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Low Density Lipoprotein Binding and Uptake by Human and Rat Islet β Cells*

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ABSTRACT

Abnormalities in lipoprotein metabolism are common in diabetes. It is unknown whether variations in form or concentration of lipoproteins influence the function of pancreatic β cells. This study investigates whether low density lipoproteins (LDL) exhibit specific interactions with islet β cells. Radioactively labeled LDL (^{125}I-LDL) and fluorescently labeled LDL (DiI-LDL) were used as tracers. Rat islet cells express high affinity LDL binding sites (K_d = 9 nM), which are also recognized by very low density lipoproteins and which are down-regulated by LDL. Binding of LDL appears restricted to the β cells, as it was not detected on islet endocrine non-β cells. At 37 C, LDL is taken up and lysosomally degraded by islet β cells but not by islet non-β cells. Human islet cells were also found to present LDL binding, uptake, and degradation. Compared with rat islet cells, human islet cells exhibit 10-fold less binding sites (2.10^7 *vs.* 2.10^8 per 10^3 cells) with a 2-fold lower K_d value (5 nM) and an equal sensitivity to LDL-induced down-regulation. In conclusion, human and rat islet β cells express LDL receptors that can internalize the lipoprotein. This pathway should be examined for its potential role in (dys)regulating pancreatic β cell functions. (*Endocrinology* **138:** 4064–4068, 1997)

FAILURE OF the pancreatic β cell population to maintain its hormonal control on nutrient metabolism is expected to lead to a rise in circulating nutrients, some of which are to be considered as potential causes for a further deterioration of β cell functions. Chronically elevated glucose levels have been found to reduce the acute cellular responses to glucose (1). High concentrations of free fatty acids inhibit β cell functions such as glucose-induced insulin secretion (2–5). Whether variations in circulating lipoproteins can affect β cells is still unknown. It has been demonstrated that a deficiency in insulin results in elevated levels of both very low density (VLDL) and low density (LDL) lipoproteins (6–10). In the present study, we examine whether pancreatic β cells exhibit an LDL-recognition and processing mechanism similar to that described for other cell types (11). Experiments are undertaken to assess LDL-binding, uptake, and degradation by intact β cells. Identification of an LDL-receptor-mediated endocytotic uptake can raise an additional pathway through which nutrients exert (dys)regulatory actions on the pancreatic β cell population.

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Materials and Methods

Preparation of islet cells

Adult male Wistar rats were housed, fed, and cared for according to the guidelines of the Belgian Regulations for Animal Care. The protocol was approved by the Ethical Committee for Animal Experiments of the Free University of Brussels (V.U.B.). Rats were sedated and killed with CO_2 followed by decapitation. Islets were isolated by collagenase digestion and dissociated in a calcium-free medium containing trypsin and DNase (both from Boehringer Mannheim, Mannheim, Germany) (12). Purified β cells and non-β cells were obtained by autofluorescence-activated cell sorting as described previously (12). The β cell population was at least 95% pure. The non-β cell preparation consisted of minimally 75% α cells and less than 10% β cells. Cells were cultured in Ham's-F10 medium with 10 mmol/liter glucose (13).

Human islets were isolated from donor pancreata procured by European hospitals affiliated with β Cell Transplant, a European Concerted Action on islet cell transplantation in diabetes (14). Islets were prepared in the Central Unit of this multicenter program (Medical Campus, Vrije Universiteit Brussel, Brussels, Belgium). After collagenase digestion and Ficoll gradient purification, the islet-enriched interface was harvested, washed, and suspended in Ham's F10 medium supplemented as described (15).

The islet cell preparations were cultured for 48 h in polylysine-coated (10 μg/ml, Sigma Chemical Co., St. Louis, MO) wells (Falcon, Franklin Lakes, NJ) at a density of 10^5 cells/ml or in tissue culture chamber slides (four wells; Nunc, Denmark) containing 2×10^4 cells/well.

Preparation of LDL

Human lipoproteins were obtained from serum of healthy volunteers, after an overnight fast. The VLDL and LDL fractions were isolated by ultracentrifugation (16), with one additional run for LDL. The electroforetic mobility of LDL on 0.75% agarose exhibited an R_f of 0.24 ± 0.02 (SEM, n = 7). LDL was radiolabeled with ^{125}I (IMS-30, Amersham international, Buckinghamshire, UK) following the ICl method (17), modified as described (18, 19). The specific activity of the ^{125}I-LDL varied between 200–450 cpm/ng LDL protein. Acetylated LDL was obtained by repeated additions of acetic anhydride (19) and characterized by an increased relative electroforetic mobility (R_f = 0.55 ± 0.02 SEM, n = 4). Fluorescent LDL was prepared with DiI (Molecular Probes, Eugene, OR) (20) and isolated by an additional ultracentrifugation. All lipoprotein

preparations were filtered through a 22-μm filter (Millipore) before use. Their protein concentration was determined with the Pierce BCA kit using BSA as standard.

Incubations of cells with LDL

Experiments with ^{125}I- LDL were carried out in 300-μl culture medium containing 1.8 mM $CaCl_2$, 2% BSA, 20 mM HEPES (GIBCO, Paisley, Scotland, UK). Temperature was 4 C for studies on binding and 37 C on association and degradation. After incubation, culture plates were placed on ice and medium was collected for measurements of degradation (21). Wells were washed five times with ice-cold isolation medium (for composition, see Ref. 21) and once with PBS without BSA. The cells were then dissolved in 0.1 N NaOH and cell-associated radioactivity determined. In one series of experiments, chloroquine and NH_4Cl (both from Sigma Chemical Co., St Louis, MO) were added to the incubation medium as inhibitors of lysosomal degradation.

Down-regulation was assessed after a 22-h culture period with various LDL concentrations. At the end of this period, cells were extensively washed and then examined for their LDL binding. The viability of cells was determined at the start and at the end of each assay using a vital staining method with neutral red (12).

Dissociation of cell-bound LDL was examined by first incubating the cells at 4 C in the presence of 10 μg ^{125}I-LDL protein/ml, followed by washing in a lipoprotein-free medium and by a second incubation in the presence of unlabeled LDL (10 μg protein/ml) for different time periods. After washing the cells, their receptor-bound LDL as well as their nonspecific LDL binding was determined following an incubation with heparin (10 mg/ml, Sigma) during 1 h at 4 C (22, 23).

Tissue culture chamber slides were used for all experiments with the fluorescent probe DiI-LDL. Rat fibroblasts were used as positive controls.

Determination of binding characteristics

The equilibrium dissociation constant for LDL binding was derived from binding studies under equilibrium conditions with ^{125}I-LDL at concentrations from 0.5–20 nM. Receptor-bound LDL was determined as the difference in cell-bound ^{125}I-LDL counts in conditions with and without an excess of unlabeled LDL. Graphpad Prism 2.0 software (San Diego, CA) was used to plot binding curves and calculate the equilibrium dissociation constant (K_d) and maximum amount of lipoprotein bound (B_{max}). Competition of ^{125}I-LDL binding by unlabeled LDL provided values for EC_{50} at which LDL binding was half-maximally inhibited. Competition and saturation data shown are representative for two experiments.

The rate constants k_{+1} and k_{-1} for the LDL-interaction with cell surface receptors were derived from the time course of LDL binding at three LDL concentrations. The time needed for half-maximal binding [$t_{1/2}$(assoc.)] at each concentration was calculated with Graphpad Prism 2.0 and k_{+1} and k_{-1} values obtained from the plot of [ln $2/t_{1/2}$(assoc.)] *vs.* the concentration of lipoprotein [slope = k_{+1}; y-intercept = k_{-1}] (22). The ratio k_{-1}/k_{+1} can be used as another index of K_d.

Results

LDL binding and uptake by rat islet cells

^{125}I-labeled LDL binds specifically to rat pancreatic islet cells during a 6 h incubation at 4 C. Binding increases linearly with cell number in the range of 2.5×10^4 to 1.5×10^5 cells per condition (Fig. 1). This is also the case with purified β cell preparations, although these cells exhibit 50 percent lower binding (Fig. 1). Addition of glucagon (10^{-8} M) to the medium in which the β cells are cultured before binding partially reduced this difference (20 percent higher specific binding on β cells in the presence of glucagon, $P < 0.05$). The non-β cell population is characterized by low specific binding values (Fig. 1)

Fluorescently labeled LDL is incorporated by islet β cells during a 3-h incubation at 37 C. The compound appears located in dispersed dots (Fig. 2). No uptake is observed in islet non-β cells.

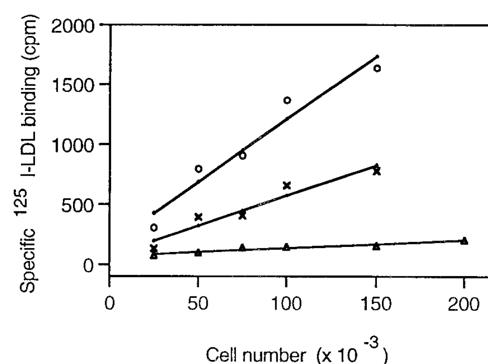

FIG. 1. Specific binding of ^{125}I-LDL to rat islet cells (○), islet β cells (x), and islet non-β cells (△) as a function of cell number. Six-hour incubations are carried out at 4 C with 10 μg ^{125}I-LDL protein/ml. Data are corrected for nonspecific binding. Each *point* represents the average of duplicate determinations.

Characteristics of LDL binding to rat islet cells

Binding equilibrium is obtained after 4 h incubation at 4 C with 10 μg ^{125}I-LDL protein/ml. LDL binding to rat islet cells is saturable in the range of 0.5–20 nM labeled LDL (Fig. 3). Fitting of binding data indicates the existence of one homogenous pool of receptors rather than two pools with different affinity. The high affinity LDL binding sites exhibit an equilibrium dissociation constant of 6 nM for a total of 2.10^8 binding sites per 10^3 cells. Competition experiments with 1 to 1000 nM unlabeled LDL yielded an equilibrium dissociation constant of 13 nM (Fig. 3). Binding analysis indicates again a best fit for one homogenous pool of binding sites.

The time required for half-maximal association [$t_{1/2}$(ass.)] was obtained at the three tracer concentrations by fitting the binding data using least squares analysis. Data were best fitted by an algorithm for one phase exponential association. Linear regression analysis of the values of [ln$2/t_{1/2}$(ass.)] *vs.* lipoprotein concentration resulted in $k_{+1} = 2.3 \times 10^4$ M^{-1} s^{-1} and $k_{-1} = 2.1 \times 10^{-4}$ s^{-1}. The equilibrium dissociation constant (K_d) calculated from these rate constants is 9 nM.

^{125}I-LDL binding was reversible as evidenced by the time-dependent decrease in cell-bound radioactivity and the parallel increase in medium radioactivity. Dissociation from the receptor was characterized by $t_{1/2}$ of 2 h and k_{-1} (= 0.693/$t_{1/2}$) of 9.6×10^{-5} s^{-1}. When islet cells were preincubated for 22 h with increasing amounts of LDL, subsequent ^{125}I-LDL binding decreased dose dependently. At 100 μg LDL protein/ml, binding represented 20 percent of control values.

^{125}I-LDL binding was inhibited by VLDL concentrations that were similar (25 to 100 μg/ml) to the inhibitory LDL concentrations (Fig. 4). No competition was observed with acetylated LDL, tested at 5- to 20-fold higher concentrations than the ^{125}I-LDL tracer. Addition of VLDL (50 μg/ml) also blocked cellular uptake of fluorescently labeled LDL.

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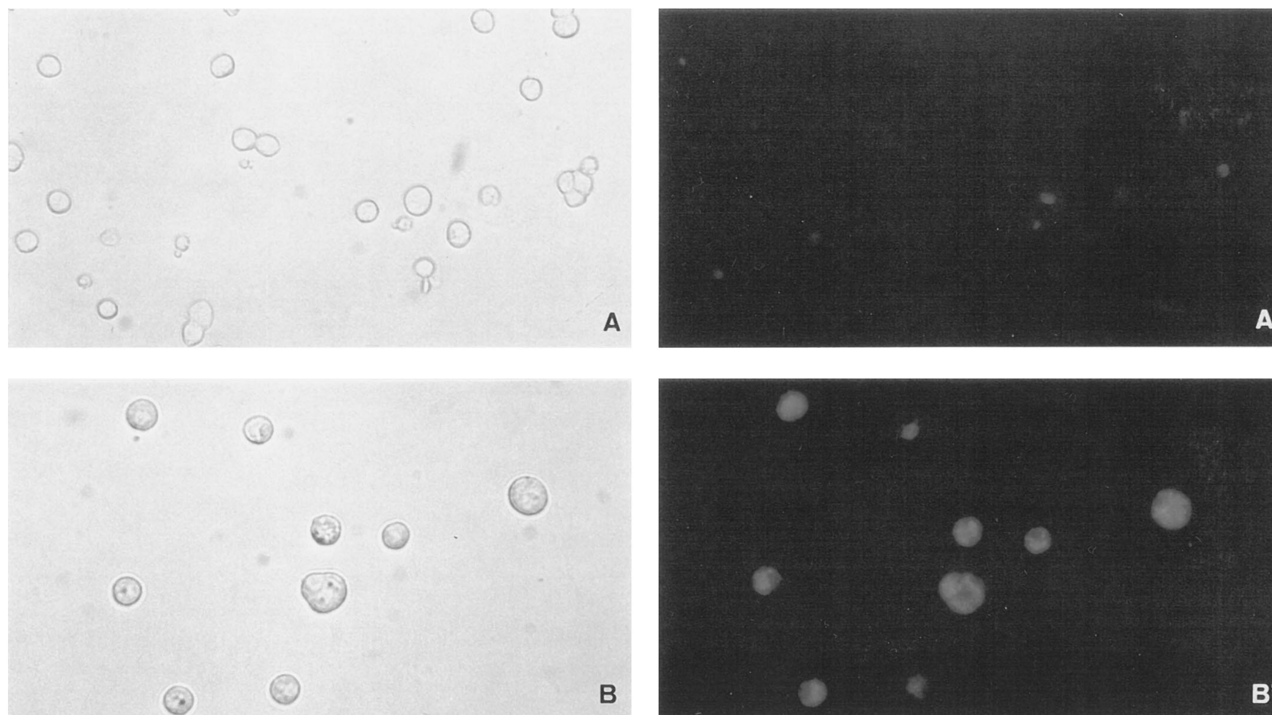

FIG. 2. Uptake of fluorescently labeled LDL by rat islet non-β (A, A′, *upper panels*) and β cells (B, B′ *lower panels*). Cells were incubated at 37 C with 10 μg DiI-LDL protein/ml for 3 h. The preparations are shown in direct light (A and B) and in fluorescence (530 nm; A′ and B′). Magnification 356×.

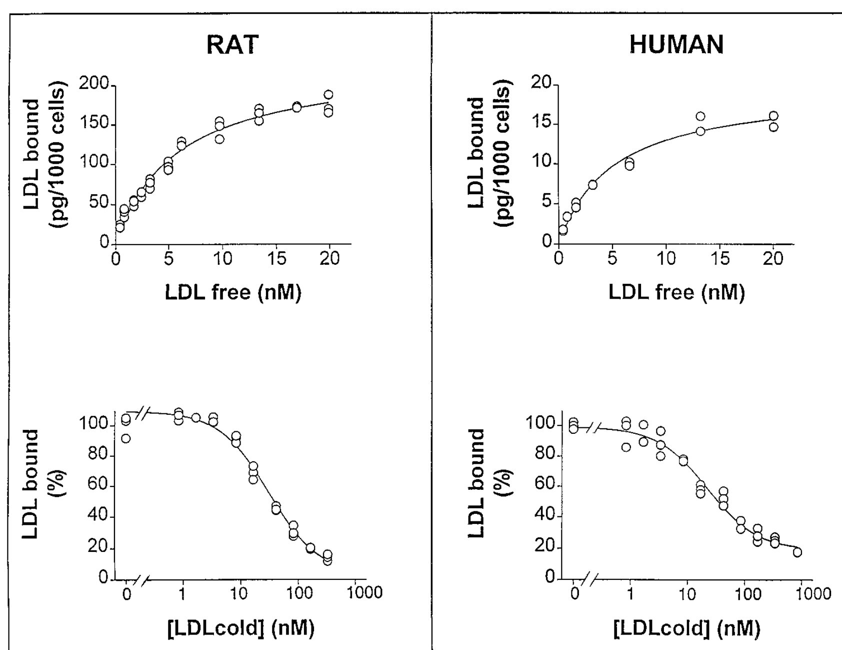

FIG. 3. Rat and human islet cells were incubated for 4 h at 4 C with the indicated concentrations of iodinated (saturation, *upper panels*) and native (competition, *lower panels*) low density lipoproteins. Competition of LDL binding was measured in the presence of 10 μg ^{125}I-LDL protein/ml. Saturation data were corrected for nonspecific binding.

Degradation of LDL by rat islet cells

Binding incubations at 37 C resulted in the appearance of degraded ^{125}I-LDL in the medium. Degradation does not occur at 37 C in the absence of cells and is therefore considered secondary to cellular uptake of ^{125}I-LDL. At the end of a 3-h incubation at 37 C, the amount of degraded LDL in the medium, measured as TCA-soluble radioactivity, represents one-third of the cell-associated ^{125}I-LDL. Addition of chlo-

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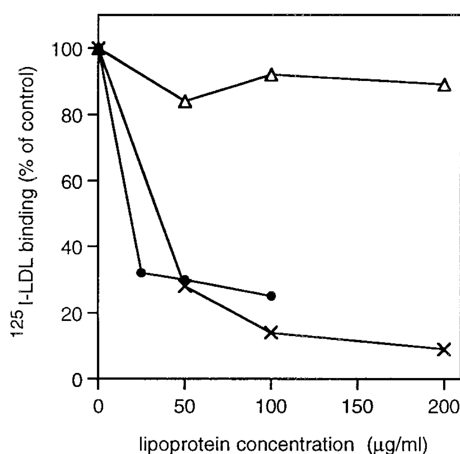

FIG. 4. Competition with ^{125}I-LDL binding to rat islet cells. Cells were incubated for 4 h at 4 C with 10 μg ^{125}I-LDL protein/ml and with the indicated amounts of unlabeled LDL (x), VLDL (●) or acetyl-LDL (△). ^{125}I-LDL binding is expressed as a percentage of the radioactivity obtained in the absence of unlabeled lipoprotein.

roquine or NH_4Cl inhibits degradation, while cell-associated radioactivity was not reduced (Table 1).

LDL binding, uptake, and degradation by human islet cells

Human islet cells also exhibited saturable LDL binding sites (Fig. 3). Binding characteristics are compatible with the existence of one homogenous pool of high affinity receptors. The saturation plot indicates an equilibrium dissociation constant of 5 nM and a density of 2.10^7 sites per 10^3 cells (Fig. 3). Competition with native LDL yielded an equilibrium dissociation constant of 4 nM. Binding was down-regulated for 90% by preincubation for 22 h (see *Materials and Methods*) with 100 μg LDL protein/ml.

Human islet cell preparations were also found to incorporate ^{125}I or fluorescently labeled LDL at 37 C and to degrade the lipoprotein. Degradation was suppressed by 80–90% after addition of NH_4Cl or chloroquine to the incubation medium (Table 1).

Discussion

The present study demonstrates that rat pancreatic β cells exhibit LDL binding sites that can also interact with VLDL but not with acetylated LDL. These binding sites are thus specific for the apoB or apoE proteins present in LDL and VLDL lipoproteins. The LDL binding characteristics indicate the existence of one single class of high affinity receptors with a K_d of 9 nM. LDL binding can be down-regulated by prior exposure to LDL, which makes the recognition mechanism in islet cells comparable with that in fibroblasts and other extra hepatic cells but not to that in human liver parenchymal cells which are less efficiently down-regulated (24).

LDL binding to β cells is probably responsible for the uptake of lipoproteins by the cells. This process occurs at 37 C and can be observed with both the radioactively and fluorescently labeled ligands. Degradation of the lipoproteins incorporated by the β cells is caused by lysosomal activity because it can be suppressed by chloroquine and NH_4Cl (25, 26). The fraction of cell-associated ^{125}I-LDL was enhanced after incubation in the presence of these compounds, probably due to accumulation of undegraded ^{125}I-LDL in the cells (27).

TABLE 1. Effect of chloroquine and NH_4Cl on the association and degradation of ^{125}I-LDL by human and rat islet cells

		Chloroquine		NH_4Cl
		50 μM	100 μM	10 mM
Rat	Association	227	249	185
	Degradation	33	12	39
Human	Association	140	139	93
	Degradation	20	10	24

Cells were incubated 3 h at 37 C with 10 μg ^{125}I-LDL protein/ml in the presence of 50 μM chloroquine, 100 μM chloroquine, or 10 mM NH_4Cl. ^{125}I-LDL association and degradation are expressed as a percentage of the radioactivity measured in the absence of chloroquine or NH_4Cl.

No LDL binding and uptake was detected in the islet non-β cell preparations. In dissociated islet cells and islet β cells, specific binding was already detectable in the condition with 5.10^4 cells. It is unlikely that the smaller surface (2- to 3-fold) of the non-β cells (28) is responsible for a detection problem because the use of 4-fold higher cell concentrations did not result in any measurable binding. Furthermore, no uptake was microscopically observed when these cells were exposed to fluorescently labeled LDL. Because α cells are the predominant cell type in the islet non-β cell preparation (12), it can be concluded that rat α cells do not express high affinity LDL binding sites and fail to incorporate this lipoprotein. The increased LDL binding by β cells that have been cultured in the presence of glucagon may suggest that α cells can regulate the LDL interaction with insulin-producing β cells, if locally released glucagon reaches the pancreatic β cells *in situ*. On the other hand, adenylcyclase activation in β cells, by glucagon or other peptides, is known to increase the functional activity and viability of islet β cells (29, 30) and may thus be involved in receptor up-regulation in isolated β cell preparations.

Human islet cells were also found to exhibit both LDL binding sites and an LDL uptake process. Because these properties were not present in rat islet non-β cells, it can be speculated that the data obtained in human islet cell preparations express characteristics of human β cells. The LDL binding sites on human islet cells appear also to correspond to one class of high affinity receptors that can be down-regulated by LDL. Compared with the rat, human islet cells contain 10-fold less LDL-binding sites (2.10^7 *vs.* 2.10^8 sites per 10^3 cells) with a 2-fold lower K_d value (5 *vs.* 9 nM). It should be noted that these LDL binding site concentrations were determined in unpurified islet cell preparations and could hence be underestimated by a factor up to 2, if binding is indeed confined to the β cell population, which is known to represent only 50% of the human islet cell population (15). LDL uptake in human islet cells was also visualized with the fluorescent ligand, and LDL degradation was again suppressed by inhibitors of lysosomal activity.

It is concluded that islet β cells express LDL binding sites that fulfill the properties of the LDL receptors originally described by Brown and Goldstein (11, 25). Their molecular

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characterization is needed to unambiguously determine their identity as "classical LDL receptor." It is unknown whether the observed receptor-mediated uptake of LDL influences the functional properties of the pancreatic β cells, and, if so, whether such finding has any physio(patho)logic significance. It is still unclear whether circulating LDL reaches the pancreatic β cells so that changes in circulating lipoprotein levels are reflected in the interstitial medium of the endocrine pancreas.

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