the inhibition of endogenous SHIP2 and PTEN by phosphatase-defective mutant expression protects against the impairment of insulin-induced phosphorylation of Akt, GSK3, and Akt substrate 160 (AS160), and glucose uptake by pretreatment with TNF-α.

MATERIALS AND METHODS

Materials. Human crystal insulin was provided by Novo Nordisk Pharmaceutical (Copenhagen, Denmark). 2-[^{3}H]deoxyglucose (2DG; 3,330 GBq/mM) was purchased from NEN Life Science Products (Boston, MA). Human recombinant TNF-α was obtained from Pepro Tech (Rocky Hill, NJ). The two polyclonal anti-SHIP2 antibodies were described previously (12). A monoclonal anti-phosphotyrosine antibody (PY20) was purchased from Transduction Laboratories (Lexington, KY). A polyclonal anti-Thr$^{308/309}$ phospho-specific Akt antibody and a polyclonal anti-Ser$^{473/474}$ phospho-specific Akt antibody, a polyclonal anti-Akt antibody, a polyclonal anti-Akt2 antibody, a polyclonal anti-Ser$^{21/9}$ phospho-specific GSK3α/β antibody, a polyclonal anti-GSK3α/β antibody, an a polyclonal anti-Ser/Thr-phospho-specific Akt substrate antibody, and a polyclonal anti-AS160 antibody were from Cell Signaling (Beverly, MA). A monoclonal anti-Akt1 antibody and a monoclonal anti-PTEN antibody were from Santa Cruz Biotechnology (Santa Cruz, CA). A polyclonal anti-IRS-1 antibody was from Upstate Biotechnology (Lake Placid, NY). Enhanced chemiluminescence reagents were from GE Healthcare Bio-Science (Tokyo, Japan). DMEM was from GIBCO-BRL Japan (Tokyo, Japan). All other reagents were of analytical grade and purchased from Sigma Chemical (St. Louis, MO) or Wako Pure Chemical Industries (Osaka, Japan).

Adenoviral vectors. Adenoviral vectors encoding wild-type SHIP2 (WT-SHIP2), a phosphatase-defective mutant SHIP2 (ΔIP-SHIP2) containing Pro^{687} to Ala, Asp^{691} to Ala, and Arg^{692} to Gly changes (38), wild-type PTEN (WT-PTEN), and a phosphatase-defective mutant PTEN (ΔIP-PTEN) containing Cys^{124} to Ser change (23) were described previously.

Cell culture and infections with adenovirus. 3T3-L1 fibroblasts were grown and passaged in DMEM supplemented with 10% donor calf serum. Cells at 2–3 days postconfluence were used for differentiation. The differentiation medium contained 10% FBS, 250 nM dexamethazone, 0.5 mM IBMX, and 500 nM insulin. After 3 days, the differentiation medium was replaced with postdifferentiation medium containing 10% FBS and 500 nM insulin. After 3 more days, the postdifferentiation medium was replaced with DMEM including 25 mM glucose supplemented with 10% FBS. SHIP2 and PTEN were transiently expressed in differentiated 3T3-L1 adipocytes by means of adenovirus-mediated gene transfer. A multiplicity of infection (MOI) of 10–40 plaque-forming units (PFU)/cell was used to infect 3T3-L1 adipocytes in DMEM containing 2% FBS, with the virus being left on the cells for 16 h before removal. Subsequent experiments were conducted 24–48 h after initial addition of the virus (38). The efficiency of adenovirus-mediated gene transfer of SHIP2 and PTEN was ~95%.

Insulin and TNF-α treatment. 3T3-L1 adipocytes grown in six-well multiplates were incubated with DMEM containing 0.1% FBS without or with 17 nM insulin at 37°C for 2- to 120-min periods. For experiments with TNF-α treatment, 20 nM TNF-α were added for 16 h and then treated with 17 nM insulin for 10 or 120 min.

Immunoprecipitation and Western blotting. The cells were lysed in a buffer containing 20 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 2.5 mM sodium deoxycholate, 1 mM β-glycerophosphate, 1% NP40, 1 mM PMSF, 1 mM Na_3VO_4, 50 mM sodium fluoride, 10 μg/ml of aprotinin, and 10 μM leupeptin, pH 7.4, for 30 min at 4°C. The lysates were centrifuged to remove insoluble materials. The supernatants (100 μg of protein) were immunoprecipitated with antibodies for 2 h at 4°C. The precipitates or the lysates were then separated by 7.5% SDS-PAGE and transferred onto polyvinylidene difluoride membranes using a Bio-Rad Transblot apparatus. The membranes were blocked in a buffer containing 50 mM Tris, 150 mM NaCl, 0.1% Tween 20, and 2.5% BSA or 5% nonfat milk, pH 7.5, for 2 h at 20°C. They were then probed with antibodies for 2 h at 20°C or for 16 h at 4°C. After the membranes were washed in a buffer containing 50 mM Tris, 150 mM NaCl, and 0.1% Tween 20, pH 7.5, the blots were incubated with a horseradish peroxidase-linked secondary antibody and subjected to enhanced chemiluminescence detection using ECL reagent according to the manufacturer's instructions (GE Health Science Bio-Science; Ref. 38). Densitometric analysis was conducted directly from the blotted membrane by utilizing LAS-4000 lumino-image analyzer system (Fujifilm, Tokyo, Japan). The relative phosphorylation level of each protein was calculated as the ratio of phosphorylated to total protein level.

Measurement of 2-deoxyglucose uptake. 3T3-L1 adipocytes grown in six-well multiplates were pretreated with TNF-α and serum starved for 2 h. The cells were washed twice with PBS and incubated with Krebs-Ringer phosphate-HEPES buffer, 10 nM HEPES, 131.2 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, and 2.5 mM NaH_2PO_4, containing 1% BSA, pH 7.4, for 1 h at 37°C. The cells were subsequently stimulated with various concentrations of insulin. After a 15-min insulin treatment, 3.7 kBq of 2-[^{3}H]deoxyglucose (2-DG) were added for 4 min. The reaction was stopped by the addition of 10 μmol/l cytochalasin B. The cells were washed three times with PBS and solubilized with 0.2 mM SDS-0.2 N NaOH (38). The radioactivity incorporated into the cells was measured by liquid scintillation counting.

Statistical analysis. Data are expressed as means ± SE. P values were determined by one-way ANOVA with Bonferroni's correction test, and $P < 0.05$ was considered significant.

RESULTS

Expression of lipid phosphatases in 3T3-L1 adipocytes. SHIP2 (140-kDa) is a 5′-lipid phosphatase and PTEN (54-kDa) is a 3′-lipid phosphatase, both of which are known to be involved in the negative regulation of insulin signaling (22, 23, 30, 38). Endogenous SHIP2 and PTEN were clearly observed in control 3T3-L1 adipocytes. Consensus amino acids located within the catalytic domain of lipid phosphatases were mutated to generate phosphatase-defective SHIP2 (ΔIP-SHIP2) and PTEN (ΔIP-PTEN) (23, 38). Wild-type and phosphatase-defective lipid phosphatases were transiently expressed in 3T3-L1 adipocytes by means of adenovirus-mediated gene transfer. By transfecting with these lipid phosphatase genes at an MOI of 10 PFU/cell (Fig. 1, *A* and *B*) and 40 PFU/cell (Fig. 1, *C* and *D*), we observed a 2.5- and 5-fold increase in expression levels of SHIP2 and a 4- and 7-fold increase in expression levels of PTEN, respectively, over the endogenous level in 3T3-L1 adipocytes. Since the obtained results with expression at an MOI of 10 and 40 PFU/cell were similar, the after analyses were shown at an MOI of 40 PFU/cell.

Effect of expression of wild-type lipid phosphatases on insulin-induced phosphorylation of Akt. Akt is one of the downstream target molecules of PI3-kinase and has been shown to mediate the metabolic actions of insulin (5, 38). We compared the effect of WT-SHIP2 and WT-PTEN expressions on the phosphorylation of Akt1 and Akt2 after insulin stimulation for up to 120 min in 3T3-L1 adipocytes. Overexpression

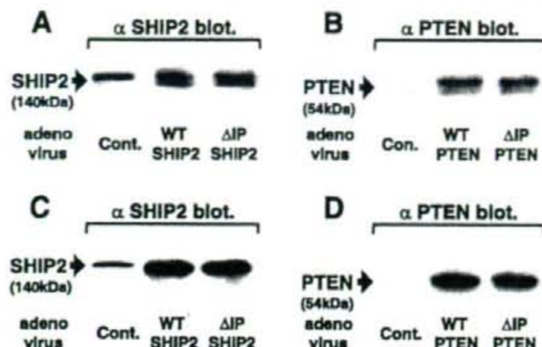

Fig. 1. Expression of lipid phosphatases in 3T3-L1 adipocytes. 3T3-L1 adipocytes were transfected with LacZ, wild-type SH2-containing inositol 5′-phosphatase 2 (WT-SHIP2), phosphatase-defective mutant SHIP2 (ΔIP-SHIP2), wild-type phosphatase and tensin homologs deleted on chromosome 10 (WT-PTEN), and ΔIP-PTEN at a multiplicity of infection (MOI) of 10 plaque-forming units (PFU)/cells (*A* and *B*) and 40 PFU/cells (*C* and *D*). After infection, cells were lysed and subjected to immunoblot analysis with anti-SHIP2 antibody (*A* and *C*) and anti-PTEN antibody (*B* and *D*). Results represent 3 separate experiments.

of WT-SHIP2 mildly inhibited the phosphorylation of Akt1 at Thr^{308} after insulin stimulation for up to 120 min (13.0% reduction at 5 min; Fig. 2*A*). On the other hand, overexpression of WT-SHIP2 greatly inhibited Akt2 phosphorylation at Thr^{309} after insulin treatment for up to 15 min (30.9% reduction at 5 min; Fig. 2*C*). Overexpression of WT-PTEN significantly inhibited insulin-induced Akt1 phosphorylation at 2 and 5 min by 26.6 and 16.5%, respectively (Fig. 2*B*). Insulin-induced phosphorylation of Akt2 at 2 and 5 min was also inhibited by 29.1 and 20.0%, respectively, by the overexpression of WT-PTEN (Fig. 2*D*). Similar results were obtained concerning Akt phosphorylation at $Ser^{473/474}$ residue and GSK3 phosphorylation at $Ser^{21/9}$ residue (data not shown).

Effect of expression of phosphatase-defective (ΔIP) lipid phosphatases on insulin-induced phosphorylation of Akt. Expression of ΔIP-SHIP2 enhanced Akt1 phosphorylation at Thr^{308}, and phosphorylation was significantly enhanced by 70.4% after 120 min of insulin treatment (Fig. 3*A*). The enhancing effect was more apparent in Akt2 phosphorylation at Thr^{309}. Phosphorylation of Akt2 at 2, 5, and 120 min of insulin treatment was significantly augmented by 20.7, 16.6, and 93.7%, respectively, by the expression of ΔIP-SHIP2 (Fig. 3*C*). Expression of ΔIP-PTEN enhanced long-term insulin-stimulated Akt1 phosphorylation, which was significantly augmented by 43.9% after 120 min of insulin treatment (Fig. 3*B*). Phosphorylation of Akt2 at only 2 min of insulin stimulation was slightly enhanced by 17.7% by the expression of ΔIP-PTEN (Fig. 3*D*). Similar results were again obtained concerning the phosphorylation of Akt at $Ser^{473/474}$ residue and GSK3 at $Ser^{21/9}$ residue (data not shown).

Expression of phosphatase-defective lipid phosphatases ameliorates insulin-induced phosphorylation of Akt after TNF-α treatment. We next examined the ameliorative effect of the expression of phosphatase-defective lipid phosphatases on the decreased insulin-induced phosphorylation of Akt by pre-

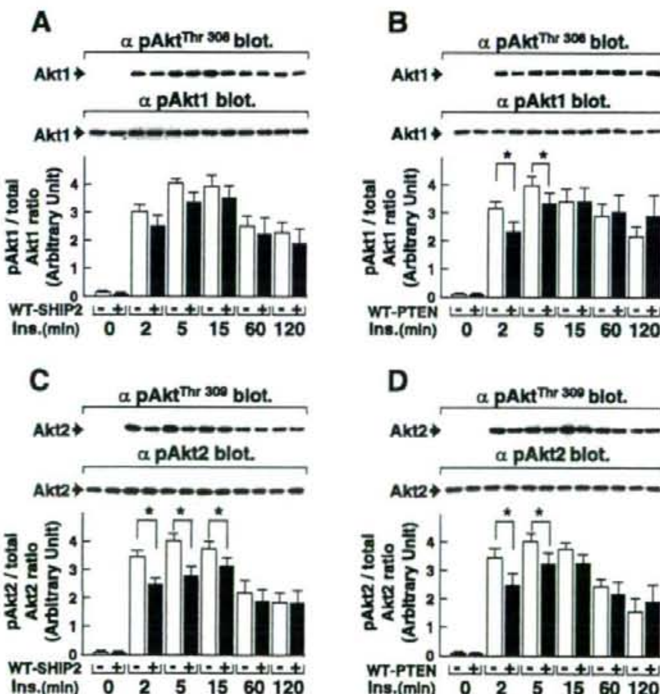

Fig. 2. Effect of wild-type SHIP2 and PTEN overexpression on insulin-induced phosphorylation of Akt. 3T3-L1 adipocytes were transfected with WT-SHIP2 (*A* and *C*) and WT-PTEN (*B* and *D*) at an MOI of 40 PFU/cell. Cells were serum starved for 16 h and subsequently treated with 17 nM insulin at 37°C for indicated times. Cells were immunoprecipitated with anti-Akt1 antibody (*A* and *B*) or anti-Akt2 antibody (*C* and *D*). Precipitates were separated by 7.5% SDS-PAGE and immunoblotted with anti-$Thr^{308/309}$-phospho-specific Akt antibody, anti-Akt1 antibody, or anti-Akt2 antibody. Amount of Akt phosphorylated at $Thr^{308/309}$ corrected for total protein level was quantitated by densitometry. Results are means ± SE of 5 separate experiments. $*P < 0.05$ vs. amount of phosphorylated Akt in LacZ-transfected cells with respective insulin treatment.

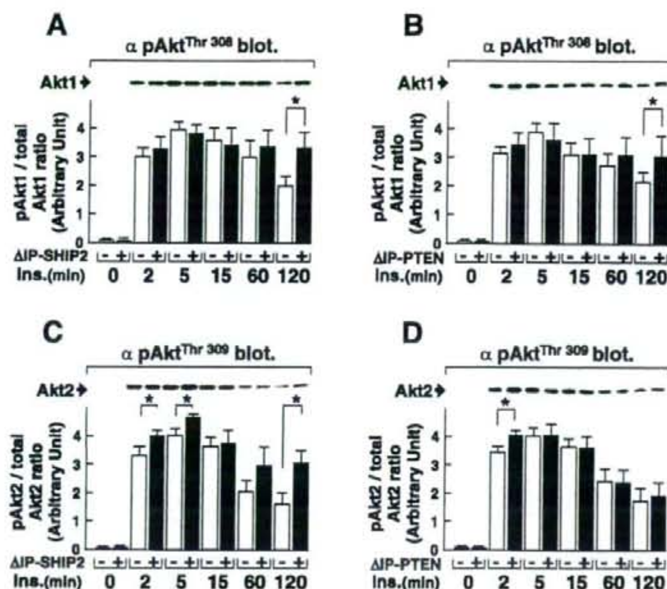

Fig. 3. Effect of phosphatase-defective SHIP2 and PTEN expression on insulin-induced phosphorylation of Akt. 3T3-L1 adipocytes were transfected with ΔIP-SHIP2 (*A* and *C*) and ΔIP-PTEN (*B* and *D*) at an MOI of 40 PFU/cell. Cells were serum starved for 16 h and subsequently treated with 17 nM insulin at 37°C for indicated times. Cells were immunoprecipitated with anti-Akt1 antibody (*A* and *B*) or anti-Akt2 antibody (*C* and *D*). Precipitates were separated by 7.5% SDS-PAGE and immunoblotted with anti-Thr$^{308/309}$-phospho-specific Akt antibody. Amount of Akt phosphorylated at Thr$^{308/309}$ corrected for total protein level was quantitated by densitometry. Results are means ± SE of 5 separate experiments. *$P < 0.05$ vs. amount of phosphorylated Akt in LacZ-transfected cells with respective insulin (Ins.) treatment.

treatment with TNF-α (Fig. 4). Pretreatment with TNF-α decreased the phosphorylation of Akt1 at Thr^{308} after insulin stimulation for 10 min (*lane 5* vs. *7*) but not for 120 min (*lane 9* vs. *11*). The TNF-α-induced decrease in insulin-induced phosphorylation of Akt1 at 10 min was not apparently affected by the expression of either ΔIP-SHIP2 (Fig. 4*A*) or ΔIP-PTEN (Fig. 4*B*). In contrast, the expression of either phosphatase-defective mutant enhanced insulin-induced phosphorylation of Akt1 at 120 min even after pretreatment with TNF-α (Fig. 3, *A* and *B*, and Fig. 4, *A* and *B*). On the other hand, pretreatment with TNF-α markedly inhibited the insulin-induced phosphorylation of Akt2 (Thr^{309} residue) at 10 min (*lane 5* vs. *7*) and also at 120 min (*lane 9* vs. *11*). The decreased phosphorylation of Akt2 by pretreatment with TNF-α was effectively ameliorated by the expression of ΔIP-SHIP2 (Fig. 4*C*) but not by the expression of ΔIP-PTEN (Fig. 4*D*). Similar results were ob-

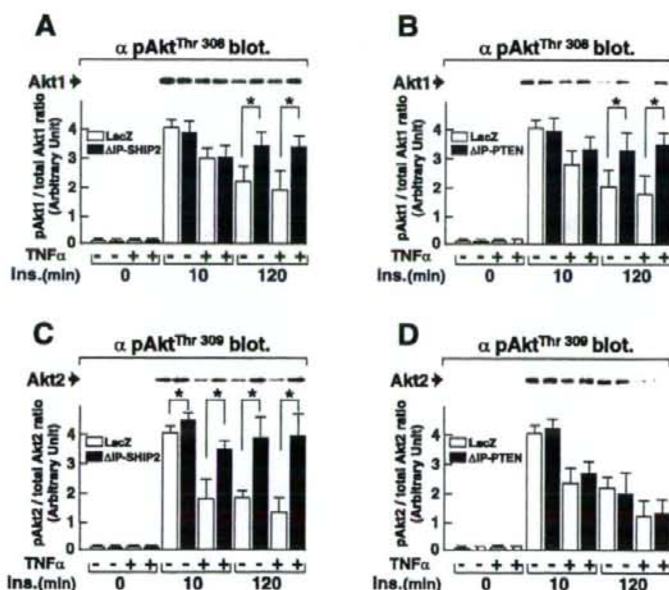

Fig. 4. Effect of phosphatase-defective SHIP2 and PTEN expression on TNF-α-induced decrease in phosphorylation of Akt. 3T3-L1 adipocytes were transfected with ΔIP-SHIP2 (*A* and *C*) and ΔIP-PTEN (*B* and *D*) at an MOI of 40 PFU/cell. Serum-starved transfected cells preincubated with 20 nM TNF-α for 16 h were treated with 17 nM insulin for 10 and 120 min. Cells were immunoprecipitated with anti-Akt1 antibody (*A* and *B*) or anti-Akt2 antibody (*C* and *D*). Precipitates were separated by 7.5% SDS-PAGE and immunoblotted with anti-Thr$^{308/309}$-phospho-specific Akt antibody. Amount of phosphorylated Akt at Thr$^{308/309}$ corrected for total protein level was quantitated by densitometry. Results are means ± SE of 4 separate experiments. *$P < 0.05$ vs. amount of phosphorylated Akt in LacZ-transfected cells with respective insulin treatment.

tained by the reciprocal immunoblotting studies with anti-Akt1 antibody or anti-Akt2 antibody after immunoprecipitation with anti-$Thr^{308/309}$ phospho-specific Akt antibody (data not shown). In addition, similar findings were again observed concerning the phosphorylation of Akt at $Ser^{473/474}$ residue (data not shown).

Expression of phosphatase-defective lipid phosphatases ameliorates insulin-induced phosphorylation of GSK3 and AS160 after TNF-α treatment. GSK3 and AS160 are important downstream molecules of Akt implicated in the metabolic action of insulin (3, 28, 37). We therefore investigated the ameliorative effect of the expression of phosphatase-defective lipid phosphatases on the decreased insulin-induced phosphorylation of GSK3 and AS160 by pretreatment with TNF-α (Fig. 5). Pretreatment with TNF-α decreased the phosphorylation of GSK3 and AS160 after insulin stimulation for 10 min (*lane 5* vs. *7*) and for 120 min (*lane 9* vs. *11*). TNF-α-induced decrease in insulin-induced phosphorylation of both GSK3 and AS160 was relatively more ameliorated by the expression of ΔIP-SHIP2 (Fig. 5, *A* and *C*) than that of ΔIP-PTEN (Fig. 5, *B* and *D*). These results indicate that the change of GSK3 and AS160 phosphorylations is relatively correlated with the phosphorylation of Akt2 rather than Akt1 by the expression of ΔIP-SHIP2.

Expression of phosphatase-defective lipid phosphatases does not affect insulin-induced degradation and tyrosine phosphorylation of IRS-1 after TNF-α treatment. We further examined whether expression of phosphatase-defective lipid phosphatases affects insulin-induced degradation and tyrosine phosphorylation of IRS-1 after TNF-α treatment, since TNF-α is an important adipokine that causes insulin resistance by facilitating the degradation of IRS-1 in 3T3-L1 adipocytes (14). As a result, insulin-induced tyrosine phosphorylation of IRS-1 is decreased after pretreatment with TNF-α (14). Consistent with these findings, pretreatment with TNF-α caused the degradation of IRS-1, and the subsequent tyrosine phosphorylation of IRS-1 after insulin treatment for 10 and 120 min was decreased. The expression of neither ΔIP-SHIP2 (Fig. 6*A*) nor ΔIP-PTEN (Fig. 6*B*) affected TNF-α- and insulin-induced degradation of IRS-1. Similarly, the suppressing effect of TNF-α on insulin-induced tyrosine phosphorylation of IRS-1 at 10 and 120 min was not affected by the expression of either ΔIP-SHIP2 (Fig. 6*C*) or ΔIP-PTEN (Fig. 6*D*). These results indicate that the expression of ΔIP-SHIP2 and ΔIP-PTEN ameliorates the decreased Akt-dependent signaling without affecting degradation of IRS-1. It is of note that treatment with TNF-α itself also did not affect the expression of endogenous SHIP2 and PTEN (data not shown).

Effect of expression of phosphatase-defective lipid phosphatases on insulin-induced glucose uptake after TNF-α treatment. We finally examined the effect of the expression of phosphatase-defective lipid phosphatases on insulin-induced 2-DG uptake in the presence or absence of TNF-α (Fig. 7). The expression of either ΔIP-SHIP2 or ΔIP-PTEN enhanced insulin-induced 2-DG uptake, although the former augmented it greater than the latter. Treatment with TNF-α inhibited 1.7- and 17-nM insulin-induced 2-DG uptake by 42.7 and 19.0%,

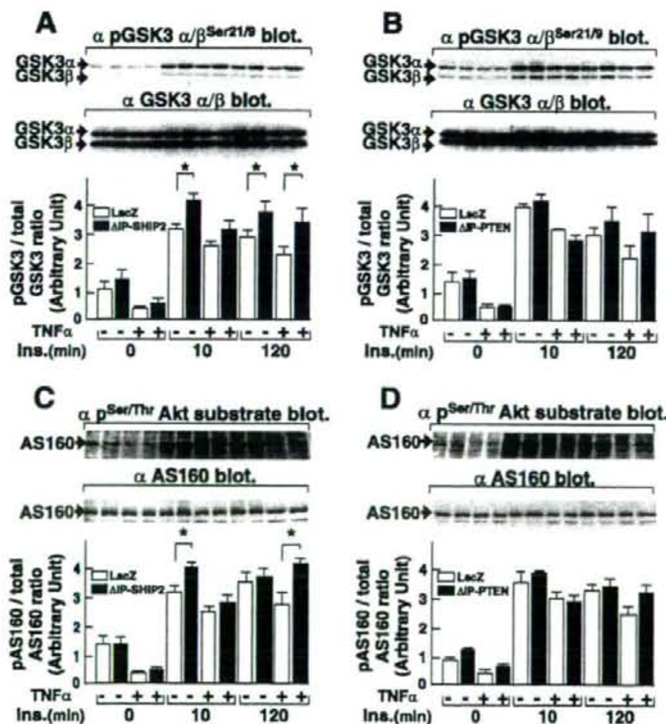

Fig. 5. Effect of phosphatase-defective SHIP2 and PTEN expression on TNF-α-induced decrease in phosphorylation of GSK3 and AS160. 3T3-L1 adipocytes were transfected with ΔIP-SHIP2 (*A* and *C*) and ΔIP-PTEN (*B* and *D*) at an MOI of 40 PFU/cell. Serum-starved transfected cells preincubated with 20 nM TNF-α for 16 h were treated with 17 nM insulin for 10 and 120 min. Total cell lysates were separated by 7.5% SDS-PAGE and immunoblotted with anti-$Ser^{21/9}$-phospho-specific GSK3α/β antibody (*A* and *B*), anti-GSK3α/β antibody (*A* and *B*), anti-Ser/Thr-phospho-specific Akt substrate antibody (*C* and *D*), or anti-AS160 antibody (*C* and *D*). Amount of phosphorylated GSK3 and AS160 corrected for total GSK3 and AS160 levels, respectively, was quantitated by densitometry. Results are expressed as means ± SE of 4 separate experiments. $^*P < 0.05$ vs. amount of phosphorylated GSK3 or AS160 in LacZ-transfected cells with respective insulin treatment.

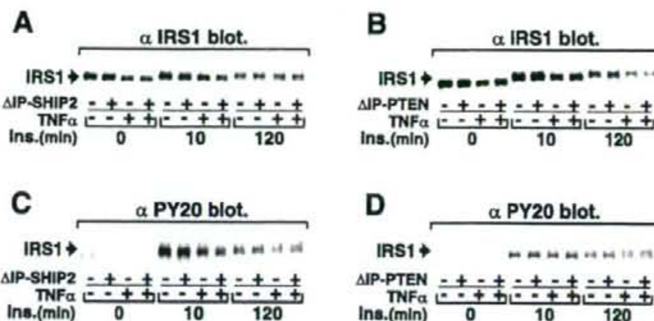

Fig. 6. Effect of phosphatase-defective SHIP2 and PTEN expression on insulin-induced degradation and tyrosine phosphorylation of IRS-1 after pretreatment with TNF-α. 3T3-L1 adipocytes were transfected with ΔIP-SHIP2 (*A* and *C*) and ΔIP-PTEN (*B* and *D*) at an MOI of 40 PFU/cell. Cells were serum starved for 16 h and subsequently pretreated with 20 ng/ml of TNF-α for 16 h. They were then treated with 17 nM insulin at 37°C for 10 and 120 min. Cells were lysed and separated by 7.5% SDS-PAGE and immunoblotted with anti-IRS-1 antibody (*A* and *B*) or anti-phosphotyrosine antibody (*C* and *D*). Amount of IRS protein and tyrosine-phosphorylated IRS-1 was quantitated by densitometry. Results are means ± SE of 4 separate experiments. $*P < 0.05$ vs. amount of IRS in LacZ-transfected cells with respective insulin treatment.

respectively. Interestingly, the expression of either ΔIP-SHIP2 or ΔIP-PTEN ameliorated the reduced 2-DG uptake by pretreatment with TNF-α to the control level. The amount of GLUT4 protein was not altered by treatment with TNF-α or the expression of both ΔIP-SHIP2 and ΔIP-PTEN (data not shown).

DISCUSSION

SHIP2 and PTEN are lipid phosphatases known to be involved in the negative regulation of insulin signaling in vivo and/or in vitro (4, 18, 20, 23, 33–35, 38, 39); however, the possible difference in characteristics and properties among these lipid phosphatases after short- vs. long-term insulin treatment is still unknown. Our previous reports (31) showed that SHIP2 predominantly regulates the phosphorylation of Akt2, but not Akt1, after short-term insulin treatment. Again, our current results showed that overexpression of WT-SHIP2 inhibited insulin-induced phosphorylation of Akt2 rather than Akt1 only after insulin treatment for up to 15 min. Interestingly, the effect was diminished after 60 and 120 min of insulin stimulation. Furthermore, the expression of ΔIP-SHIP2 enhanced insulin-induced phosphorylation of Akt2 after both short- and long-term insulin treatments, whereas phosphorylation of Akt1 was augmented only after 120 min of insulin treatment by the expression; therefore, the present results further clarified the characteristics of the regulation of insulin signaling by SHIP2, indicating that SHIP2 predominantly regulates the phosphorylation of Akt2 rather than Akt1 in a time-specific manner.

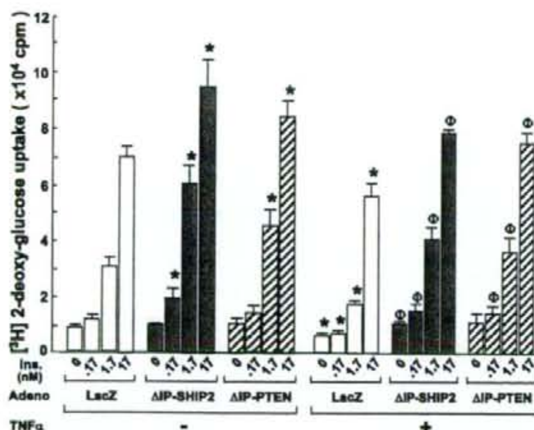

Fig. 7. Effect of phosphatase-defective SHIP2 and PTEN expression on TNF-α-induced inhibition of glucose uptake. 3T3-L1 adipocytes were transfected with ΔIP-SHIP2 or ΔIP-PTEN at an MOI of 40 PFU/cell. Serum-starved transfected cells pretreated with 20 nM TNF-α for 16 h were incubated in glucose-free medium for 30 min. After the cells had been stimulated with 10 nM insulin for 15 min, 3.7 kBq of 2-[^{3}H]deoxyglucose (2-[^{3}H]DG) were added for 4 min. Reaction was stopped by the addition of 10 μM cytochalasin B. Cells were washed 3 times with PBS and solubilized with 0.2 mM SDS-0.2 N NaOH. Radioactivity incorporated into the cells was measured with a liquid scintillation counter. Results are means ± SE of 5 separate experiments. $*P < 0.05$ vs. 2-[^{3}H]DG uptake in LacZ-transfected control cells with respective insulin treatment. $\Phi P < 0.05$ vs. 2-[^{3}H]DG uptake in TNF-α-treated cells with respective insulin treatment.

PTEN is a 3′-lipid phosphatase hydrolyzing $PI(3,4,5)P_3$ to $PI(4,5)P_2$ (20). It is reported that overexpression of WT-PTEN inhibited short-term insulin-induced activation of Akt, although the isoform-specific difference was not examined (22, 23, 35). On the other hand, the expression of ΔIP-PTEN did not affect short-term insulin-induced metabolic signaling, whereas the amount of $PI(3,4,5)P_3$ was increased in 3T3-L1 adipocytes (23). In contrast, depletion of PTEN protein by siRNA-mediated gene silencing enhanced short-term insulin-induced phosphorylation of Akt (35). The present studies showed that overexpression of WT-PTEN inhibited the phosphorylation of Akt1 and Akt2 after insulin stimulation for up to 5 min and that the effect was diminished thereafter. Our results clearly indicate that the effect of WT-PTEN expression is not specific to either Akt1 or Akt2 and is seen only after short-term insulin treatment. In addition, the expression of ΔIP-PTEN enhanced Akt2 phosphorylation, and only had a mild impact on augmenting the phosphorylation of Akt1 after 2 min of insulin treatment. Furthermore, ΔIP-PTEN expression enhanced the phosphorylation of Akt1, but not Akt2, after 120 min of insulin treatment. Taken together, PTEN appears to be implicated in the regulation of both short- and long-term insulin treatment-induced phosphorylation of Akt, whereas the Akt isoform-specific difference during regulation is ambiguous. SHIP2 is reported to translocate from cytosol to plasma membrane whereby phosphorylation of Akt2 is preferentially regulated upon insulin treatment (31), whereas the redistribution of PTEN is uncertain in 3T3-L1 adipocytes. The possible difference of subcellular redistribution between SHIP2 and PTEN may be a reason to cause an alteration in the isoform and temporal specific effects of Akt phosphorylation. Alternatively, it is possible that the stronger effect of SHIP2 on Akt2 and the

equivalent effects of PTEN on Akt1 and Akt2 reflect different properties of the pleckstrin homology (PH) domain of isoforms. Along this line, the PH domain of Akt2 may have higher affinity to $PI(3,4,5)P_3$ than $PI(3,4)P_2$, whereas the PH domain of Akt1 has similar affinities to both $PI(3,4,5)P_3$ and $PI(3,4)P_2$.

SHIP2 appears to be implicated in insulin resistance as a cause of type 2 diabetes in addition to the control of glucose homeostasis (4, 15, 16, 21, 33). SHIP2 knockout mice demonstrated enhanced phosphorylation of Akt in the skeletal muscle and liver, whereas whole body glucose homeostasis is not altered in mice fed a normal chow diet (33). However, the mice were protected from obesity and insulin resistance caused by a high-fat diet (33). Consistent with the report, the liver-specific inhibition of endogenous SHIP2 via the adenovirus-mediated expression of ΔIP-SHIP2 ameliorated glucose metabolism and insulin resistance in diabetic *db/db* mice and $KK\text{-}A^y$ mice (8, 9). In addition, muscle denervation is known to cause insulin resistance characterized by a decrease in the ability of insulin to stimulate glucose uptake and glycogen synthesis in rats (1). A reduction of SHIP2 expression using an antisense oligonucleotide against SHIP2 mRNA ameliorated insulin resistance in rats (1). Furthermore, insulin resistance caused by chronic insulin treatment was effectively ameliorated by the expression of ΔIP-SHIP2 (29). Taken together, inhibition of endogenous SHIP2 appears to be valuable in the amelioration of insulin resistance in type 2 diabetes. Concerning the pathological impact of PTEN in glucose homeostasis (10, 13), heterozygous deletion of the PTEN gene in IRS-2 knockout mice conferred protection from insulin resistance, although homozygous disruption of the PTEN gene in mice resulted in embryonic lethality (19). Antisense oligonucleotide-mediated inhibition of endogenous PTEN expression in the liver led to the amelioration of elevated glucose levels and decreased insulin sensitivity in diabetic *ob/ob* and *db/db* mice (2). Adipose tissue-specific knockout of PTEN is known to protect against streptozotocin-induced diabetes (18). Muscle-specific knockout of PTEN resulted in the amelioration of decreased insulin-induced phosphorylation of Akt in the soleus caused by high-fat feeding (39). Although tissue-specific inhibition of PTEN may also appear to be a therapeutic target in the treatment of type 2 diabetes with insulin resistance, the main role of PTEN is the regulation of cell growth and tumor suppressor (7, 18, 34, 39); therefore, care should be taken when inhibiting PTEN for therapeutic usage because of possible tumor formation. Taken together, it is important to clarify the different molecular mechanisms by which inhibition of these lipid phosphatases ameliorates the state of insulin resistance.

TNF-α is an important adipokine causing insulin resistance by impairing insulin signaling (14, 27, 40). In the present study, we clarified that treatment with TNF-α impaired insulin-induced phosphorylation of Akt2 more profoundly than Akt1 in 3T3-L1 adipocytes. Interestingly, the expression of dominant-negative SHIP2 and PTEN enhanced the phosphorylation of Akt1 induced by insulin treatment for 120 min together with TNF-α. The present results indicate that enhancement of PI3-kinase-dependent insulin signaling by the inhibition of either lipid phosphatase is sufficient to ameliorate insulin-induced phosphorylation of Akt. Interestingly, inhibition of SHIP2, but not PTEN, effectively restored the impaired phosphorylation of Akt2 caused by TNF-α treatment. Similarly, the impaired phosphorylation of GSK3 and AS160 was ameliorated relatively more effectively by inhibition of SHIP2 than that of PTEN. Since Akt2 rather than Akt1 is closely related to the control of glucose metabolism (5), inhibition of SHIP2 rather than PTEN might be a more suitable approach to ameliorate decreased metabolic signaling of insulin in the state of insulin resistance; however, inhibition of either SHIP2 or PTEN ameliorated reduced insulin-induced glucose uptake caused by TNF-α to the same extent. It is uncertain why the extent of amelioration in glucose uptake is similar, whereas inhibition of SHIP2 improved the phosphorylation of Akt2 more than PTEN. Amelioration of Akt1 phosphorylation may be sufficient to improve glucose uptake, at least under our experimental conditions. Alternatively, the $PI(3,4,5)P_3$-mediated pathway independent of Akt may regulate glucose uptake. It might be necessary to examine the effect of glucose uptake more precisely to dissect the ameliorative role of inhibition between SHIP2 and PTEN.

In summary, the present results indicate that SHIP2 predominantly regulates insulin-induced phosphorylation of Akt2 rather than Akt1 in a time-specific manner and that PTEN regulates insulin-induced phosphorylation of both Akt1 and Akt2, whereas isoform specificity is ambiguous in the regulation in 3T3-L1 adipocytes. In addition, the expression of the dominant-negative SHIP2 effectively ameliorated decreased phosphorylation of Akt2 caused by pretreatment with TNF-α, whereas the reduced phosphorylation of Akt1 was restored by the expression of either of these phosphatase-defective lipid phosphatases without affecting the degradation of IRS-1. Our results further extend the knowledge that inhibition of both SHIP2 and PTEN is an attractive approach to ameliorate the metabolic action of insulin in the state of insulin resistance, whereas inhibition of SHIP2 appears to have more impact than PTEN on the amelioration of Akt2 phosphorylation.

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Inter-organ metabolic communication involved in energy homeostasis: Potential therapeutic targets for obesity and metabolic syndrome

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Abstract

The global rate of obesity is rising alarmingly, exerting a major adverse impact on human health by increasing the prevalences of disorders, such as diabetes, hypertension and heart disease. To maintain systemic energy homeostasis, metabolic information must be communicated among organs/tissues. Obesity-related disorders can be thought of as resulting from dysregulation of this vital inter-tissue communication. Remarkable advances in obesity research during this decade have shown humoral factors manufactured and secreted by adipose tissue (adipocytokines) to be of great importance. In addition to these humoral factors, such as nutrients (glucose, fatty acids and amino acids) and hormones (insulin, adipocytokines and so on), the functional significance of the autonomic nervous system has recently attracted research attention. Autonomic nerves are essential components of the endogenous system for maintaining energy homeostasis, making them potential therapeutic targets for obesity-related disorders. This review focuses on the therapeutic possibilities of targeting inter-organ communication systems.
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Keywords: Obesity; Metabolic syndrome; Inter-organ communication; Energy homeostasis; Autonomic nervous system; Central nervous system

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1. Introduction

The incidence of obesity, a major risk factor for numerous disorders, including diabetes, hypertension and heart disease, is rising at an alarming rate in much of the world (Flier, 2004). Body weight is generally accepted to be determined by the balance between energy intake and expenditure. Normal weight individuals are reportedly protected against the expansion of body fat stores induced by overfeeding (Leibel et al., 1995), indicating the existence of biological mechanisms which protect against weight gain, as well as weight loss,

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at least in normal weight individuals. Energy homeostasis, maintained by multiple mechanisms, involves collecting information on systemic nutritional status and responding appropriately, both behaviorally and metabolically, to changes in fuel availability. Humoral factors, including insulin and adipocytokines, are known to be very important for this inter-organ/tissue communication. In addition, we and other investigators have recently demonstrated the autonomic nervous system to have a key role in transmitting metabolic information. Employing these systems, the brain gathers information on peripheral metabolic status, processes it, and then sends signals which regulate metabolism in the periphery. The hypothalamus, in particular, is a primary site of convergence and integration for redundant energy status signaling, which encompasses both central and peripheral neural inputs as well as hormonal and nutritional factors. These inter-tissue communication pathways are summarized in (Fig. 1; Yamada & Katagiri, 2007).

All but the most severe obesity cases can be successfully managed, solely through lifestyle modifications, i.e., improvements in diet and promotion of greater physical activity. However, low compliance with these strategies has generated interest in alternative effective therapies, including gastrointestinal bypass surgery (efficacious and long-lasting, but limited in use because of associated risks and costs) and pharmacological interventions. The market for safe and efficacious drugs is therefore potentially enormous, though the value of currently approved therapies does not reflect this potential, due to the limited efficacies and side-effect profiles of these treatments. This review summarizes our current understanding of the roles of inter-tissue communication in energy homeostasis and suggests potential therapeutic targets for obesity and the metabolic syndrome.

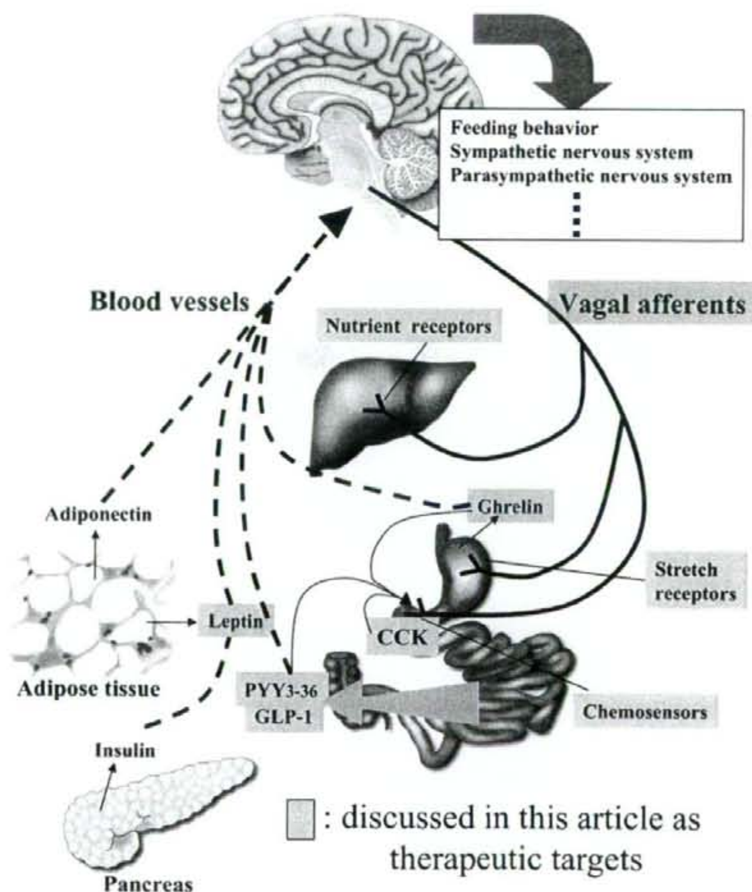

Fig. 1. Schematic presentation of intertissue communication (quoted with slight modification from Yamada & Katagiri, 2007). The brain receives various forms of metabolic information from peripheral organs/tissues through humoral and neuronal pathways. These inputs are probably integrated and processed in the brain, leading to appropriate systemic responses. Several signals, as therapeutic targets, are discussed in this article.

2. Neuroendocrine regulation of body weight and therapeutic implications for obesity

2.1. Brain inputs — humoral factors

2.1.1. Nutrients

The brain senses and then responds to nutrient-related signals arising from changes in intracellular energy contents or in either the availability or metabolism of substrates, such as free fatty acids. Some of these signals are generated in response to decreases in substrates, while others represent responses to nutrient excesses.

2.1.1.1. Glucose. In addition to serving as the primary fuel source for the brain, glucose metabolism in a subset of neurons (so-called "glucose-responsive" and "glucose-sensitive" neurons) generates signals that regulate membrane potential and neuronal firing. In glucose-responsive neurons, the molecular mechanism underlying this glucose effect resembles that, whereby glucose stimulates insulin secretion from pancreatic β cells, resulting in increased firing rates (Rowe et al., 1996). Such neurons have been characterized mainly in the ventromedial hypothalamic (VMH) nucleus and the arcuate (ARC) nucleus (Levin et al., 2004). Glucose metabolism in these cells activates KATP channels, allowing K^+ efflux, and thereby hyperpolarize the cells. KATP channel activity is a key step in converting metabolic changes into the electrical activity of ARC and VMH neurons (Wang et al., 2004). Interestingly, KATP channel activation by glucose in ARC glucose-responsive neurons is attenuated by insulin and leptin via a phosphatidylinositol 3OH kinase (PI3K)-dependent mechanism (Spanswick et al., 2000). Neither the underlying mechanism nor the extent to which these glucose-sensing neurons contribute to the actions of insulin and leptin, in neuroendocrine control of energy homeostasis, has as yet been determined.

In contrast, in glucose-sensitive neurons, firing is suppressed by glucose (Levin et al., 1999). Membrane potential effects mediated by tandem-pore K^+ (K_{2P}) channels were recently reported to be involved in glucose-induced inhibition of orexin neurons, a subset of glucose-sensitive neurons (Burdakov et al., 2006). In these neurons (in this case), glucose metabolism to ATP is not required. Thus, several types of potassium channels, including KATP and K_{2P} channels, are likely to play important roles in glucose sensing in a variety of neurons. However, the molecular mechanisms by which glucose suppresses firing in glucose-sensitive neurons is still largely unknown.

2.1.1.2. Free fatty acids. The access of circulating free fatty acids to cerebrospinal fluids is generally proportional to the plasma fatty acid concentration (Miller et al., 1987; Rapoport, 1996), indicating the brain to possibly acquire information about the peripheral metabolic state via cerebrospinal fluid fatty acid levels. Fatty acid-sensitive neurons have been identified in the hypothalamus. As an example, an *in vitro* patch clamp study (Wang et al., 2006) demonstrated 13% of arcuate neurons to show increased electrical activity, while 6% showed decreased activity, when oleic acid was applied. Several recent studies have examined the role of cerebrospinal fluid fatty acids in energy metabolism. Intracerebroventricular (i.c.v.) administration of oleic acid reportedly inhibits both hepatic glucose production and food intake (Obici et al., 2002). In addition, hypothalamic inhibition of carnitine/palmitoyl-coenzyme A transferase-1 (CPT-1), an important mitochondrial enzyme for transfer of long-chain fatty acyl-coenzyme A (LCFA-CoA) into mitochondria, decreases food intake and suppresses endogenous glucose production (EGP) in the liver (Obici et al., 2003). Efferent vagal nerve signals from the brain to the liver are also reportedly involved in hepatic gluconeogenesis in these experimental settings (Pocai et al., 2005a, 2005b). Hu et al. found that central administration of C75, a potent fatty acid synthase (FAS) inhibitor, decreased food intake (Hu et al., 2003). Since FAS inhibition increases malonyl-CoA and thereby suppresses CPT1 activity, LCFA-CoA in hypothalamic neurons would appear to be increased. These results, taken together, indicate the cytoplasmic LCFA-CoA concentration in hypothalamic neurons to play an important role in energy homeostasis. Further studies are needed to clarify the mechanisms regulating the neuronal LCFA-CoA content, its relationship to plasma free fatty acid (FFA) levels and the intracellular mechanism whereby a change in the LCFA-CoA content alters neuronal function.

2.1.1.3. Amino acids. Amino acids also apparently transmit energy status information from the periphery. Amino acids are reportedly transported across the blood–brain barrier (Choi et al., 2001). Therefore, the amino acid levels in cerebrospinal fluids appear to reflect those in peripheral blood. Centrally administered leucine increases hypothalamic mammalian target of rapamysin (mTOR) activity, thereby decreasing both food intake and body weight (Cota et al., 2006). mTOR is a highly conserved serine/threonine kinase found in organisms from yeast to mammals. mTOR activity sensitive to branched chain amino acid levels, especially that of L-leucine (Proud, 2002; Meijer & Dubbelhuis, 2004). Thus, mTOR is known to be among the energy sensors for amino acids conserved throughout evolution and, in mammals, hypothalamic mTOR signaling apparently plays an important role in regulating systemic energy metabolism. Leptin increases hypothalamic mTOR activity, and inhibition of mTOR signaling suppresses leptin's anorectic effect (Cota et al., 2006). However, further studies are needed to fully clarify mTOR's role in energy homeostasis.

2.1.2. Hormonal signals

2.1.2.1. Insulin. Insulin, produced by pancreatic β cells, is the master metabolic switch between fed and fasted states, mediating metabolic fuel disposition and use. Some investigators speculate that insulin itself might signal fuel status to the brain, but the actual mechanisms by which insulin would exert such effects have long eluded clarification.

An electrophysiological study showed inhibitors of PI3K to block the capacity of insulin to hyperpolarize hypothalamic "glucose-responsive" neurons (Spanswick et al., 2000). A subsequent in vivo study showed i.c.v. infusion of PI3K inhibitors to effectively prevent insulin-induced anorexia (Niswender et al., 2003). Furthermore, activation of insulin signaling in the ARC

alone, in the absence of elevated systemic insulin, is sufficient to decrease not only food intake but also blood glucose levels, by markedly inhibiting EGP in the liver (Plum et al., 2006; Prodi & Obici, 2006). A recent study revealed the central effects of insulin on this hepatic EGP suppression to be mediated by KATP channel activation through the insulin receptor (IR)–insulin receptor substrate 2 (IRS2)–PI3K pathway in the ARC (Pocai et al., 2005a, 2005b). Thus, intracellular insulin signal transduction in the brain, particularly in the hypothalamic ARC nucleus, plays an important role in regulating food intake, as well as in systemic glucose metabolism.

2.1.2.2. Leptin. Leptin was identified by positional cloning using the *ob/ob* mouse model (Zhang et al., 1994) as a key molecule in the regulation of both body weight and energy balance. Leptin is produced mainly by adipocytes in proportion to fat stores. Adipocyte leptin expression is transcriptionally regulated, being determined mainly by adipocyte size. Adequate leptin levels communicate the status of energy stores in white adipose tissue (WAT) to the central nervous system (especially the hypothalamus), suppressing food intake and permitting energy expenditure via sympathetic stimulation of several tissues (Haynes et al., 1997; Friedman & Halaas, 1998). As an example, when energy stores increase, the energy balance is negatively regulated by decreased food intake and increased energy expenditure (Friedman & Halaas, 1998). Leptin binds to the leptin receptor Ob-Rb in the hypothalamus, thereby activating the JAK-STAT (Bjorbaek et al., 1997; Bates & Myers, 2004) and IRS2-PI3K (Niswender et al., 2001) pathways. Leptin also suppresses hypothalamic AMPK activity and thus reduces food intake (Minokoshi et al., 2004). As described above, leptin also activates mTOR signaling in the hypothalamus. Thus, there appear to be complicated interactions among the (at least) 4 pathways, JAK-STAT, IRS2-PI3K, AMPK and mTOR, involved in leptin signaling.

In most individuals with ordinary obesity, circulating leptin is elevated, but the body does not adequately respond to higher leptin levels by reducing food intake. This lack of responsiveness to leptin in most forms of obesity raises the possibility that obesity is a state of relative leptin resistance. Leptin resistance is thought to be an important mechanism for maintaining the obese state.

2.2. Brain inputs — afferent nerve signals

2.2.1. Innervation

2.2.1.1. Intra-abdominal innervation without white adipose tissues. Innervation of intra-abdominal tissues warrants an explanation. Intra-abdominal tissues are innervated by both splanchnic (sympathetic) and vagal (parasympathetic) nerves. These nerve bundles consist of both efferent and afferent fibers. Detailed fiber count studies have revealed abdominal vagal and splanchnic nerves to be comprised of approximately 75% and 50% afferent fibers, respectively. Vagal afferents respond to specific chemical stimuli, the degree of physiological gut distention and nutrients, whereas splanchnic afferents carry information about noxious stimuli (Badman & Flier, 2005).

2.2.1.2. Innervation of intra-abdominal adipose tissues. WAT is also innervated by both efferent and afferent nerve fibers. Numerous reports have described the important metabolic roles, including lipolysis and β oxidation (Shimazu, 1981; Bartness & Bamshad, 1998; Imai et al., 2006), of efferent sympathetic fibers. Efferent parasympathetic innervation of WAT is controversial (Kreier et al., 2002; Giordano et al., 2006). On the other hand, afferent nerves from WAT have been demonstrated by several methods. Sensory innervation of WAT was directly demonstrated using a neuroanatomical approach with application of an anterograde tract tracer, True Blue, to WAT, resulting in labeling of neurons in rat dorsal root ganglia (Fishman & Dark, 1987). More recently, afferent innervation of epididymal WAT was demonstrated by another group using the pseudorabies virus as a retrograde neuronal tracer (Kreier et al., 2006).

2.2.2. Signals transmitted by afferent autonomic nerve fibers

2.2.2.1. Signals from the gut. Many peptides are synthesized and released by the gastrointestinal tract. Several of these peptides, such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and ghrelin, have been shown to modulate eating behaviors, (Woods & Gibbs, 1989; Stanley et al., 2004; Woods, 2004). Several of these peptides have direct access to brain regions involved in regulating food intake, such as the ARC of the hypothalamus and the area postrema, via the circulation. These peptides also function outside the CNS, influencing the activities of neurons, e.g., the vagal afferent nerve which projects to the nucleus of the solitary tract (NTS) in the brain stem. Further research is needed to determine the weighting and integration of each of these different signals.

2.2.2.1.1. CCK. CCK, produced by mucosal enteroendocrine cells of the duodenum and jejunum, is secreted in response to the presence of food in the gut lumen. Satiating effects of CCK have been confirmed based on the carboxy-terminal octapeptide of CCK reducing meal size and duration (Pi-Sunyer et al., 1982). Pharmacologic and genetic experiments have yielded evidence that the CCK1 receptor mediates CCK-induced satiation (Moran et al., 1998; Kopin et al., 1999). Sulfated CCK, which preferentially binds to CCK1R on vagal afferent neurons, signals satiety to the brain; this explains why vagotomy inhibits the anorectic effect of CCK (Smith et al., 1981). However, CCK1R is also expressed in both the hindbrain and the hypothalamus. Lesioning the hindbrain area postrema attenuates CCK-induced satiation (Edwards et al., 1986) and CCK microinjections into several hypothalamic nuclei lead to decreased food intakes (Blevins et al., 2000). Collectively, these observations suggest that CCK might relay satiation signals to the brain both directly and indirectly.

Continuously administering intraperitoneal CCK to rats results in reduced meal sizes, but this reduction is offset by increased meal frequency, such that there is no effect on body weight (West et al., 1984). In human subjects, food intake and gastric emptying were acutely reduced by CCK infusions (Muurahainen et al., 1988), but these anorectic effects disappeared after only 24 hr of continuous infusion (Crawley & Beinfeld, 1983). Therefore, although CCK clearly plays a role in terminating individual

meals, it appears to have little impact on long-term body weight regulation and thus seems unlikely to be an antiobesity drug target.

2.2.2.1.2. PYY3-36. The secretion of PYY3-36 from enteroendocrine L cells is triggered by luminal nutrients. Sugars activate L cells via the closure of ATP-sensitive potassium channels, which in turn leads to depolarization of the L cells via a mechanism analogous to insulin secretion from β cells (Reimann & Gribble, 2002; Gribble et al., 2003). PYY1-36 binds to all known Y receptors with similar affinities. In contrast, most circulating PYY immunoreactivity is in the amino-terminally truncated form, PYY3-36, which preferentially binds to Y2 receptors (Y2R). In the hypothalamus, Y2R is a presynaptic autoinhibitory receptor on orexigenic neurons expressing both NPY and agouti-related protein (AgRP), known as NPY/AgRP neurons. This has lead to the proposal that circulating PYY3-36 reduces food intake by inhibiting NPY/AgRP neurons through Y2R, thereby activating anorectic melanocortin-producing cells, which are inhibited by NPY/AgRP neurons (Batterham et al., 2002). Consistent with this model, the anorectic effects of PYY3-36 can be abolished by either pharmacologic or genetic ablation of Y2R (Batterham et al., 2002; Scott et al., 2005; Talsania et al., 2005). Though these lines of evidence support a hypothalamic mechanism of action for peripherally administered PYY3-36, Y2R is also expressed by vagal-afferent terminals (Koda et al., 2005), and some investigators have speculated that vagal mediation also exists. Supporting this hypothesis, the anorectic effects and arcuate neuronal activation induced by peripheral PYY3-36 were demonstrated to be eliminated by either subdiaphragmatic vagotomy or transection of hindbrain-hypothalamic pathways (Abbott et al., 2005; Koda et al., 2005). Assessed collectively, these observations suggest that PYY3-36 might also relay satiation signals, both direct and indirect, to the brain.

Therapeutic possibilities. Peripheral administration of PYY3-36 reduces food intake, and thereby body weight, in rodents, and these effects may be even more robust in primates (Moran et al., 2005), apparently due to activation of autoinhibitory Y2R on hypothalamic orexigenic NPY/AgRP neurons. In addition, recent evidence indicates vagal mediation of one component of PYY3-36-induced anorexia as described above. Intravenous infusion of a single dose (Batterham et al., 2003), as well as graded infusions (Degen et al., 2005) of PYY3-36, reportedly reduce appetite and food consumption by >30% in lean and obese subjects, although several investigators have encountered difficulties in attempting to reproduce these effects (Boggiano et al., 2005). It is noteworthy that obese subjects show normal sensitivity to the anorectic effects of PYY3-36, and circulating PYY levels are not elevated in the obese, in contrast to those of leptin (Batterham et al., 2003). The injectable PYY3-36 analogue AC-162352 was tested in phase I studies, with limited success due to nausea (Halford, 2006). In a phase I clinical trial as a nasally administered obesity treatment, on the other hand, PYY3-36 was both safe and well tolerated, and there was evidence of reduced caloric intake, appetite moderation and a tendency for weight loss in human subjects (Halford, 2006).

2.2.2.1.3. Glucagon-like peptide-1. GLP-1 is produced mainly by L cells located in the distal small intestine and colon, where it is colocalized with PYY. Ingested nutrients, especially fats and carbohydrates, stimulate GLP-1 secretion by indirect, duodenally activated neurohumoral mechanisms, as well as via direct contact within the distal intestine (Brubaker & Anini, 2003). The 2 equipotent bioactive forms, GLP-17-36 amide and GLP-17-37, are rapidly inactivated in the circulation by dipeptidyl peptidase-IV (DPP-IV; Orskov et al., 1993). GLP-1 has been shown to suppress food intake in several species (Turton et al., 1996; Donahey et al., 1998), including humans (Verdich et al., 2001). The mechanisms underlying GLP-1-induced anorexia are not fully understood but are believed to involve vagal and possibly direct central pathways. The GLP-1 receptor is expressed by organs/tissues, including the gut, pancreas, brainstem, hypothalamus and vagal-afferent nerves (Drucker, 2006). The anorectic effects of peripheral GLP-1 administration were shown to be abolished by vagotomy (Abbott et al., 2005; Talsania et al., 2005). Thus, peripheral GLP-1 also signals satiety to the brain via the vagal afferent pathway.

Therapeutic possibilities. Chronic subcutaneous GLP-1 administration for 6 weeks to obese subjects with type 2 diabetes led to a 1.9-kg body weight loss, on average (Zander et al., 2002). Given that the native GLP-1 peptide undergoes rapid enzymatic inactivation, DPP-IV-resistant GLP-1 analogues have attracted considerable attention as potential treatments for type 2 diabetes complicated by obesity. Exendin-4 is a naturally occurring 39 amino acid GLP-1 receptor agonist originally isolated from the venom of the *Heloderma suspectum* lizard (Eng et al., 1992). Exendin-4 has a glycine residue, which confers resistance to cleavage by DPP-IV, at position 2. In clinical trials, twice-daily subcutaneous administration of exendin-4 in patients with type 2 diabetes produced a dose-dependent weight loss of 1.8 kg during a period of 28 days (Poon et al., 2005) and 2.8 kg over 30 weeks (DeFronzo et al., 2005). Thus, in addition to promoting insulin secretion (incretin effects), the anorectic effects of GLP-1 agonists have attracted attention as possible diabetes treatments. This is because the improvements in glycemic control achieved with other oral glucose-lowering drugs typically promote weight gain.

2.2.2.1.4. Ghrelin. Ghrelin, a peptide recently found to be produced by the stomach, acts on a previously identified orphan receptor (growth hormone [GH] secretagogue receptor), the activation of which in the hypothalamus triggers the pituitary gland to release GH (Kojima et al., 1999). Ghrelin increases food intake in diverse species (Tschop et al., 2000), including humans (Wren et al., 2001). Date et al. (2002) reported gastric vagal afferent blockade to abolish ghrelin-induced feeding increases, GH secretion and the activations of NPY- and GH-releasing hormone (GHRH)-producing neurons. The ghrelin receptor is also expressed in vagal afferent terminals, and ghrelin suppresses vagal afferent firing. These findings, taken together, indicate gastric vagal afferent involvement in conveying signals regarding starvation, as well from the gut to the brain.

Therapeutic possibilities. In humans, feelings of hunger and food intake are both increased by either intravenous infusion or subcutaneous injection of ghrelin (Kojima & Kangawa, 2005). Therefore, blocking ghrelin signaling with ghrelin receptor antagonists has attracted interest as a possible strategy for preventing obesity. A ghrelin receptor antagonist reportedly

reduced food intake in fasted mice and an RNA Spiegelmer (an L-oligonucleotide designed to bind specifically to a particular molecule) inhibited ghrelin action both in vitro and in vivo (Cummings, 2006). It was recently demonstrated that vaccinating rats against ghrelin can suppress weight gain (Zorrilla et al., 2006). However, in obese individuals,ghrelin levels are low but rise in response to weight loss (Kojima & Kangawa, 2005). This is apparently part of a compensatory response that promotes weight regain. Therefore, the most clinically useful application of ghrelin receptor blockade might be in the prevention of rebound after weight reduction, which has been achieved by other means, rather than for initiating weight reduction de novo.

2.2.2.2. Signals from the liver

2.2.2.2.1. Hepatoportal glucose sensor. Sensor of short-term alterations in energy status. Blood glucose levels rise postprandially and decrease while fasting. Therefore, blood glucose concentrations reflect short-term energy status alterations. Glucose absorbed from the gut enters the portal vein, thereby reaching the liver directly. Thus, given its anatomical location, it is reasonable to assume that the liver functions as a glucose sensor. The hepatoportal glucose sensor is as yet incompletely defined, but reportedly consists of several components including GLUT2 (Burcelin et al., 2000a), as well as the GLP-1 receptor (Burcelin et al., 2001). Glucose entry into the hepatoportal vein triggers the activation of glucose sensors (Hevener et al., 1997), which can induce anorexia (Russek, 1963, 1970) and stimulate glucose uptake by the liver (Gardemann et al., 1986; Cardin et al., 1999), muscle, heart and brown adipose tissue (Burcelin et al., 2000b). Raising the portal vein glucose concentration decreases vagal afferent discharges reaching the NTS nuclei (Thorens & Larsen, 2004), indicating signals regarding portal glucose elevation to be carried along afferent vagal pathways. Hypoglycemic signals from the hepatoportal system, in contrast, involve splanchnic afferents. Reportedly, a counter-regulatory response to moderate systemic hypoglycemia, i.e., sympathetic efferent activation, is attenuated by clamping the liver at euglycemic levels and is blocked when splanchnic (but not vagal) afferents from the hepatic portal structure are interrupted (Donovan et al., 1991; Fujita & Donovan, 2005).

These observations, when considered collectively, indicate that the afferent autonomic nervous system, including both vagal and splanchnic nerves, from the hepatoportal circulation plays important roles in conveying information about peripheral glucose levels to the brain.

2.2.2.2.2. Peroxisome proliferator-activated receptors. Sensor of long-term alterations in energy status. Lipid mediators have key roles in metabolic control, and the peroxisome proliferator-activated receptor (PPAR) have emerged as the master transcriptional regulators of long-term lipid and carbohydrate metabolism (Desvergne et al., 2006). Saturated and unsaturated long-chain fatty acids and their eicosanoid derivatives are natural activators of this important subclass of nuclear receptors (Feige et al., 2006). Studies using mice with tissue-specific knockout of PPARγ have shown these receptors, in a number of organs, to function as a sensor of long-term energy status alterations. Notably, liver-specific disruption of PPARγ in ob/ob mice prevented hepatic steatosis, although a gradual increase in peripheral adiposity as well as decreases in the insulin sensitivities of muscle and adipose tissue were observed (Matsusue et al., 2003). In addition, hepatic expression of PPARγ, especially that of PPARγ2, is functionally enhanced in a number of obesity models (Chao et al., 2000; Rahimian et al., 2001). Therefore, hepatic PPARγ appears to play important roles not only in hepatic lipid storage but also in the regulation of both peripheral lipid metabolism and insulin sensitivity. The mechanism underlying this inter-organ/tissue communication between the liver and peripheral tissues, including muscle and fat, was recently revealed to involve autonomic nerve circuits (Uno et al., 2006).

The roles of hepatic PPARγ2 in peripheral metabolism were confirmed experimentally. Adenovirus-mediated PPARγ2 expression in the liver was shown to acutely induce severe hepatic steatosis, while peripheral adiposity was greatly reduced due to enhanced lipolysis. Systemic metabolic rates rose, such peripheral insulin sensitivity, and glucose tolerance showed marked improvements. These remote effects were attributable to increased sympathetic outflow into muscle and adipose tissues. Selective hepatic branch vagotomy significantly reversed both the peripheral adiposity reduction and the enhanced energy expenditure. Furthermore, pharmacological deafferentation of the vagus blocked the hepatic PPARγ2 expression-induced decrease in WAT weights. These findings indicate that hepatic PPARγ2 expression and/or hepatic lipid accumulation triggers the communication of metabolic information to the brain via afferent vagal nerve fibers, leading to antiobesity and antiinsulin-resistant effects in both muscle and adipose tissue (Uno et al., 2006).

Lipid storage in the liver changes dynamically according to the systemic energy balance and is known to be associated with several features of the metabolic syndrome. The liver may convey information regarding excess long-term energy storage to the central nervous system via the afferent vagus. This neuronal system is likely to underlie so-called chronic "adaptive thermogenesis," protecting the organism against metabolic perturbation induced by excessive energy storage (Fig. 2). The brain receives information regarding this excess energy storage, via leptin from adipose tissues as well as via the afferent vagus from the liver, activates the sympathetic nervous system to enhance energy expenditure and lipolysis, and thereby maintains energy homeostasis (Uno et al., 2006). A similar autonomic nerve circuit was recently shown to play an essential role in the development of glucocorticoid-induced insulin resistance and hypertension (Bernal-Mizrachi et al., 2007).

In totality, these observations highlight the importance of the vagal afferent pathway not only in short-term nutrient status alterations, such as blood glucose concentrations, but also in long-term energy storage status alterations.

Therapeutic possibilities. A recent study found a low resting metabolic rate to predict susceptibility to obesity (Buscemi et al., 2005). Therefore, enhancing energy expenditure is a promising strategy for treating obesity. Physiological sympathetic activation might thus be feasible, because it leads to relatively selective loss of fat, followed by improvements in insulin sensitivity beyond what would be expected from body weight reduction.

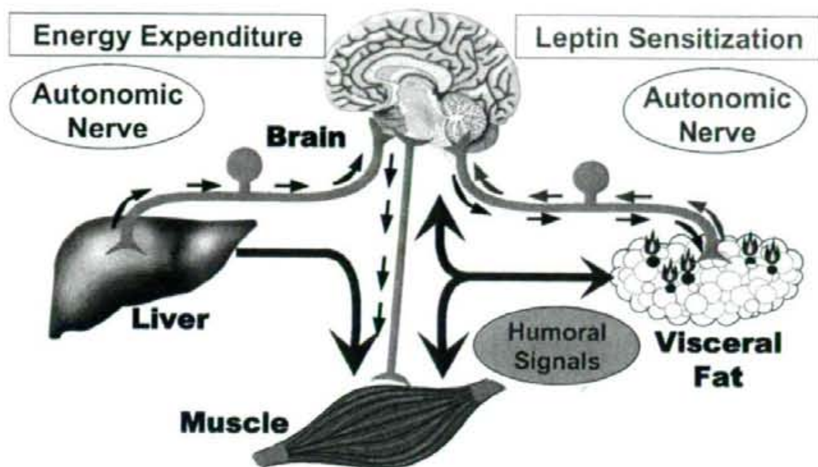

Fig. 2. Scheme of the neuronal pathways involved in energy homeostasis. Neuronal signals from WAT modulate hypothalamic leptin sensitivity, thereby regulating feeding behavior. In addition, the liver transmits information regarding excess energy to the brain via the afferent vagus, thereby activating the sympathetic nervous system which in turn enhances energy expenditure and lipolysis. The autonomic nervous system, as exemplified by these neuronal circuits, plays important roles in regulating energy metabolism.

Sibutramine, a serotonin-norepinephrine reuptake inhibitor, which is used clinically as an antiobesity drug, apparently increases sympathetic activity, though whether this would contribute in any way to weight loss in humans is unclear (Luque & Rey, 2002). As noted above, the autonomic nerve circuit consisting of the afferent vagus nerve and efferent sympathetic nervous system has a physiological antiobesity function exerted through enhanced energy expenditure. Therefore, in addition to reagents acting directly on the brain, activators of this peripheral nervous system are potential antiobesity drug targets. Elucidation of the underlying molecular mechanisms, including mediators influencing vagal activity, could lead to new therapeutic approaches to obesity and the metabolic syndrome. However, sibutramine raises both heart rate and blood pressure. Caution is necessary when any novel agents raises energy expenditure, because these effects may simply be due to an overall increase in systemic sympathetic activity.

The sympathetic nervous system reportedly activates uncoupling protein-1 (UCP-1) expression and activity. UCP-1 is most abundantly expressed in brown adipose tissue and dissipates energy as heat. In addition, transgenic overexpression of UCP-1 in WAT has been reported to exert preventive effects against the development of both genetic and dietary obesity, as well as the associated insulin resistance, in mice (Kopecky et al., 1995, 1996). Furthermore, hepatic induction of UCP-1 protein in mice with dietary obesity improves both diabetes and obesity by exerting local effects in the liver as well as remote effects in adipose tissues, muscle and the hypothalamus (Ishigaki et al., 2005). In contrast, in lean mice fed a standard diet, hepatic UCP-1 expression had little impact on either glucose or lipid metabolism and no cachectic phenotypes were observed (Ishigaki et al., 2005). These observations suggest UCP-1 to possibly be an attractive therapeutic target for both obesity and the metabolic syndrome.

2.2.2.3. Signals from adipose tissues. Few reports have focused on afferent nerve signals from adipose tissues. Niijima (1998) and Tanida et al. (2000) used electrical firing measurements to demonstrate that leptin induces functional activation of afferent nerve fibers from epididymal WAT. The functional roles of these afferent nerves in food intake regulation has been recently shown (Yamada et al., 2006).

Fat accumulation in intra-abdominal adipose tissue plays a major role in development of the metabolic syndrome, which is associated with insulin as well as leptin resistance. As described above, leptin resistance is induced by excessive adiposity and, in turn, is an important mechanism for maintaining the obese state. UCP-1 induction in restricted portions of epididymal adipose tissue, even at very low levels, dramatically improves hypothalamic leptin resistance without altering adiposity and thereby decreases food intake. Locally dissecting nerves from the epididymal fat pad and pharmacological deafferentation blunted these anorectic effects of UCP-1 expression in adipose tissue. Thus, afferent nerve signals originating in epididymal fat pads were shown to modulate hypothalamic sensitivity to leptin (Yamada et al., 2006; Fig. 2). In addition, the involvement of afferent nerves from WAT in adiposity was suggested by the observation that localized selective sensory denervation, achieved by microinjecting capsaicin bilaterally into epididymal WAT of Siberian hamsters, produced increases in other intraabdominal fat masses (Shi & Bartness, 2005).

Adipose tissues were long regarded as simply being passive fuel storage sites. However, the discovery of various adipocytokines, with leptin being the most important example, has raised adipose tissue to the status of a versatile endocrine gland. In addition, these aforementioned studies provide further evidence that adipose tissue serves as a base, sending out neuronal signals regulating feeding and energy storage.

Therapeutic possibilities. From the therapeutic perspective, the mechanism underlying leptin resistance is an important issue awaiting clarification, though two hypotheses have received considerable attention. One involves a failure of circulating leptin to arrive at its targets in the brain. Leptin is normally transported across the blood brain barrier by a saturable transport system, and the activity of this system has been shown to be impaired in obese subjects (Schwartz et al., 1996). Intranasal delivery of leptin can reportedly overcome this barrier and thereby produce weight loss in rats (Fliedner et al., 2006).

Another important observation is that, independently of blood–brain transit, intracellular leptin-receptor signaling is blunted in brain areas critical to energy homeostasis in the setting of diet-induced obesity, such that neuronal leptin responsiveness is diminished even when leptin is directly injected into the brain (El-Haschimi et al., 2000). In fact, several studies support potential roles of two molecules, suppressor of cytokine signaling-3 (SOCS3; Bjorbaek et al., 1998) and protein tyrosine phosphatase-1B (PTP1B; Cheng et al., 2002; Zabolotny et al., 2002), in the inhibitory regulation of Ob-Rb signaling both in vitro and in vivo. Although hypothalamic PTP1B levels do not appear to be altered in obesity, SOCS3 expression is increased in several rodent models of leptin-resistant obesity, which is consistent with the potential role of SOCS3 in leptin resistance (Bjorbaek et al., 1998; Munzberg et al., 2004). Moreover, ablation of SOCS3 activity by employing neuron-specific conditional knockout increases leptin-induced activation of intracellular signaling events and catabolic neuropeptide expressions, associated with enhancement of the weight-reducing effects of leptin and resistance to diet-induced obesity (Howard et al., 2004; Mori et al., 2004). PTP1B also inactivates the leptin receptor via dephosphorylation of its key tyrosine residues, which are phosphorylated in response to ligand binding (Cheng et al., 2002; Zabolotny et al., 2002). Global and neuron-specific PTP1B knockout mice are lean, resistant to diet-induced obesity, and insulin sensitive, all of which result more from increased energy expenditure than decreased food intake (Elchebly et al., 1999; Klaman et al., 2000; Bence et al., 2006). Therefore, SOCS3 and PTP1B are potential therapeutic targets for leptin resistance. Caution is warranted, however, since SOCS3 and PTP1B regulate more than just leptin signaling.

As stated above, neuronal signals from intraabdominal adipose tissue modulate hypothalamic leptin sensitivity (Yamada et al., 2006). Activation of this novel neuronal pathway is a possible therapeutic strategy against obesity and the metabolic syndrome. Elucidating the molecular mechanism(s) underlying this pathway, including identification of the neurotransmitters involved and their receptors, might lead to the development of novel therapeutic strategies, tackling the metabolic syndrome via improved leptin resistance.

3. Epilogue

Metabolism is not a process carried on independently in different organs/tissues, but rather is coordinated and regulated throughout the body. The coordination of metabolic regulation among organs/tissues, which requires communication among these organs/tissues, is apparently essential for maintaining the homeostasis of systemic metabolism, especially glucose and energy metabolism. In addition, disturbances of this coordinated control system may be involved in the development of metabolic disorders, including obesity, type 2 diabetes, hyperlipidemia, and the metabolic syndrome.

Recent research advances have revealed the complex and important roles played by the central nervous system. The brain obtains an abundance of metabolic information from peripheral organs/tissues through humoral and neuronal avenues. In addition, these signals interact, as exemplified by adiponectin expressions being regulated by sympathetic activity (Imai et al., 2006). These inputs are most likely integrated and processed in the brain, leading to the transmission of regulatory signals, which then induce appropriate systemic metabolic responses (Katagiri et al., 2007). Elucidation of these regulatory systems, in far greater detail, may reveal the mechanisms underlying metabolic homeostasis and thereby allow us to understand the complex metabolic disorders that result from perturbation of these systems.

Although life-style change is widely accepted as the first-line treatment for obesity and the metabolic syndrome, in the actual clinical setting, the multiple risks associated with obesity do not normalize with efforts aimed at life-style changes alone. Unfortunately, the existing pharmacological treatments for obesity, which might be used to ameliorate the risks associated with obesity, provide limited efficacy. This lack of efficacy is often further compounded by unacceptable side-effects. Concern about the safety of centrally acting drugs is one reason that pharmaceutical companies are currently seeking alternative obesity treatments. Targeting inter-tissue/organ communication in energy homeostasis might offer advantages in exploiting natural regulatory circuits while minimizing unwanted side effects. Diet and exercise remain the undisputed cornerstones of obesity therapy. However, more effective medications, designed to augment the impacts of these efforts, would be welcomed by obese individuals.

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Cell Metabolism
Short Article

ATF4-Mediated Induction of 4E-BP1 Contributes to Pancreatic β Cell Survival under Endoplasmic Reticulum Stress

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SUMMARY

Endoplasmic reticulum (ER) stress-mediated apoptosis may play a crucial role in loss of pancreatic β cell mass, contributing to the development of diabetes. Here we show that induction of 4E-BP1, the suppressor of the mRNA 5′ cap-binding protein eukaryotic initiation factor 4E (eIF4E), is involved in β cell survival under ER stress. 4E-BP1 expression was increased in islets under ER stress in several mouse models of diabetes. The *Eif4ebp1* gene encoding 4E-BP1 was revealed to be a direct target of the transcription factor ATF4. Deletion of the *Eif4ebp1* gene increased susceptibility to ER stress-mediated apoptosis in MIN6 β cells and mouse islets, which was accompanied by deregulated translational control. Furthermore, *Eif4ebp1* deletion accelerated β cell loss and exacerbated hyperglycemia in mouse models of diabetes. Thus, 4E-BP1 induction contributes to the maintenance of β cell homeostasis during ER stress and is a potential therapeutic target for diabetes.

INTRODUCTION

Recent studies have shown decreased pancreatic β cell mass to be a common feature of subjects with type 2 diabetes mellitus (Butler et al., 2003). Susceptibility to stress-induced apoptosis may underlie β cell loss. Translational regulation is an essential strategy by which cells cope with stress conditions (Clemens, 2001). Translation of eukaryotic mRNA is regulated primarily at the level of initiation. Translational initiation begins with formation of a ternary complex composed of the methionine-charged initiator tRNA, eukaryotic initiation factor 2 (eIF2), and GTP (Holcik and Sonenberg, 2005). The ternary complex then binds to the 40S ribosomal subunit and several other initiation factors, generating the 43S preinitiation complex. The mRNA 5′ cap-binding protein eIF4E associates with eIF4A and eIF4G to form the eIF4F complex and interacts with the 5′ cap structure of the mRNA. The eIF4F complex then recruits the 43S preinitiation complex to the mRNA, allowing the complex to scan toward the initiator AUG codon. The two best characterized regulatory steps in this translational control are formation of the ternary complex and assembly of the eIF4F complex. Phosphorylation of the α subunit of eIF2 (eIF2α) prevents ternary complex formation and thereby suppresses global translation. In addition, eIF4E-binding proteins (4E-BPs) inhibit eIF4F assembly by competitively displacing eIF4G from eIF4E. Global translational suppression through eIF2α phosphorylation is a mechanism shared among different stress-response pathways. Depending on the nature of the stress stimulus, eIF2α can be phosphorylated by four different kinases (Holcik and Sonenberg, 2005). Global attenuation of protein biosynthesis then paradoxically increases expression of several proteins, including the transcription factor ATF4 (Harding et al., 2000).

Because of their high insulin secretory activity, β cells are vulnerable to endoplasmic reticulum (ER) stress, a condition of disrupted ER homeostasis due to accumulation of misfolded proteins (Schroder and Kaufman, 2005). Cells respond to ER stress by activating an adaptive cellular response known as the unfolded protein response (UPR). Under ER stress conditions, global translation is suppressed through eIF2α phosphorylation by an ER-resident kinase, PERK. The importance of PERK-mediated translational suppression has been demonstrated in infancy-onset diabetes and skeletal defects caused by loss of PERK in humans (Delepine et al., 2000) and mice (Harding et al., 2001; Zhang et al., 2002). However, the roles of translational control through inhibition of eIF4F assembly by 4E-BPs under stress conditions, including ER stress, have yet to be fully clarified. Herein, we have studied roles of 4E-BP1,