

**Insulin facilitates the hepatic clearance of plasma amyloid β -peptide (1-40) by
intracellular translocation of LRP-1 to the plasma membrane in hepatocytes**

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Insulin facilitates LRP-1 mediated hepatic A β clearance

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A β , amyloid β -peptide; AD, Alzheimer's disease; α 2M, α 2-macroglobulin; DM, diabetes mellitus; EC₅₀, 50% effective concentration; FITC, fluorescein-4-isothiocyanate; LDL, low-density lipoprotein; LRP-1, LDL receptor-related protein 1; LUI, liver uptake index; PBS, phosphate-buffered saline; PPAR γ , peroxisome proliferator activated receptor γ ; RAP, receptor-associated protein

ABSTRACT

The hepatic clearance of amyloid β -peptide (1-40) ($A\beta(1-40)$) from plasma, which is largely mediated by low-density lipoprotein receptor-related protein (LRP-1), is suggested to play a role in preventing $A\beta(1-40)$ accumulation in the brain. Epidemiologic investigations suggest a high incidence of cerebral $A\beta$ 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 $A\beta(1-40)$. 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 non-treated controls after a 10-min infusion, while the expression in whole lysate was not affected by insulin treatment. The apparent hepatic uptake of [125 I] $A\beta(1-40)$ was also induced by insulin in a time-dependent manner. The increase in [125 I] $A\beta(1-40)$ uptake by insulin was concentration-dependent ($EC_{50} = 230$ pM), and was completely abolished by receptor-associated protein (2 μ M), 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 $A\beta(1-40)$ 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.

INTRODUCTION

Amyloid β -peptide ($A\beta$), a 39-43 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 $A\beta$, $A\beta(1-40)$, is the predominant form of cerebrovascular amyloid (Castano et al., 1996) and accounts for approximately 90% of the $A\beta$ present in the circulation (Seubert et al., 1992).

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

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 (Leibson et al., 1997; Ott et al., 1999; Yoshitake et al., 1995). Although the exact pathogenesis remains unclear, several mechanisms to explain the high incidence of AD in patients with type II DM 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 while 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 A β , since 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 A β by the liver, leading to cerebral A β 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 also on the hepatic clearance of A β (1-40) from the circulation.

MATERIALS AND METHODS

Animals

Sprague-Dawley 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, Japan.

Reagents

[¹²⁵I]Aβ(1-40) (2,200 Ci/mmol), Na¹²⁵I (17 Ci/mg) and [³H]water (1 mCi/g) were purchased from PerkinElmer Life Sciences (Boston, MA, USA). Human recombinant insulin, α₂-macroglobulin (α₂M) and xylazine hydrochloride were purchased from Sigma (St. Louis, MO, USA). Ketamine hydrochloride was purchased from Sankyo (Tokyo, Japan). Receptor-associated protein (RAP) was purchased from Oxford Biomedical Research (Oxford, MI, USA). 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). Briefly, native α₂M (8.6 mg/ml) was incubated with 200 mM methylamine in 50 mM sodium phosphate, 150 mM NaCl, pH 7.4, for 1 h at room temperature. Methylamine-activated α₂macroglobulin (α₂M*) was then dialyzed extensively against

100 mM NaHCO₃, 500 mM NaCl, pH 8.3, using a Centricon® centrifugation unit (YM-10; Millipore, Billerica, MA, USA).

α2M* (10 μg) was iodinated with Na¹²⁵I (250 μCi) in 1 mg/ml chloramine-T, 250 mM sodium phosphate, pH 7.5, for 1 min at 25 °C. Unincorporated ¹²⁵I was removed by passing the reaction mixture through a D-Salt™ dextran desalting column (Pierce, Rockford, IL, USA) equilibrated with phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin. [¹²⁵I]α2M* (17.9 μCi/μg) was collected in the void volume of the column effluent. The percentage of precipitable [¹²⁵I]α2M* by trichloroacetic acid was 99.1%.

Liver uptake index (LUI) method

The unidirectional uptake of [¹²⁵I]α2M* and [¹²⁵I]Aβ(1-40) was determined by injection into a rat portal vein, as described 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 Ringer's Hepes buffer (pH 7.4), which contained both 0.2 μCi iodinated compound and 4.0 μCi [³H]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 (Packard Instruments, Meriden, CT, USA). For the inhibition study, 200 μL 2 μM RAP or saline was administered via a portal vein 30 s prior to the tracer injection if required. Levels of ¹²⁵I and ³H in the liver and in the injection solutions were measured using a gamma counter (ALC300; ALOCA, Tokyo, Japan) and a liquid scintillation counter (TRI-CARB

2050CA; Packard Instruments), respectively.

Determination of the LUI

The *LUI* is defined in Equation 1 and was determined using Equation 2:

$$LUI = E_T / E_R \quad (\text{Eq. 1})$$

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

Here, E_T and E_R are the fractions of the iodinated compound and [^3H]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. Since the E_R value of [^3H]water is reported as $65 \pm 4\%$ (Pardridge and Mietus, 1979), the following equation is valid:

$$E_T = (LUI) \times 0.65 \quad (\text{Eq. 3})$$

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:

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

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 $13 \pm 3\%$ (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 $\mu\text{l}/(\text{min}\cdot\text{g}$ of liver) and a concentration of 420 nM, except in concentration-dependence shown in Fig. 4C. The insulin concentration in the portal blood flow ($C_{\text{insulin, portal}}$) was estimated as follows:

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

Here, I_0 and F are the infusion rate of insulin ($\text{pmol}/(\text{min}\cdot\text{g}$ of liver)) and the portal blood flow rate ($\text{ml}/(\text{min}\cdot\text{g}$ of liver)), respectively. In this calculation, we used the reported F value of 1.4 $\text{ml}/(\text{min}\cdot\text{g}$ of liver) (Pardridge and Mietus, 1979).

The insulin concentration in rat plasma was determined by using Mercodia Ultrasensitive Rat Insulin ELISA (Mercodia AB, Uppsala, Sweden).

Western blot analysis

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

Proteins were electrophoresed under non-reducing conditions on 7.5% Tris/glycine SDS-polyacrylamide gel (BIO-RAD, Hercules, CA, USA). 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, 0.1% Tween 20, pH 8.0) for 1 h and diluted antibody against β -chain of LRP-1 (0.1 $\mu\text{g}/\text{ml}$; American Diagnostica, Greenwich, CT, USA), Na^+ , K^+ -ATPase $\alpha 1$ subunit (1:10,000; Upstate Biotechnology, Lake Placid, NY, USA) or β -actin (1:2,000; 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 (1:5,000; ICN, Aurora, OH, USA) 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, USA). 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, USA).

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, Carpinteria, MA, USA). 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 $\mu\text{g}/\text{ml}$) was applied to the sections, and incubated for 16 h at 4 °C. The sections were washed with Tris-buffered saline containing

1 mM CaCl₂ and then incubated with fluorescein-4-isothiocyanate (FITC)-conjugated anti-mouse IgG (1:200; ICN) for 1 h at room temperature. Nuclei were stained with 1.5 μM propidium iodide (Molecular Probes, Eugene, OR, USA) and sections were mounted using Vectashield mounting medium (Vector Laboratories, Burlingame, CA, USA). 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 Equation 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 10min of treatment, while LRP-1 expression in the whole lysate was not affected (Fig. 1A, B). Neither the level of β -actin nor Na^+ , K^+ -ATPase protein, which was a loading control 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 \pm 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, while the expression in the whole lysate was not affected by nutrient intake (Fig. 2A, B). The intracellular localization of LRP-1 in the liver was examined by immunohistochemical analysis (Fig 2C-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. 2C, 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. 2E, F).

Plasma insulin increases LRP-1-mediated uptake of $\alpha 2M^*$ in the liver

To clarify the effect of plasma insulin on the function of LRP-1 in the liver, the hepatic uptake of $\alpha 2M^*$, which is a ligand of LRP-1, was determined by the LUI method. Under fed conditions, the extravascular extraction of [^{125}I] $\alpha 2M^*$ was $32.4 \pm 4.6\%$ (Fig. 3A). The hepatic uptake of [^{125}I] $\alpha 2M^*$ was significantly inhibited by 76.4% following pre-treatment with RAP, an LRP-1-interacting molecule, indicating the major involvement of LRP-1 in the hepatic uptake of $\alpha 2M^*$. Under fasted conditions, the hepatic uptake of [^{125}I] $\alpha 2M^*$ was 62.0% lower than that under fed conditions, and no significant inhibition by RAP was observed.

The hepatic uptake of [^{125}I] $\alpha 2M^*$ under fasted conditions was significantly increased by insulin treatment in a time-dependent manner (Fig. 3B). The uptake of [^{125}I] $\alpha 2M^*$ was increased 2.39-fold following 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 $A\beta(1-40)$ by the liver.

Under fed conditions, the extravascular extraction of [^{125}I] $A\beta(1-40)$ was $69.5 \pm 3.1\%$ (Fig. 4A). The hepatic uptake of [^{125}I] $A\beta(1-40)$ was significantly inhibited by 47.8% following pre-treatment with RAP. Under fasted conditions, the hepatic uptake of

[¹²⁵I]Aβ(1-40) was 42.8% lower than that under fed conditions, and no significant inhibition by RAP was observed.

The hepatic uptake of [¹²⁵I]Aβ(1-40) under fasted conditions was significantly increased by insulin treatment in a time-dependent manner (Fig. 4B; open symbol), as in the case of [¹²⁵I]α2M* (Fig. 3B). The uptake of [¹²⁵I]Aβ(1-40) by the liver was increased 1.70-fold following a 10 min insulin treatment and then reached steady-state. The insulin-dependent increase in [¹²⁵I]Aβ(1-40) uptake was completely suppressed by pre-treatment with RAP (Fig. 4B; closed symbol). Facilitation of [¹²⁵I]Aβ(1-40) uptake was insulin concentration-dependent, and the uptake was increased at the estimated insulin concentration in the portal blood flow above 0.1 nM and reached a plateau at concentrations above 1 nM (Fig. 4C). A half-maximal response was determined at an estimated plasma insulin concentration of 230 pM (50% effective concentration; EC₅₀).

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 A β (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 acute regulation of LRP-1 occurred at the posttranscriptional level, since 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 α 2M* 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 [¹⁴C]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 4-5 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 likely stimulates the rate of LRP-1 translocation from the intracellular pool to the plasma membrane since 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 and protease-inhibitor complexes as well as A β (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. As the physiological function of insulin regulation of LRP-1 in the liver, increasing plasma insulin in fed conditions is likely to facilitate the hepatic clearance of the dietary lipids, which are increased by nutrient intake, for maintaining systemic lipid homeostasis. This also could explain the hyperlipidemia associated with DM following the attenuation of LRP-1-dependent uptake.

We have previously identified LRP-1 as a major receptor responsible for the hepatic uptake of plasma A β (1-40) (Tamaki et al., 2006). The present study demonstrates that the hepatic uptake of A β (1-40) is induced by insulin in a time- and concentration-dependent manner (Fig. 4). As in the case of α 2M*, the induction of the uptake is RAP-sensitive and 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 A β (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 EC₅₀ 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 A β (1-40) could be dynamically regulated by diet-dependent changes in the plasma insulin level. However, in type II DM patients, it is conceivable that the hepatic clearance of plasma A β (1-40) failed to be induced by plasma insulin, since LRP-1 translocation to the plasma membrane would be impaired due to attenuation of the insulin signaling response. This impaired induction of hepatic A β (1-40) clearance is likely to be one of the causes of A β accumulation in the brain. Aged SD rats exhibit features of insulin resistance (Barzilai and Rossetti, 1996; Field and Gibbons, 2000; Narimiya et al., 1984). Our previous report demonstrated that the hepatic clearance of A β (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 A β (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 type II DM patients.

The present study proposes that induction of LRP-1 function 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 (PPAR γ) ligand, stimulates mRNA transcription and functional expression of LRP-1 (Gauthier et al., 2003). PPAR γ 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 PPAR γ ligands allow recovery of the attenuated LRP-1 function in the liver of type II DM patients. Interestingly, recent evidence suggests that PPAR γ ligand treatment lowers the brain A β 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 also in mild-to-moderate AD patients (Risner et al., 2006). Therefore, thiazolidinediones could be especially effective for AD treatment in type II DM by attenuating insulin resistance and also stimulate LRP-1 mRNA transcription, both leading to the activation of LRP-1-mediated clearance of A β (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 A β (1-40) mediated by LRP-1 has been shown to be regulated by plasma insulin, suggesting a causal relationship between reduced hepatic A β (1-40) clearance and insulin resistance. The present findings increase our understanding of the role of insulin in the regulation of plasma A β (1-40) clearance mediated by LRP-1, and also of the link between AD and type II DM.

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FOOTNOTE

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LEGEND FOR FIGURES

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. 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 (A). The relative band intensities in whole lysate (LRP-1/ β -actin; open column) and plasma membrane fraction (LRP-1/Na⁺, K⁺-ATPase; closed column) were determined by densitometry (B; mean \pm S.E.M., n = 3). ** Significant difference from relevant control, $p < 0.01$.

Fig. 2. Diet-dependent induction of LRP-1 expression in the plasma membrane of the liver. (A, 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; open column) and plasma membrane fraction (LRP-1/Na⁺, K⁺-ATPase; closed column) were determined by densitometry (B; mean \pm S.E.M., n = 3). *** Significant difference from relevant control, $p < 0.001$. NS, no significant differences. (C, D, E, F) Localization of LRP-1 in fed (C, D) and fasted (E, F) rat liver was analyzed by immunohistochemical analysis. Anti-LRP-1 antibody was used as a primary antibody and binding was detected with an FITC-conjugated secondary antibody (C, D, E, F; green). Nuclei are labeled in red (D, F; red). Scale bar = 20 μ m.

Fig. 3. Diet- and insulin-dependent induction of α 2M* uptake by the liver. (A) The extravascular extraction of [¹²⁵I] α 2M* was determined in fed and fasted rats. Saline

(open column) or 2 μ M RAP (closed column) in 200 μ L was administered 0.5 min prior to the tracer injection. (B) Fasted rats received an infusion of 420 nM insulin for the indicated times and the extravascular extraction of [125 I] α 2M* was determined. Each value represents the mean \pm 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 A β (1-40) uptake by the liver. (A) The extravascular extraction of [125 I]A β (1-40) was determined in fed and fasted rats. Saline (open column) or 2 μ M RAP (closed column) in 200 μ L was administered 0.5 min prior to the tracer injection. (B) Fasted rats received an infusion of 420 nM insulin for the indicated times and the extravascular extraction of [125 I]A β (1-40) was determined. Saline (open symbol) or 2 μ M RAP (closed symbol) in 200 μ L was administered 0.5 min prior to the tracer injection. (C) Insulin at various concentrations was infused for 10 min. The insulin concentration in the portal vein was estimated using Equation 6. Each piece of data represents the mean \pm S.E.M. (n = 4-5). ** Significant difference from relevant control, $p < 0.01$. NS; no significant differences.

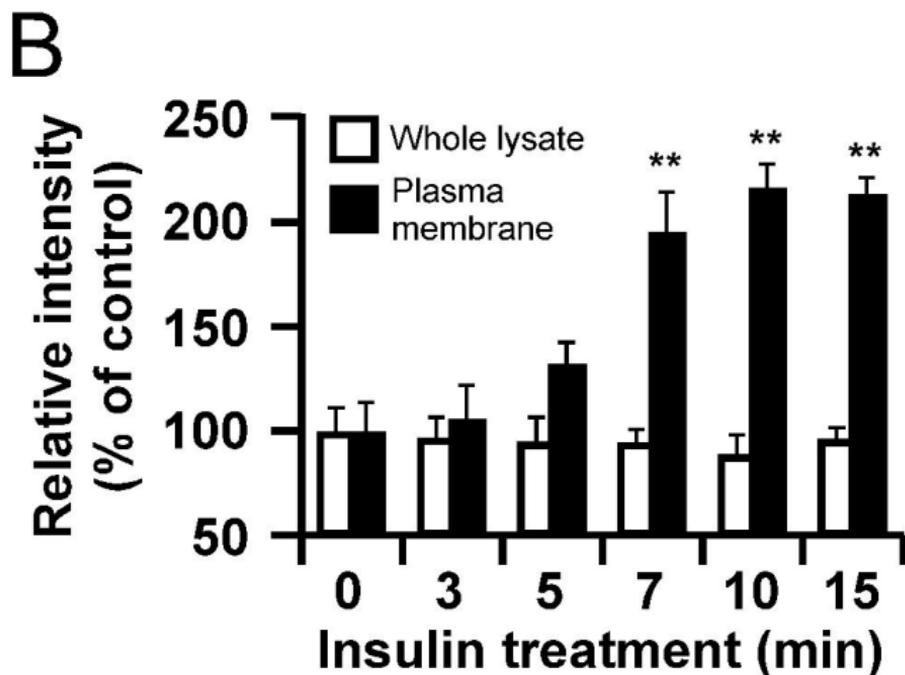
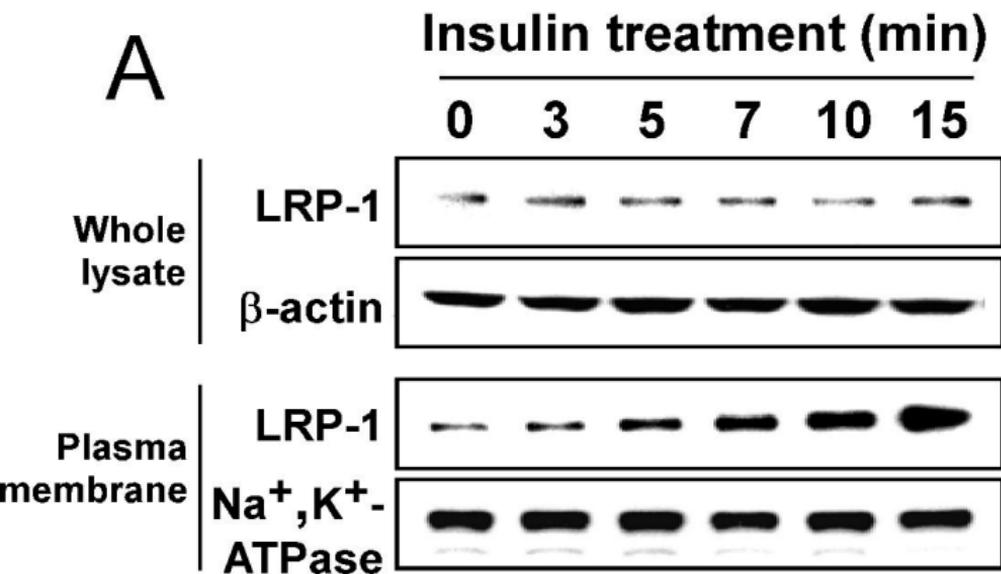


Figure 1

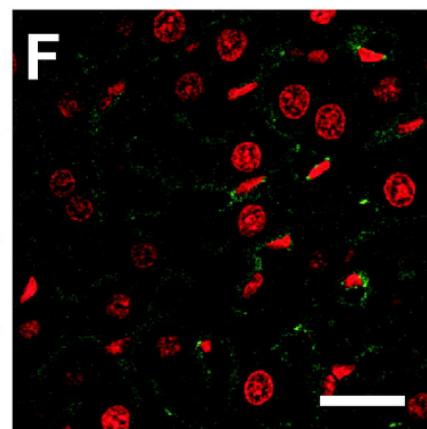
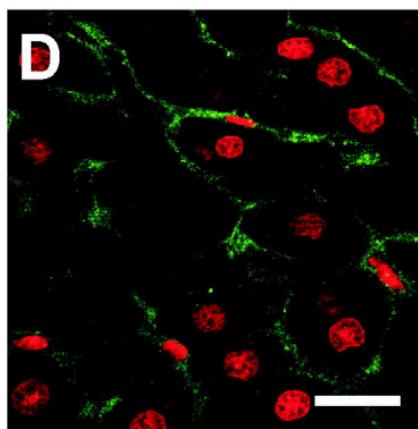
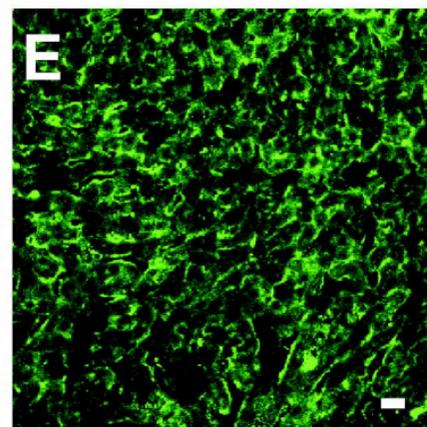
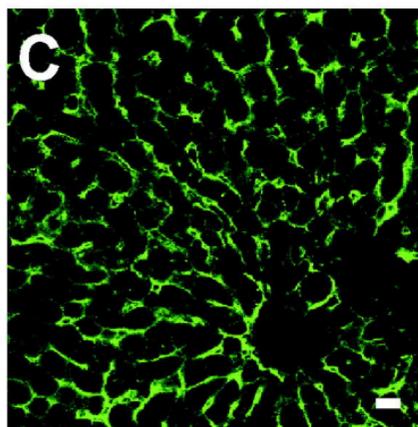
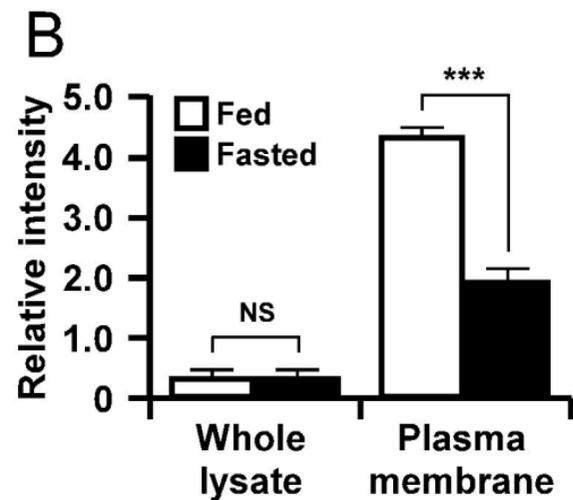
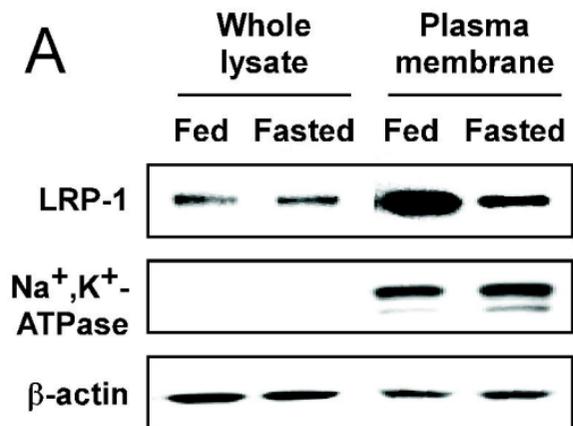


Figure 2

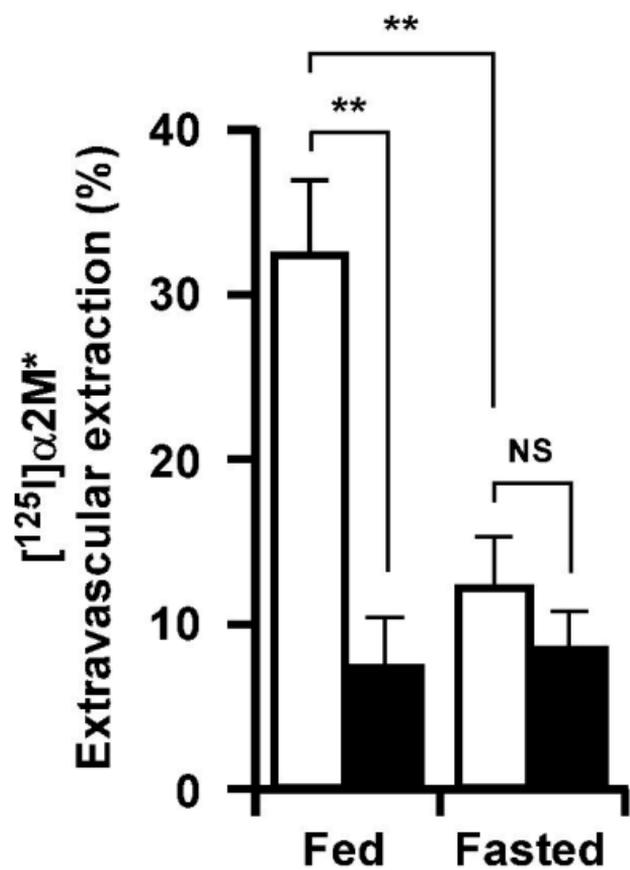
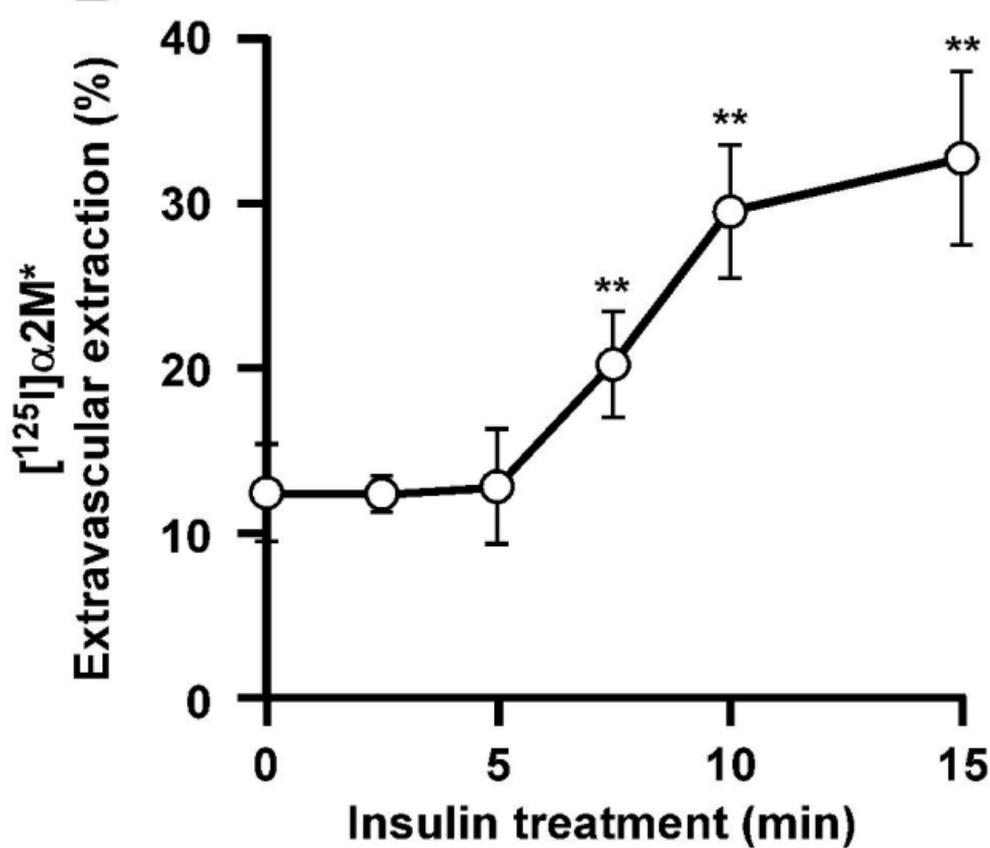
A**B**

Figure 3

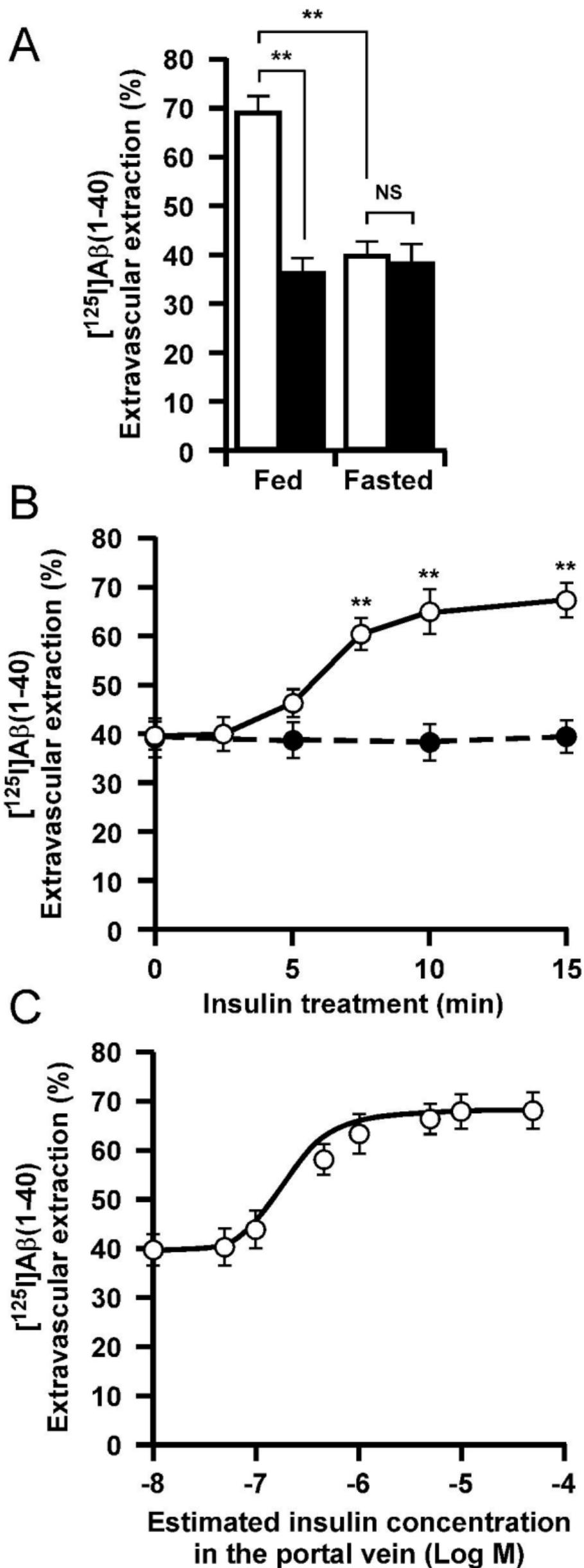


Figure 4