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Insulin Facilitates the Hepatic Clearance of Plasma Amyloid β-Peptide (1–40) by Intracellular Translocation of Low-Density Lipoprotein Receptor-Related Protein 1 (LRP-1) to the Plasma Membrane in Hepatocytes

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ABSTRACT

The hepatic clearance of amyloid β-peptide (1–40) [Aβ(1–40)] from plasma, which is largely mediated by low-density lipoprotein receptor-related protein (LRP-1), is suggested to play a role in preventing Aβ(1–40) accumulation in the brain. Epidemiological investigations suggest a high incidence of cerebral Aβ deposition in insulin-resistant type II diabetes mellitus. The purpose of this study was to clarify the effect of insulin on the hepatic clearance of Aβ(1–40). LRP-1 expression on the hepatic plasma membrane was increased in a time-dependent manner by portal infusion of insulin and was 2.2-fold greater than that in nontreated controls after a 10-min infusion, whereas the expression in whole lysate was not affected by insulin treatment. The apparent hepatic uptake of [^{125}I]Aβ(1–40) was also induced by insulin in a time-dependent manner. The increase in [^{125}I]Aβ(1–40) uptake by insulin was concentration-dependent (EC_{50} = 230 pM) and was completely abolished by receptor-associated protein (2 μM), an LRP-1 inhibitor. In conclusion, plasma insulin facilitates LRP-1 translocation to the hepatic plasma membrane from the intracellular pool, resulting in significant enhancement of hepatic Aβ(1–40) uptake from the circulating blood. The presently proposed mechanism would explain the epidemiological results demonstrating that type II diabetes mellitus is a risk factor of Alzheimer's disease.

Amyloid β-peptide (Aβ), a 39- to 43-amino acid peptide derived from the proteolytic cleavage of amyloid precursor protein, is the main constituent of brain parenchymal and cerebrovascular amyloid deposits in Alzheimer's disease (AD). The 40-amino acid form of Aβ, Aβ(1–40), is the predominant form of cerebrovascular amyloid (Castaño et al., 1996) and accounts for approximately 90% of the Aβ present in the circulation (Seubert et al., 1992).

Under physiological conditions, plasma Aβ(1–40) is rapidly cleared from the circulation with a half-life of 1.2 to 15 min in rodents (Poduslo et al., 1997; Hone et al., 2003; Ghiso et al., 2004; Kandimalla et al., 2005). This rapid clearance of plasma Aβ(1–40) is largely mediated by the liver (Hone et al., 2003; Ghiso et al., 2004), and low-density lipoprotein receptor-related protein (LRP-1) is the major receptor responsible for the hepatic Aβ(1–40) uptake (Tamaki et al., 2006). A reduction in the plasma Aβ(1–40) clearance by systemic organs has been shown to be associated with an increase in Aβ(1–40) accumulation in the brain (Mackic et al., 1998, 2002), indicating that the systemic clearance of Aβ(1–40), especially by the liver, could play an important role in determining the levels of plasma Aβ(1–40) available for transport into the brain across the blood-brain barrier (Mackic et al., 1998, 2002).

Type II diabetes mellitus (DM), which is characterized by an attenuated response to insulin signaling in peripheral tissues (insulin resistance), is associated with an increased risk of both vascular dementia and AD (Yoshitake et al., 1995; Leibson et al., 1997; Ott et al., 1999). Although the exact pathogenesis remains unclear, several mechanisms to explain the high incidence of AD in patients with type II DM

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ABBREVIATIONS: Aβ, amyloid β-peptide; AD, Alzheimer's disease; α2M, α2-macroglobulin; α2M*, methylamine-activated α2 macroglobulin; DM, diabetes mellitus; LRP-1, low-density lipoprotein receptor-related protein 1; LUI, liver uptake index; PBS, phosphate-buffered saline; PPARγ, peroxisome proliferator activated receptor γ; RAP, receptor-associated protein; SD, Sprague-Dawley.

have been proposed, including ischemic damage and damage through toxic glucose metabolites and advanced glycation of functional proteins (Biessels and Kappelle, 2005).

Recent increasing evidence highlights the direct role of insulin in the link between AD and type II DM. Intravenous infusion of insulin whereas keeping plasma glucose at a fasting baseline level produces striking memory enhancement for patients with AD (Craft et al., 1996). Insulin is also likely to affect the clearance process of Aβ, because it has been reported that plasma levels of amyloid precursor protein, one of the LRP-1 ligands, is inversely correlated with plasma insulin levels (Boyt et al., 2000). This suggests a direct association between plasma insulin and LRP-1-mediated clearance of amyloid precursor protein in the periphery and raises the possibility that peripheral insulin resistance in type II DM causes impaired LRP-1-mediated clearance of Aβ by the liver, leading to cerebral Aβ accumulation. Therefore, the purpose of the present study was to clarify the effect of plasma insulin on the expression and localization of LRP-1 in hepatocytes and on the hepatic clearance of Aβ(1–40) from the circulation.

Materials and Methods

Animals. Sprague-Dawley (SD) rats were supplied by Charles River (Yokohama, Japan). Animals were allowed ad libitum access to tap water and a standard pelleted diet (CRF-1; Oriental Yeast, Tokyo, Japan). For the fasted condition, animals were fasted for 48 h with free access to water. The investigations described in this report conformed to the requirements of the Animal Care Committee, Graduate School of Pharmaceutical sciences, Tohoku University (Sendai-shi, Japan).

Reagents. [^{125}I]Aβ(1–40) (2200 Ci/mmol), Na[^{125}I] (17 Ci/mg), and [^{3}H]water (1 mCi/g) were purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). Human recombinant insulin, α2-macroglobulin (α2M), and xylazine hydrochloride were purchased from Sigma (St. Louis, MO). Ketamine hydrochloride was purchased from Sankyo (Tokyo, Japan). Receptor-associated protein (RAP) was purchased from Oxford Biomedical Research (Oxford, MI). All other chemicals were commercial products of reagent grade.

Preparation and Iodination of Methylamine-Activated α2M. α2M was activated with methylamine as described previously (Ashcom et al., 1990). In brief, native α2M (8.6 mg/ml) was incubated with 200 mM methylamine in 50 mM sodium phosphate and 150 mM NaCl, pH 7.4, for 1 h at room temperature. Methylamine-activated α2 macroglobulin (α2M*) was then dialyzed extensively against 100 mM $NaHCO_3$ and 500 mM NaCl, pH 8.3, using a Centricon centrifugation unit (YM-10; Millipore, Billerica, MA).

α2M* (10 μg) was iodinated with Na[^{125}I] (250 μCi) in 1 mg/ml chloramine-T, and 250 mM sodium phosphate, pH 7.5, for 1 min at 25°C. Unincorporated ^{125}I was removed by passing the reaction mixture through a D-salt dextran desalting column (Pierce, Rockford, IL) equilibrated with phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin. [^{125}I]α2M* (17.9 μCi/μg) was collected in the void volume of the column effluent. The percentage of precipitable [^{125}I]α2M* by trichloroacetic acid was 99.1%.

Liver Uptake Index Method. The unidirectional uptake of [^{125}I]α2M* and [^{125}I]Aβ(1–40) was determined by injection into a rat portal vein, as descried previously (Pardridge and Mietus, 1979). Rats were anesthetized by intramuscular injection of ketamine (125 mg/kg) and xylazine (1.25 mg/kg), and their rectal temperatures were maintained at 37°C using a heating pad. The hepatic artery was ligated, and 200 μl of Ringer's HEPES buffer, pH 7.4, which contained both 0.2 μCi of iodinated compound and 4.0 μCi of [^{3}H]water as a highly diffusible internal reference was rapidly injected into the portal vein. Eighteen seconds after injection, the right major lobe was excised from the liver and solubilized in Solvable (PerkinElmer Life and Analytical Sciences). For the inhibition study, 200 μl of 2 μM RAP or saline was administered via a portal vein 30 s before the tracer injection if required. Levels of ^{125}I and ^{3}H in the liver and in the injection solutions were measured using a γ counter (ALC300; ALOCA, Tokyo, Japan) and a liquid scintillation counter (TRI-CARB 2050CA; PerkinElmer), respectively.

Determination of the LUI. The LUI is defined in eq. 1 and was determined using eq. 2:

$$LUI = E_T/E_R \quad (1)$$

$$LUI(\%) = ([^{125}I]/[^{3}H]) \text{ counts in the liver}/([^{125}I]/[^{3}H]) \text{ counts in the injection solution} \times 100 \quad (2)$$

Here, E_T and E_R are the fractions of the iodinated compound and [^{3}H]water, respectively, extracted by the liver on a single pass. The value of E_T can be estimated when LUI and E_R are determined experimentally. Because the E_R value of [^{3}H]water is reported as 65 ± 4% (Pardridge and Mietus, 1979), the following equation is valid:

$$E_T = (LUI) \times 0.65 \quad (3)$$

The apparent fractional extractions consist of intracellular uptake, distribution to the interstitial space, and retention in the vascular space. Therefore, the extravascular extraction of the iodinated compound (E), which is only due to the intracellular uptake, was obtained as follows:

$$E = (E_T - E_{ns})/(100 - E_{ns}) \quad (4)$$

Here, E_{ns} represents the fractional extraction for distribution in the vascular and extracellular space. In this calculation, we used the reported E_{ns} value of 13 ± 3% (Pardridge and Mietus, 1979).

Insulin Treatment of Rats. Rats were anesthetized with an intramuscular injection of ketamine and xylazine, and insulin was infused into the portal vein at 5 μl/(min · g of liver) and a concentration of 420 nM, except in concentration-dependence shown in Fig. 4C. The insulin concentration in the portal blood flow ($C_{insulin,\ portal}$) was estimated as follows:

$$C_{insulin,\ portal} = I_0/F \quad (5)$$

Here, I_0 and F are the infusion rate of insulin (in picomoles per minute per gram of liver) and the portal blood flow rate (in milliliters per minute per gram of liver), respectively. In this calculation, we used the reported F value of 1.4 ml/(min · g of liver) (Pardridge and Mietus, 1979). The insulin concentration in rat plasma was determined by using Mercodia Ultrasensitive Rat Insulin enzyme-linked immunosorbent assay (Mercodia AB, Uppsala, Sweden).

Western Blot Analysis. Whole lysate was obtained from the supernatant (1000*g*, 10 min) of homogenates of rat liver in hypotonic lysis buffer (10 mM Tris, 10 mM NaCl, and 1.5 mM $MgCl_2$, pH 7.4). To obtain the plasma membrane fraction, homogenates were centrifuged at 10,000*g* for 10 min at 4°C, and the supernatant was then centrifuged at 100,000*g* for 1 h at 4°C. Pellets were resuspended in 10 mM HEPES and 250 mM sucrose, pH 7.4, and overlaid on 38% sucrose solution and then centrifuged at 100,000*g* for 40 min at 4°C using a swing rotor (SW40Ti; Beckman Coulter, Fullerton, CA). The turbid layer was collected and centrifuged at 100,000*g* for 1 h at 4°C, and the plasma membrane fraction was obtained from the pellets.

Proteins were electrophoresed under nonreducing conditions on 7.5% Tris/glycine SDS-polyacrylamide gel (Bio-Rad Laboratories, Hercules, CA). Separated proteins were subsequently electrotransferred to a nitrocellulose membrane (Toyo Roshi, Tokyo, Japan), and the membrane was treated with blocking solution (25 mM Tris, 125 mM NaCl, 5% skimmed milk, and 0.1% Tween 20, pH 8.0) for 1 h and diluted antibody against β-chain of LRP-1 (0.1 μg/ml; American Diagnostica, Greenwich, CT), Na^+,K^+-ATPase α1 subunit (1:10,000; Upstate Biotechnology, Lake Placid, NY) or β-actin (1:2000; Sigma)

for 16 h at 4°C. The membrane was washed with 0.1% Tween 20 in PBS and then incubated with horseradish peroxidase-conjugated anti-mouse IgG (1:5000; MP Biomedicals, Irvine, CA) for 1 h. Immunoreactivity was visualized with an enhanced chemiluminescence kit (Supersignal west pico chemiluminescent substrate; Pierce) and exposed to an X-OmatAR film (Eastman Kodak, Rochester, NY). Relative band intensities in the whole lysate (LRP-1/β-actin) and in the plasma membrane fraction (LRP-1/Na^+,K^+-ATPase) were determined from densitometric images using Scion Image Beta WIN 4.02 software (Scion Corporation, Frederick, MD).

Immunohistochemical Analysis. Rats were anesthetized with an intramuscular injection of ketamine and xylazine and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (40 ml/min, 10 min). The liver was isolated and immersed in PBS containing 500 mM sucrose for 16 h at 4°C to protect against freezing. Frozen sections (10 μm thickness) were made using a cryostat (CM1900; Leica, Heidelberg, Germany) and then mounted onto silanized slide glasses (Dako North America, Inc., Carpinteria, CA). After sections were incubated with 1% goat serum (Nichirei, Tokyo, Japan) for 1 h at room temperature, diluted antibody against the β-chain of LRP-1 (0.1 μg/ml) was applied to the sections and incubated for 16 h at 4°C. The sections were washed with Tris-buffered saline containing 1 mM $CaCl_2$ and then incubated with fluorescein isothiocyanate-conjugated anti-mouse IgG (1:200; MP Biomedicals) for 1 h at room temperature. Nuclei were stained with 1.5 μM propidium iodide (Invitrogen, Carlsbad, CA) and sections were mounted using Vectashield mounting medium (Vector Laboratories, Burlingame, CA). The sections were analyzed using a laser-scanning confocal fluorescence microscope (TSC SP; Leica).

Statistical Analysis. All data are presented as the mean ± S.E.M. The statistical significance of differences between the means was determined using the unpaired two-tailed Student's *t* test for two groups and by one-way analysis of variance followed by the Dunnett's test for more than two groups.

Results

Plasma Insulin Increases LRP-1 Expression on the Plasma Membrane of the Liver. To clarify the effect of plasma insulin on the LRP-1 expression and localization in the liver, fasted rats received an insulin infusion of 2.1 pmol/(min · g of liver) into the portal vein for each of the time periods indicated in Fig. 1, and the expression of LRP-1 in the liver was determined by Western blot analysis. The insulin concentration in the portal blood flow was estimated to be 1.5 nM from eq. 5. As shown in Fig. 1, the expression of LRP-1 in the plasma membrane fraction was increased by insulin infusion in a time-dependent manner, and a 2.16-fold increase was obtained after 10 min of treatment, whereas LRP-1 expression in the whole lysate was not affected (Fig. 1, A and B). Neither the level of β-actin nor of Na^+,K^+-ATPase protein, which were loading controls for the whole lysate and plasma membrane fraction, respectively, was affected by insulin treatment.

Nutrient Intake Affects LRP-1 Expression on the Plasma Membrane of the Liver. Under our experimental conditions, the plasma insulin concentrations were 250 ± 22 and 60 ± 17 pM in fed and fasted rats, respectively (mean ± S.E.M., $n = 3$). The expression of LRP-1 in the plasma membrane fraction was 2.22-fold greater in the liver of fed rats compared with that of fasted rats, whereas the expression in the whole lysate was not affected by nutrient intake (Fig. 2, A and B). The intracellular localization of LRP-1 in the liver was examined by immunohistochemical analysis (Fig. 2, C–F). Under fed conditions, the green fluorescence derived from LRP-1 was mainly detected on the plasma membrane of the hepatocytes and Kupffer's cells (Fig. 2, C and D). In the hepatocytes, LRP-1 was localized on the sinusoidal membrane, and no significant fluorescence was observed on the canalicular membrane. Under fasted conditions, the green fluorescence was detected in the cytoplasmic compartment of the hepatocytes (Fig. 2, E and F).

Plasma Insulin Increases LRP-1-Mediated Uptake of α2M* in the Liver. To clarify the effect of plasma insulin on the function of LRP-1 in the liver, the hepatic uptake of α2M*, which is a ligand of LRP-1, was determined by the LUI method. Under fed conditions, the extravascular extraction of [^{125}I]α2M* was 32.4 ± 4.6% (Fig. 3A). The hepatic uptake of [^{125}I]α2M* was significantly inhibited by 76.4% after pretreatment with RAP, an LRP-1-interacting molecule, indicating the major involvement of LRP-1 in the hepatic uptake of α2M*. Under fasted conditions, the hepatic uptake of [^{125}I]α2M* was 62.0% lower than that under fed conditions, and no significant inhibition by RAP was observed.

The hepatic uptake of [^{125}I]α2M* under fasted conditions was significantly increased by insulin treatment in a time-dependent manner (Fig. 3B). The uptake of [^{125}I]α2M* was increased 2.39-fold after a 10-min insulin treatment and then began to level off, which correlates with the increase in the LRP-1 expression on the plasma membrane fraction of the liver (Fig. 1).

Plasma Insulin Induces LRP-1-Mediated Uptake of Aβ(1–40) by the Liver. Under fed conditions, the extravascular extraction of [^{125}I]Aβ(1–40) was 69.5 ± 3.1% (Fig. 4A). The hepatic uptake of [^{125}I]Aβ(1–40) was significantly inhibited by 47.8% after pretreatment with RAP. Under fasted conditions, the hepatic uptake of [^{125}I]Aβ(1–40) was 42.8% lower than that under fed conditions, and no significant inhibition by RAP was observed.

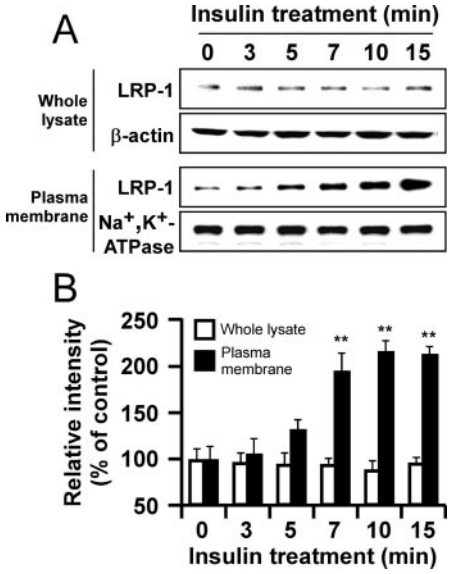

Fig. 1. Insulin-dependent induction of LRP-1 expression in the plasma membrane of the liver. Rats fasted for 48 h received an infusion of 420 nM insulin for the indicated times. A, the expression of LRP-1, Na^+,K^+-ATPase, and β-actin proteins in whole lysate, and plasma membrane fraction was determined by Western blot analysis. B, the relative band intensities in whole lysate (LRP-1/β-actin; □) and plasma membrane fraction (LRP-1/Na^+,K^+-ATPase; ■) were determined by densitometry (mean ± S.E.M., $n = 3$). **, significant difference from relevant control, $p < 0.01$.

The hepatic uptake of [^{125}I]Aβ(1–40) under fasted conditions was increased significantly by insulin treatment in a time-dependent manner (Fig. 4B, ○), as in the case of [^{125}I]α2M* (Fig. 3B). The uptake of [^{125}I]Aβ(1–40) by the liver was increased 1.70-fold after a 10-min insulin treatment and then reached steady state. The insulin-dependent increase in [^{125}I]Aβ(1–40) uptake was completely suppressed by pretreatment with RAP (Fig. 4B; ●). Facilitation of [^{125}I]Aβ(1–40) uptake was insulin concentration-dependent, and the uptake was increased at the estimated insulin concentration in the portal blood flow greater than 0.1 nM and reached a plateau at concentrations greater than 1 nM (Fig. 4C). A half-maximal response was determined at an estimated plasma insulin concentration of 230 pM (EC_{50}).

Discussion

The present study demonstrates that plasma insulin induces the subcellular translocation of LRP-1 to the plasma membrane in hepatocytes and increases the apparent transport function of LRP-1. In addition, LRP-1-mediated hepatic uptake of Aβ(1–40) from the circulation was shown to be under the regulation of plasma insulin.

By infusing fasted rats with insulin via a portal vein, the LRP-1 expression in the hepatic plasma membrane fraction was increased in a time-dependent manner and reached steady state in 10 min (Fig. 1), suggesting that insulin could facilitate the translocation of LRP-1 from the intracellular pool to the plasma membrane in hepatocytes. This short-term regulation of LRP-1 occurred at the post-transcriptional level, because the expression in the whole lysate was not affected by insulin treatment, and LRP-1 was translocated to the plasma membrane under fed conditions (Figs. 1 and 2). Furthermore, the increase in hepatic α2M* uptake by insulin treatment correlates with the increase in LRP-1 expression on the hepatic plasma membrane (Fig, 2). These results clearly demonstrate that plasma insulin induces subcellular translocation of LRP-1 to the plasma membrane, leading to an increase in the function of LRP-1 in the liver. The extraction of [^{14}C]carboxyl-inulin was not affected either by nutrient intake or insulin treatment (data not shown), suggesting that insulin does not affect the fractional extraction for distribution in the vascular and extracellular space.

The regulation mechanism of LRP-1 translocation by insulin has been studied in adipocytes. The binding of RAP to the plasma membrane of adipocytes was increased by treating rats with insulin, and binding reached a maximum after 4- to 5-min treatment (Descamps et al., 1993). It has been reported that increased cell surface localization of LRP-1 is associated with activation of phosphatidylinositide 3-kinase, and insulin probably stimulates the rate of LRP-1 translocation from the intracellular pool to the plasma membrane because the internalization rate of LRP-1 was not reduced by insulin treatment (Ko et al., 2001). It has been also reported that insulin treatment induces the caveolar localization of LRP-1, which may increase the stable localization on the plasma membrane (Zhang et al., 2004). LRP-1 may share these mechanisms in the liver, whereas further analysis is required for a deeper understanding of the mechanism of insulin-regulated localization of LRP-1.

In the liver, LRP-1 mediates the bulk clearance of lipoproteins, plasma proteases, protease-inhibitor complexes, and Aβ(1–40) shown in this study (Nykjaer and Willnow, 2002). For example, LRP-1 mediates the hepatic uptake of chylomicron remnants, the lipoprotein that shuttles dietary lipids from the intestine to the liver. Therefore, insulin regulation of LRP-1 is likely to play a physiological role in maintenance of systemic lipid homeostasis in response to fed conditions by facilitating the hepatic clearance of the dietary lipids, which are increased by nutrient intake. This also could explain the hyperlipidemia associated with DM after the attenuation of LRP-1-dependent uptake.

We have identified previously LRP-1 as a major receptor responsible for the hepatic uptake of plasma Aβ(1–40) (Tamaki et al., 2006). The present study demonstrates that the hepatic uptake of Aβ(1–40) is induced by insulin in a time- and concentration-dependent manner (Fig. 4). As in the case of α2M*, the induction of the uptake is RAP-sensitive and

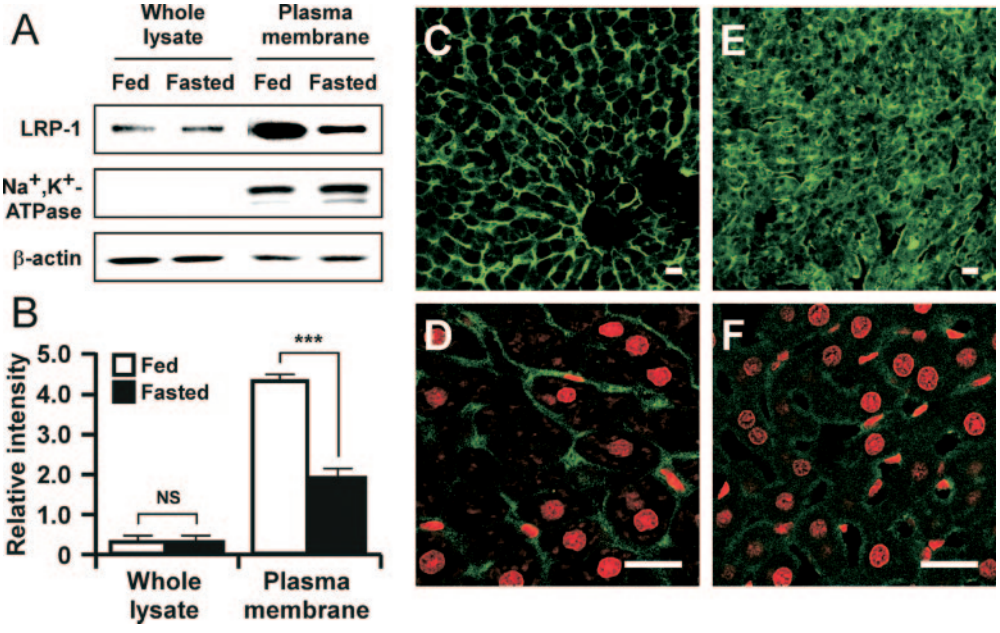

Fig. 2. Diet-dependent induction of LRP-1 expression in the plasma membrane of the liver. A and B, the expression of LRP-1, Na^+,K^+-ATPase and β-actin proteins in whole lysate and plasma membrane fraction obtained from fed and fasted rat liver was determined by Western blot analysis (A). The relative band intensities in whole lysate (LRP-1/β-actin) and plasma membrane fraction (LRP-1/Na^+,K^+-ATPase) were determined by densitometry (B; mean ± S.E.M., n = 3). ***, significant difference from relevant control, $p < 0.001$. NS, no significant differences. C to F, localization of LRP-1 in fed (C and D) and fasted (E and F) rat liver was analyzed by immunohistochemical analysis. Anti-LRP-1 antibody was used as a primary antibody, and binding was detected with an fluorescein isothiocyanate-conjugated secondary antibody (C–F, green). Nuclei are labeled in red (D and F, red). Scale bar, 20 μm.

correlates with increased expression of LRP-1 on the hepatic plasma membrane (Fig. 1). Therefore, insulin-induced translocation of LRP-1 leads to induction of the hepatic uptake of Aβ(1–40). This mechanism explains the report that the plasma level of amyloid precursor protein, one of the LRP-1 ligands, is inversely correlated with the plasma insulin level (Boyt et al., 2000).

Analysis of the concentration-dependence revealed that the EC_{50} of insulin for the facilitation of the uptake (0.23 nM) was comparable with the plasma concentrations under fed conditions (0.25 nM). This observation indicates that the plasma insulin levels were high enough to activate the subcellular translocation of LRP-1 under fed conditions. Therefore, under physiological conditions, the hepatic clearance of plasma Aβ(1–40) could be dynamically regulated by diet-dependent changes in the plasma insulin level. However, in patients with type II DM, it is conceivable that the hepatic clearance of plasma Aβ(1–40) failed to be induced by plasma insulin, because LRP-1 translocation to the plasma membrane would be impaired due to attenuation of the insulin-signaling response. This impaired induction of hepatic Aβ(1–40) clearance is likely to be one of the causes of Aβ accumulation in the brain. Aged SD rats exhibit features of insulin resistance (Narimiya et al., 1984; Barzilai and Rossetti, 1996; Field and Gibbons, 2000). Our previous report demonstrated that the hepatic clearance of Aβ(1–40) was reduced in 13-month-old SD rats compared with 7-week-old SD rats (Tamaki et al., 2006). This evidence supports the impairment of hepatic Aβ(1–40) clearance by insulin resistance. However, further analysis is necessary to clarify the attenuation of the function and translocation of LRP-1 in the liver of patients with type II DM.

The present study proposes that induction of LRP-1 func-

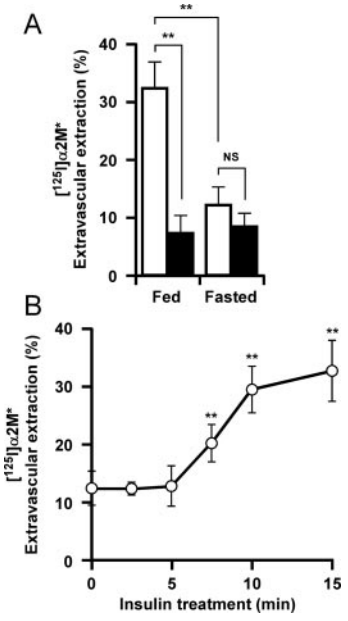

Fig. 3. Diet- and insulin-dependent induction of α2M* uptake by the liver. A, the extravascular extraction of [^{125}I]α2M* was determined in fed and fasted rats. Saline (□) or 2 μM RAP (■) in 200 μl was administered 0.5 min before the tracer injection. B, fasted rats received an infusion of 420 nM insulin for the indicated times, and the extravascular extraction of [^{125}I]α2M* was determined. Each value represents the mean ± S.E.M. (n = 4–5). **, significant difference from relevant control, $p < 0.01$. NS, no significant differences.

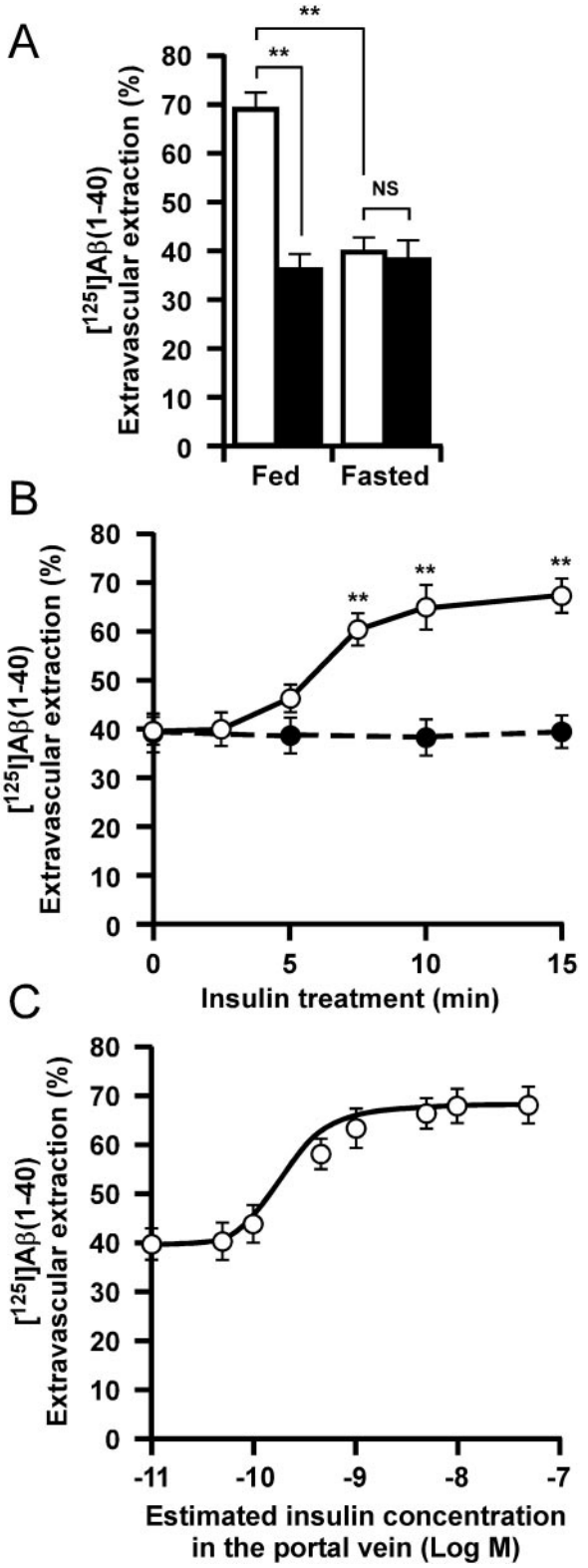

Fig. 4. Diet- and insulin-dependent induction of Aβ(1–40) uptake by the liver. A, the extravascular extraction of [^{125}I]Aβ(1–40) was determined in fed and fasted rats. Saline (□) or 2 μM RAP (■) in 200 μl was administered 0.5 min before the tracer injection. B, fasted rats received an infusion of 420 nM insulin for the indicated times, and the extravascular extraction of [^{125}I]Aβ(1–40) was determined. Saline (○) or 2 μM RAP (●) in 200 μl was administered 0.5 min before the tracer injection. C, insulin at various concentrations was infused for 10 min. The insulin concentration in the portal vein was estimated using eq. 5. Each piece of data represents the mean ± S.E.M. (n = 4–5). **, significant difference from relevant control, $p < 0.01$. NS, no significant differences.

tion in the liver would be effective for AD treatment in type II DM. Although the molecular mechanism of inducing LRP-1 translocation and function in the liver by insulin is unknown, the human LRP-1 gene has a predicted peroxisome proliferator response element, and rosiglitazone, which is a peroxisome proliferator activated receptor γ (PPARγ) ligand, stimulates mRNA transcription and functional expression of LRP-1 (Gauthier et al., 2003). PPARγ ligands, such as thiazolidinediones (rosiglitazone and pioglitazone), also increase the peripheral insulin sensitivity, and they are used for the treatment of type II DM (Peraldi et al., 1997). Therefore, it is possible that PPARγ ligands allow recovery of the attenuated LRP-1 function in the liver of patients with type II DM. It is interesting that recent evidence suggests that PPARγ ligand treatment lowers the brain Aβ levels and has a protective effect on the learning and memory deficit in AD model mice (Heneka et al., 2005; Pedersen et al., 2006) and in patients with mild-to-moderate AD (Risner et al., 2006). Therefore, thiazolidinediones could be especially effective for AD treatment in type II DM by attenuating insulin resistance and stimulate LRP-1 mRNA transcription, both leading to the activation of LRP-1-mediated clearance of Aβ(1–40) in the liver.

The present study demonstrates that plasma insulin facilitates the intracellular translocation of LRP-1 to the plasma membrane in the liver and increases the apparent transport function of LRP-1. In addition, the hepatic uptake of plasma-free Aβ(1–40) mediated by LRP-1 has been shown to be regulated by plasma insulin, suggesting a causal relationship between reduced hepatic Aβ(1–40) clearance and insulin resistance. The present findings increase our understanding of the role of insulin in the regulation of plasma Aβ(1–40) clearance mediated by LRP-1 and of the link between AD and type II DM.

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