Apolipoprotein A-V Modulates Insulin Secretion in Pancreatic β -cells Through its Interaction with Midkine

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Key Words

Apolipoprotein A-V • Insulin secretion • Pancreatic β -cells • Midkine

Abstract

Apolipoprotein A-V is an important determinant of plasma triglyceride level in both humans and mice. This study showed the physiological impact of apoA-V on insulin secretion in rat pancreatic β -cells (INS-1 cells). In order to precise the mechanism of action, binding experiments coupled to mass spectrometry were performed to identify a potential membrane receptor. Results showed an interaction between apoA-V and midkine protein. Confocal microscopy confirmed the plasma membrane co-localisation of this two-proteins after the treatment of INS-1 cells with the apoA-V recombinant protein and indicated that the cell surface midkine could be involved in apoA-V endocytosis, since these two proteins were co-translocated at the plasma membrane or in the cytosol compartment. This co-localisation is correlated with an increase in insulin secretion in a dose dependant manner during short incubation period. Reduction of midkine expression by small interfering RNA duplexes revealed a decrease in the ability of these transfected

cells to secrete insulin in presence of apoA-V. Competition experiments for the apoA-V-midkine binding at the cell surface using antibody directed against midkine is able to influence INS-1 cell function as insulin secretion. Our results showed apoA-V ability to enhance insulin secretion in β -cells and provide evidence of an internalization pathway involving the midkine as partner.

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Introduction

Type 2 diabetes is a metabolic syndrome with multifactorial origins involving both defective insulin secretion and impaired insulin sensitivity. It is associated with a marked increase in the risk of cardiovascular diseases, which is typically increased twofold in diabetic men and threefold in diabetic women [1, 2]. Lipotoxicity and alteration in glucose homeostasis in liver, muscle, and pancreatic islets plays a crucial role in the development of both β -cell dysfunction, insulin secretion and insulin resistance [3].

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Apolipoproteins play a central role in lipid homeostasis and are intimately associated with the cardiovascular disease risk [4, 5]. Recently, the gene coding for a new apolipoprotein family member, *APOA5*, was identified to be an important determinant of plasma triglyceride levels in both humans and mice [6-8]. Apolipoprotein A-V is only synthesized in liver, and data from mice overexpressing or lacking *APOA5* provide direct evidence that this protein is inversely correlated with plasma triglyceride levels [9]. In humans, polymorphisms across the *APOA5* locus have been associated with elevated plasma TG concentrations [6, 10], familial combined hypertriglyceridemia [11] and increased risk of cardiovascular disease [12, 13]. ApoA-V appears to activate lipoprotein lipase (LPL)-mediated very low density lipoprotein (VLDL)-triglyceride (TG) hydrolysis, thus accelerates VLDL clearance, and reduces plasma TG by inhibiting hepatic VLDL-TG production [14-17]. Moreover, genetically engineered mice showed that *APOA5* and *APOC3* influence independently, and in an opposite manner, the triglyceride metabolism [18]. It was demonstrated that insulin [19] and thyroid hormone [20] down-regulate the *APOA5* gene expression. Moreover, transcription factors implicated in the triglycerides metabolism [21-25] regulate *APOA5* gene. Overall, *APOA5* represents a potential therapeutic target for modulation of plasma triglyceride levels.

Midkine is a heparin-binding growth/differentiation factor encoded by a retinoic acid responsive gene [26, 27]. To act its specific functions in different cell types, midkine binds different proteins such as the low density lipoprotein receptor-related protein (LRP) [28], heparan sulfate proteoglycans and syndecans [29, 30], the receptor-like protein-tyrosine phosphatase ξ [31], or the anaplastic lymphoma kinase [32]. This protein is developmentally regulated and involved in angiogenesis, neurogenesis, cell migration and mesoderm-epithelial interactions [33, 34]. Midkine is highly expressed in many human cancers [35] and enhances the activity of plasminogen activator (PA) [36]. The signalling system of midkine corresponding to these activities is only partly understood [37, 38]. Kojima et al. suggested that dimer formation is an important step for the biological activity of midkine [39], and Salama et al. demonstrated that the binding of midkine to laminin binding protein precursor leads to nuclear translocation of the molecular complex [40].

Thus, we decide to investigate the physiological impact of apoA-V in the rat pancreatic β -cells. We provide

evidences to demonstrate the apoA-V faculty to enhance the insulin secretion after its interaction with midkine. We perform experiment to show the involvement of midkine and the co-localization with apoA-V to drive this new physiological role. Taken together, our results show the importance of the protein complex apo-AV-midkine, and reveal a new important role for apoA-V in pancreatic β -cell function and insulin secretion.

Materials and Methods

Cell Culture and Treatments

INS-1 cells were maintained in RPMI 1640 medium at 10 mM glucose supplemented with 10 mM HEPES, 10% heat-inactivated fetal calf serum (FCS), L-glutamine, sodium pyruvate, 50 μ M β -mercaptoethanol, streptomycin/penicillin in a humidified atmosphere (5% CO₂).

For secretion experiments and interaction study, INS-1 cells were culture, as described by Helleboid-Chapman et al. [41] prior to incubation with apoA-V recombinant protein. The culture medium was replaced with KRBH containing 5 mM glucose and apoA-V recombinant protein for 30 min to 3h.

Affinity binding

An affinity chromatography step with an activated CH Sepharose™ 4B column was introduced after membrane protein extraction. The amino acid sequence of the N-terminal fragment (20-114) of apoA-V used in this work was ATQARKGFWD YFSQTSGDKG RVEQIHQQKM AREPATLKDS LEQDLNNMNK FLEKLRLPSG SEAPRLPQDP VGMRRQLQEE LEEVKARLQP YMAEA. ApoA-V peptide was synthesized by Boc-benzyl chemistry with an Applied Biosystems 433A automated solid-phase peptide synthesizer. The purity of the peptide was monitored by reverse phase high pressure liquid chromatography (>95%). The peptide identity was confirmed by mass spectrometry. Resin and apoA-V peptide were mixed for 2h at 37°C. 15 mg of INS-1 membrane protein was passed through the resin equilibrated with PBS. The resin bound complex was eluted with Tris-glycine buffer pH 8 (0.2M). Eluted proteins were revealed on 10% SDS-PAGE and analyzed by mass spectrometry.

Mono-dimensional Electrophoresis and Mass spectrometry

Desalted proteins were separated by SDS-PAGE using 10% acrylamide gels containing 1.5 M Tris-HCl (pH 8.8), 10% SDS, 10% APS, Temed and run 6h at 250 V using the PROTEAN II XL cell format. Silver staining was performed using the ProteoSilver Plus Silver Stain Kit (Sigma-Aldrich, Inc.). The spots of interest were then excised for identification by mass spectrometry (MALDI-MS) as described by Coutte et al. [42] using Voyager-DE-STR (Applied Biosystem). The peptide masses were matched with the theoretical peptide masses of all proteins of the SWISS-PROT databases.

Real-time biomolecular interaction analysis using Biacore 3000

Real-time biomolecular interaction analyses were performed by surface plasmon resonance technology using a Biacore 3000 system (Biacore AB, Uppsala, Sweden). All experiments were conducted at 25°C. The running buffer used was HBS-EP (10 mM HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, and 0.005% Surfactant P20) at a flow rate of 5 µl/min. ApoA-V peptide was coupled to the dextran-modified gold surface of a CM5 sensor chip by amine coupling as described in the Biacore system manual. The immobilized level was 3500RU corresponding to 3.5 ng/mm² of peptide. Samples (30 µl) were injected over the chip at a flow rate of 5 µg/min, followed by a washing phase with HBS-EP buffer to achieve a steady baseline.

Recombinant apoA-V cloning; expression and purification

The coding sequence of human *APOA5* [NM_052968] was amplified using the forward primer 5'-ATG CGG ATC CCG GAA AGG CTT CTG GGA CTA-3' and the reverse primer 5'-GAC TAA GCT TTC AGG GGT CCC CCA GAT GGC-3'. The PCR product was subcloned into pCold I vector (Takara BIO INC) by use of *Bam*HI and *Hind*III. The plasmid was propagated in *Escherichia coli* DH5α cells. The construct contained the *APOA5* coding region without the signal peptide, but with a His₆ epitope tag insert upstream. We next transformed *Escherichia coli* BL21 (DE3) pLysS (Novagen) and propagated isolated colony overnight at 37°C with aeration in Terrific Broth (per liter, 12 g select peptone 140, 23.6 g yeast extract, 9.4 g dipotassium dihydrogen, 2.2 g potassium dihydrogen, 4 ml glycerol) supplemented with 100 mg/L ampicillin and 34 mg/L chloramphenicol. We diluted 1 ml of the overnight culture (1:300) in fresh Terrific Broth supplemented with 100 mg/L ampicillin and incubated the sample at 37°C with shaking. When the culture OD₆₀₀ reached 0.4-0.6, the culture solution was refrigerated at 15°C for 30 min and apoA-V was induced by the addition of 0.15 mM of isopropyl-β-D-thiogalactopyranoside. After 24h, cells were harvested by centrifugation, and the pellet was stored at -80°C until needed. Purification of recombinant apoA-V was conducted with Ni-NTA resin (QIAGEN) according to the manufacturer's instructions.

Insulin secretion analysis

At the end of the treatment, INS-1 cells were washed twice in KRBH. After 20 min, the solution was collected and insulin was measured in the medium by ELISA (Mercodia) using rat insulin as standard. The insulin secretion was expressed as an insulin concentration normalized using the protein.

Preparation of total protein extract and membrane rich protein sample

Total protein extract was prepared as described by Helleboid-Chapman *et al.* [41]. For membrane rich protein sample, INS-1 cells were harvested by centrifugation in lysis buffer (50 mM Tris-HCl; 50 mM NaCl; 300 mM sucrose; 1 mM iodoacetamide; 1 µM leupeptin; pH 7.4) and sonicated. After removing nuclei and unlysed cells by centrifugation at 800 g

for 10 min, this fraction was sedimented by ultracentrifugation (100000 g; 1h). Samples, isolated as pellets, were dissolved in 2% CHAPS and sonicated. After an equilibration step (10 min) at 37°C, samples were centrifuged 10 min at 10000 g. Protein concentrations were determined by Bio-Rad protein assay kit (Bio-Rad).

Confocal microscopy

After the treatment, INS-1 cells (cultured on coverslips) were fixed with 4% [v/v] paraformaldehyde at room temperature for 40 min. For visualization of midkine and apoA-V distribution, the cells were permeabilized with 0.2% of Triton X-100 in PBS for 10 min at room temperature. Before incubation with polyclonal anti-apoA-V antibody (6) at 5 µg/ml for 1h at room temperature, cells were blocked with 5% goat serum in PBS for 15 min at room temperature. Cells were then incubated with polyclonal anti-midkine antibody (Interchim) at 5 µg/ml for 1h at room temperature. Finally, the cells were incubated with Alexa Fluor 488 anti-goat IgG and Alexa Fluor 543 anti-rabbit IgG at 5 µg/ml for 1h at room temperature. Then, the cover slips were mounted on microscopy slides and analyzed by confocal microscopy (Leica TCS SP2).

Co-immunoprecipitation

Protein A/G beads slurry were incubated with monoclonal apoA-V antibody [14] at 4°C for 2h with gentle rotation. After centrifugation, a mix of apoA-V peptide and INS-1 proteins was added and incubated at 4°C for 4h with gentle rotation. The protein A/G bead were washed with HBS buffer three times and subjected to boiling in Laemmli buffer (Biorad) containing 10% dithiothreitol for 5 min to release and denature the proteins, which were then separated by SDS-PAGE for immunoblotting.

Western Blotting

Immunoblotting were performed as described by Helleboid-Chapman *et al.* [41]. For midkine detection, the polyclonal rabbit anti-midkine antibody was used at the dilution of (1:2000).

siRNA Transfection

Transfection assays of INS-1 cells were conducted with siPORT Amine (Ambion) according to the manufacturer's instructions. Cells were transfected with a Silencer Negative Control siRNA (Ambion) or with a RNA targeting the midkine sequence (only sense sequence is shown): 5'-GAC AAG GUG AAG AAG GGC ATT-3'. Cells were harvested 48h after adding siRNA duplexes and examined for kindlerin expression by PCR amplification.

Gene expression analysis

Total RNA was prepared from INS-1 cells using RNeasyTM Total RNA Kit (Qiagen, Germany), treated with DNase I RNase free (Qiagen) and reverse-transcribed with random hexamers using the Omniscript RT kit. The resulting cDNA product was subsequently PCR amplified. Primers were designed using Vector NTI Suite software (Informax) based on sequence data avail-

Fig. 1. (A) CH Sepharose 4B column separation of INS-1 membrane proteins. Fractions eluted at the void volume of the column are numbered from 1 to 4. (B) Sensorgram of interaction in real time of the purified fractions (1-4) on the immobilized apoA-V peptide. Real time biomolecular interaction analyses were performed by surface plasmon resonance technology using a Biacore 3000 system.

able through Genbank: rat *midkine* (GenBank accession no NM_030859): forward 5'-GCA GCA CCG AAG TTT CTT CC-3', reverse 5'CTG CCC TTC TTC ACC TTG TC-3'.

Statistical analyses

Statistical analysis was performed using t-tests and $p < 0.05$ was considered statistically significant. Statistical differences from controls are indicated with asterisks (*, $p < 0.05$; **, $p < 0.01$ and ***, $p < 0.001$).

Results

Interaction of apoA-V with the cell surface-expressed midkine

To identify a potential apoA-V receptor, apoA-V binding proteins were isolated by affinity chromatography on a CH Sepharose 4B column from membrane proteins. Four peaks were detected and subjected to dialyze (Fig. 1A). To examine the binding of these fractions, we analyzed the interaction of apoA-V with the membrane fractions by Biacore 3000 technology (Fig. 1B). ApoA-V peptide was found to physically interact at high affinity with the fraction 3. This fraction was analyzed by SDS-PAGE and proteins were submitted to trypsin digestion followed by MALDI TOF mass spectrometry. One major band corresponding to a molecular mass of approximately 17 kDa was identified as midkine.

ApoA-V co-immunoprecipitates with midkine

To confirm apoA-V-midkine interaction, we used co-immunoprecipitation method to verify whether apoA-V forms a complex with midkine. As a result, midkine was detected in the precipitates of total and membrane protein extract after 30 min or 2h of incubation (Fig. 2). Upon SDS-PAGE, a major 34 kDa band was isolated and we supposed that it could correspond to the dimer form of midkine. Indeed, it was shown that midkine serves as a substrate for tissue transglutaminase, which readily yielded SDS- and β -mercaptoethanol-resistant multimers (39). The proportion of midkine in the co-precipitates was more important in total protein extract than in membrane fraction. This observation could be explained by the fact

Fig. 2. Co-immunoprecipitation of apoA-V and midkine. INS-1 total and membrane protein extracts pre-incubated with apoA-V were immunoprecipitated with apoA-V monoclonal antibodies bound to protein A/G agarose and subjected to immunoblotting analysis using polyclonal midkine antibodies. Pre-incubation steps with apo A-V were performed for 30 min [lane 2] or 2h [lane 3-5] with: 500 μ g [lane 2-3] and 1 mg [lane 4] of total protein or 500 μ g of membrane protein [lane 5].

that midkine was distributed in different subcellular compartments. In conclusion, using co-immunoprecipitation, we have confirmed the interaction between apoA-V and midkine in INS-1 cells.

ApoA-V and midkine co-localize in the membrane subcellular compartment

To confirm the interaction of the exogenously added apolipoprotein A-V to the cell surface expressed midkine,

Fig. 3. Co-localization study of apoA-V with midkine using confocal microscopy. As control, the figure (A) shows the distribution of midkine [red] in INS-1 cells. Figures (B) and (C) show the distribution of midkine [red] and apoA-V [green] in INS-1 cells treated for 30s with apoA-V recombinant protein at the concentration of 400 ng/ml. Figure (D) is merge image of (B) and (C). Figure (E) shows the distribution of apoA-V after 10 min of incubation with apoA-V. Figure (F) and (G) show the new distribution of apoA-V [green] and midkine [red] in INS-1 cells treated for 1h with apoA-V at the concentration of 400 ng/ml. Figure (H) is merge image of (F) and (G).

confocal microscopy studies were performed. We demonstrated that, in INS-1 cells, endogenous midkine was mainly detectable in the cytoplasm and slightly in the plasma membrane (Fig. 3A). INS-1 cells were incubated with apoA-V peptide at 37°C for 30s before immunolocalization experiments. The results showed that midkine was immediately concentrated in distinct spots at the plasma membrane (Fig. 3B), and it co-localized with apoA-V spots (Fig. 3D). There was a slight signal of apoA-V in the cytoplasm, suggesting entry during the incubation period at 37°C (Fig. 3C). Co-localization of the two proteins is represented by yellow color (Fig. 3D) when figure 3B and 3C were merged. After 10 min, an enrichment of apoA-V in the plasma membrane was observed (Fig. 3E). After 1h exposure to apoA-V recombinant protein, we examined a marked increase in apoA-V cytoplasmic staining, indicative from the transition from the plasma membrane to the cytoplasm (Fig. 3F). After this treatment, a fraction of midkine remained in plasma membrane, but there was a notable increase in midkine expression in a more cytoplasmic staining pattern (Fig. 3G). Co-staining INS-1 cells with midkine and apoA-V antibodies revealed a differential responsiveness (Fig. 3H). After this 1h treatment with apoA-V

recombinant protein, a significant loss of coincidence of midkine and apoA-V was observed in the cytoplasmic compartment. It should be noted that no green or red signal was detected when only secondary antibody was used in these experiments (not shown).

ApoA-V stimulates insulin secretion in INS-1 cells

In order to investigate the potential physiological role of apoA-V in pancreatic β -cells, we tested the ability of apoA-V recombinant protein to stimulate insulin secretion release. ApoA-V is present in human serum and circulates at much lower concentrations than other apolipoproteins, such as apoA-I. O'Brien et al. [43] observed human serum apoA-V concentrations ranging from 24 to 406 $\mu\text{g/L}$ with a mean value in normolipidemic persons of 157 $\mu\text{g/L}$. Thus, we used two concentrations of apoA-V recombinant protein not exceeding the physiological concentrations to explore its biological function in pancreatic β -cells (Fig. 4A). Interestingly, the incubation of INS-1 cells with apoA-V recombinant protein for 30 min and 1h induced insulin secretion (respectively 3.64 and 2.59 fold-induction). However, this effect was not significant at 3h of incubation. ApoA-V recombinant protein significantly increased insulin secretion in a dose de-

Fig. 4. Effect of apoA-V recombinant protein (A, B) on insulin secretion in INS-1 cells. (A) Cells were cultured overnight in RPMI medium containing 5 mM glucose before they were stimulated with apoA-V recombinant protein at the concentrations of 40 or 400 ng/ml for different time periods [30 min; 1h or 3h]. Insulin secretion was measured by ELISA and normalized to cell protein content. (B) Dose response of insulin secretion in INS-1 cells upon 1h-exposure to apoA-V recombinant protein [filled bars, n=3] or vehicle [open bar, n=3]. Values are means $\pm$ S.E. [n=4].

pendant manner (0 to 400 ng/ml) to reach a 2.59 fold-induction at the maximal concentration (400 ng/ml) after 1h of incubation (Fig. 4B). These results showed that apoA-V could be implicated in the stimulation of insulin secretion in INS-1 cells up to 1h incubation period.

The binding of apoA-V to midkine is involved in insulin secretion in INS-1 cells

To assess the function of midkine, we employed small interfering RNA (siRNA) duplexes to reduce the expression of midkine in INS-1 cells. Midkine gene expression was reduced (approx. 0.8 fold) in INS-1 cells

Fig. 5. The effect of midkine knock-down on insulin secretion in INS-1 cells stimulated with apoA-V recombinant protein. (A) Rat midkine mRNA was analyzed by semiquantitative RT-PCR normalized to 28 S. (B) INS-1 cells were transfected with siRNA control or siRNA midkine before incubation with apoA-V recombinant protein [400 ng/ml] for 30 min in 5 mM glucose. Insulin secretion was measured by ELISA and normalized to cell protein content. (C) The effect of midkine blocking on insulin secretion in INS-1 cells stimulated with apoA-V recombinant protein.

transfected with the midkine siRNA as compared with cells transfected with a scramble siRNA as shown in Fig. 5A. 48h after transfection of the siRNA duplexes, cells were incubated with apoA-V recombinant protein (400 ng/ml) for 30 min. Insulin secretion analyses revealed a decrease in the ability of midkine siRNA cells to secrete insulin compared to scramble siRNA cells upon stimulation with apoA-V recombinant protein (Fig. 5B). We concluded that reduction in midkine gene expression level affects cell response to apoA-V. To examine whether the interaction apoA-V-midkine is involved in insulin secretion, INS-1 cells were incubated for 5 min with midkine antibody and 30 min in presence of apoA-V recombinant protein. We observed that anti-midkine antibody affected insulin release induced by apoA-V treatment (Fig. 5C). In conclusion, all together, these results suggest that apoA-V stimulates insulin secretion in INS-1 cells through binding to midkine.

Discussion

Glucose-stimulated insulin secretion and β -cell growth are important facets of pancreatic islet β -cell biology. Factors that modulate these processes are of great interest for the potential treatment of Type 2 diabetes. Dysregulation of this process as alteration in lipid metabolism can affect both insulin sensitivity and β -cell function. Apolipoprotein A-V is an important determinant of lipid metabolism. In human, the role of *APOA5* gene in plasma triglyceride concentration has been shown in several studies [6, 44-47]. However, the impact of the apoA-V on β -cell function has not been examined.

In this study, we identified a protein that interacts with apolipoprotein A-V in rat pancreatic β -cells (INS-1 cell line) and examined the role of apoA-V as insulin secretion inducer. As an approach to identify cell surface molecules which bind to the apoA-V, we used affinity chromatography in combination to mass spectrometry. Herein, we report that apoA-V binds to midkine, a heparin-binding multifunctional protein. Midkine was first identified as the product of a retinoic acid-responsive gene that is up-regulated in the differentiation system of embryonal carcinoma cells [26, 27]. Its important roles have been implicated in various aspect of biology, however the mechanisms of midkine corresponding to these activities are only partly understood.

To test the hypothesis that the apoA-V have specific effect on β -cell biology, insulin secretion was measured in INS-1 cells after incubation with apoA-V

recombinant protein. In this model, apoA-V increased insulin secretion in a dose dependant manner during 1h of incubation. Antibody directed against midkine were used to investigate competition experiments for the apoA-V-midkine binding at the cell surface. As expected, the incubation with the anti-midkine antibody decreased insulin secretion stimulated by apoA-V. These results showed that the cross-linking of midkine and apoA-V on the cell surface is able to influence INS-1 cell function such as insulin secretion. We conclude that the binding of these two proteins is functionally important. To confirm the requirement of midkine in stimulation of insulin secretion, we employed a RNA interference approach targeting midkine expression. This strategy resulted in a decrease in midkine expression of 80% after 48h, which attenuated the ability of apoA-V to stimulate insulin secretion. These experiments provide evidence that both midkine and apoA-V are required to enhance insulin secretion, this underlying the importance of their interaction in this physiological function.

The kinetic of exogenous apoA-V subcellular localization was studied by exposing INS-1 cells to apoA-V recombinant protein during 30s to 1h. After 30s of incubation, apoA-V was mainly detected on the cell surface. The amount of internalized apoA-V increased in the plasma membrane until 10 min. Moreover, this subcellular localization changed during the time course and apoA-V was translocated to the cytoplasmic compartment. The internalized apoA-V was detected not homogeneously, reflecting cytoplasmic but also probably vesicular compartment localizations. In the same experiment, we observed the membrane recruitment of midkine in response to apoA-V treatment. After 30s, apoA-V and midkine were mostly co-localized on the cell surface. However, this membrane localization disappeared. ApoA-V and midkine were progressively co-translocated to the cytoplasm. It has been reported that midkine exert its effect by binding to cell surface receptor [28, 31]. Recently, Shibata et al. [48] showed that exogenous midkine was endocytosed by cultured mouse L cells and then transported to the nucleus, and the nuclear translocation is necessary for the anti-apoptotic activity of midkine. The present findings indicate that the cell surface midkine is involved in apoA-V endocytosis, since these two proteins were co-translocated in the cytosol compartment. In INS-1 cells, midkine may provide a mechanism of cytoplasmic translocation implying signalling receptor as it was reported by Muramatsu et al. [28] for the low density lipoprotein receptor-related protein, which belongs to the low density lipoprotein (LDL) receptor family.

Here, we demonstrated that the co-localization apoA-V-midkine occurs in the pancreatic β -cell and led to a significant increase of the insulin secretion. Recent studies focused on apoA-V were investigated to better understand the mechanism of its action on the TG-rich lipoprotein hydrolysis. Fruchart-Najib et al. [14] and Schaap et al. [17] have reported in 2004 that apoA-V regulates the TG levels through an increased hydrolysis of TG-rich lipoprotein by LPL lipolysis. In accordance with these findings, Lookene et al. [49] and Merkel et al. [15] proposed in 2005 a possible mechanism explaining its inverse correlation with plasma TG levels. Indeed, authors showed that apoA-V lipid complexes bind heparin and may promote their association with cell surface heparan sulfate proteoglycans. As LPL is bound to cell surface proteoglycans in physiological conditions, they concluded that apoA-V accelerates plasma hydrolysis of TG-rich lipoproteins by interaction with proteoglycan-bound LPL. Recently, new findings suggest an additional mechanism for the modulation of TG levels, focus on a direct interaction of apoA-V with the major LDL receptor family member (LRP). Interestingly, whatever hypothesis considered, both proposed mechanisms suggest a link between apoA-V and midkine, which bind heparan sulfate proteoglycans and LRP [28-30]. This could explain the apoA-V and midkine co-localization at the plasma membrane of INS-1 and the two

protein co-immunoprecipitation in membrane protein fraction.

In 2005, Chiu et al. [50] showed that the *V150M* polymorphism in the *APOA5* gene had an independent influence on first and second phases insulin response. Although the biological consequence of this polymorphism remains to be determined, the *V150M* polymorphism in the *APOA5* gene is a genetic marker for β -cell function. Our results have led to the first described physiological role for apoA-V in pancreatic β -cells by increasing insulin secretion and provide evidence of an internalization pathway involving the midkine as partner.

Abbreviations

APOA5 (the human gene); apoA-V (the human protein); *apoa5* (the rodent gene); TG (triglyceride); LPL (lipoprotein lipase); LDL (low density lipoprotein); VLDL (very low density lipoprotein); LRP (LDL receptor family member).

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