Separate Mechanisms for the Uptake of High and Low Density Lipoproteins by Mouse Adrenal Gland in Vivo*

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The adrenal gland of the mouse exhibits uptake mechanisms for plasma high density lipoprotein (HDL) and low density lipoprotein (LDL). To study this uptake, we lowered the endogenous plasma lipoprotein level in mice by administering 4-aminopyrazolopyrimidine to the animals with tracer amounts of human $^{125}$I-HDL or $^{125}$I-LDL intravenously. Uptake of $^{125}$I-HDL and $^{125}$I-LDL in the adrenal gland was demonstrable within 2 min after injection, and the content of radioactivity reached a steady state within 30 min. The adrenal gland accumulated 20-fold more $^{125}$I radioactivity/mg of tissue than lung or kidney. Moreover, the adrenal took up 50- to 200-fold more $^{125}$I-HDL or $^{125}$I-LDL than $^{125}$I-albumin. Adrenal uptake of both lipoproteins was reduced when adrenocorticotropic hormone secretion was suppressed by dexamethasone. Uptake of either LDL or HDL raised the level of cholesterol esters in the adrenal gland and suppressed the activity of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase. The uptake mechanisms were saturable in that unlabeled lipoproteins competed with the $^{125}$I-lipoproteins for uptake. In cross-experiment experiments, unlabeled LDL competed more effectively than unlabeled HDL for $^{125}$I-HDL uptake; conversely, unlabeled HDL competed more effectively than unlabeled LDL for $^{125}$I-LDL uptake. These data suggest that two different lipoprotein uptake systems supply cholesterol to the adrenal gland of the mouse, one using LDL and another using HDL.

Recent studies have focused attention on the mechanism by which the adrenal gland obtains cholesterol from plasma lipoproteins for use in steroid hormone synthesis (reviewed in Ref. 1). Cultured steroid-secreting mouse adrenal tumor cells (Y-1 clone) (2) and cultured adult bovine adrenal cells (3) possess surface receptors for plasma low density lipoprotein. Binding of LDL to these receptors leads to the uptake of the intact lipoprotein by adsorptive endocytosis and hydrolysis of its protein and cholesteryl ester components in cellular lysosomes (1-4). The unesterified cholesterol released from the hydrolysis of LDL supplies the substrate for steroid synthesis and suppresses de novo cholesterol synthesis by suppressing the activity of the rate-controlling enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (2, 3). Excess cholesterol entering the adrenal cells through the receptor-mediated uptake of LDL is re-esterified and stored as cholesteryl esters (2, 3). LDL receptor activity in adrenal cells is enhanced by adrenocorticotropic hormone and is suppressed when the cells have obtained sufficient cholesterol from the uptake of LDL (1-3). The LDL receptor in the cultured mouse and bovine adrenal cells does not bind high density lipoprotein, the other major cholesterol-carrying lipoprotein of plasma, and hence HDL is unable to supply cholesterol to these cells (2, 3).

Study of lipoprotein-mediated regulation of cholesterol metabolism in the adrenal gland of intact animals has recently been made possible by the use of 4-aminopyrazolopyrimidine, a purine analog that blocks lipoprotein secretion from the liver (5, 6). Within 24 h after the administration of 4-APP to rats, the plasma cholesterol level falls by more than 90%. Deprived of exogenous cholesterol, the adrenal gland rapidly becomes depleted of cholesteryl esters and then develops a marked elevation in cholesterol synthesis that is mediated by an increase in HMG-CoA reductase activity (7-9). The subsequent intravenous infusion of human LDL restores the content of adrenal cholesteryl esters and suppresses the elevated level of HMG-CoA reductase activity and cholesterol synthesis (7-9). In contrast to the results with the cultured mouse and bovine adrenal cells, the uptake process for cholesterol in the adrenal gland of the 4-APP-treated rat was not restricted to LDL. The administration of human or rat LDL also restored adrenal cholesteryl esters and suppressed HMG-CoA reductase activity and cholesterol synthesis (7-9). Moreover, HDL appeared more potent than LDL in that lower plasma cholesterol levels were required to produce a given increment in adrenal sterol content when HDL was infused as compared with LDL (7-9).

The above results raised the question as to whether the adrenal gland of the 4-APP-treated rat was taking up cholesterol by binding lipoproteins at receptor sites like those of the cultured adrenal cells. If so, was the same binding system recognizing both LDL and HDL, or were two different binding mechanisms involved? In the current studies, we have begun to answer these questions by measuring the uptake of $^{125}$I-labeled lipoproteins in the adrenal gland of 4-APP-treated mice. The choice of mice was dictated by two considerations: 1) the small size of the mouse permits the use of smaller amounts of $^{125}$I-labeled lipoproteins than are required for

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§ The abbreviations used are: ACTH, adrenocorticotropic hormone; 4-APP, 4-aminopyrazolopyrimidine; HDL, high density lipoprotein; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDL, low density lipoprotein; SDS, sodium dodecyl sulfate
intravenous infusion in rats, and 2) cultured mouse adrenal cells (Y-1 tumor clone) are known to possess specific LDL receptors. The current results demonstrate that the adrenal gland of the 4-APP-treated mouse exhibits saturable uptake processes for both $^{125}$I-LDL and $^{125}$I-HDL, and that these two uptake processes appear to be distinct from one another.

**EXPERIMENTAL PROCEDURES**

**Materials**—4-Aminopyrazolopyrimidine was obtained from Sigma Chemical Co. (grade II, Lot 47C-0103). Dexamethasone acetate (Decadron-LA) was purchased from Merck, Sharp and Dohme, West Point, Pa. ACTH was obtained either as Acthar gel from Armour Pharmaceutical Co., Phoenix, Ariz., or as purified Cortrophin gel from Organon, Inc., West Orange, N.J. Human crystallized albumin (Catalogue No. 82-301) was purchased from Miles Laboratories. Bovine albumin (Catalogue No. 90b-II) was obtained from Sigma Chemical Co. DL-3-Hydroxy-3-methyl[3-$^{14}$C]glutaryl-CoA (49.5 mCi/mmol) was purchased from New England Nuclear Corp. Sodium $^{131}$Iodide (11 mCi/m)g was obtained from Amersham/Searle. A kit of molecular weight standards for polyacrylamide gel electrophoresis was purchased from Bio-Rad Laboratories. Other chemicals and chromatographic materials were obtained from sources previously reported (7, 10).

**Animals and Drugs**—Female DBA/2 mice (The Jackson Laboratories), 8 to 10 weeks of age and weighing between 20 and 25 g, were used. The mice were exposed to a light-dark cycle consisting of 12 h of light (6 a.m. to 6 p.m.) and 12 h of darkness (6 p.m. to 6 a.m.) for 1 to 3 weeks prior to use. The mice had full access to tap water and Formula Chow (Wayne Laboratory, Allied Mills, Inc., Chicago, Ill.).

4-Aminopyrazolopyrimidine was administered intraperitoneally as a solution (0.5 mg/ml) in 10 mM sodium phosphate, pH 3.5 (5, 7). ACTH gel and dexamethasone acetate were administered subcutaneously. All drugs were given simultaneously every 24 h (8 a.m.) or every 12 h (8 a.m. and 8 p.m.) as indicated in the legends. Control mice received appropriate volumes of 10 mM sodium phosphate, pH 3.5, intraperitoneally for the 4-APP treatments and 0.15 $\mu$M NaCl subcutaneously for the ACTH treatments. Throughout the period of treatment with 4-APP, the mice had free access to Formula Chow and water that was supplemented with 1% (w/v) sodium chloride and 2% (w/v) glucose. Experiments were begun between 1 and 6 h after the last injection of drugs.

**Lipoproteins**—Human lipoprotein fractions were prepared from single 500 ml. units of blood collected in 0.1% EDTA from individual healthy subjects (10). Human LDL (density 1.019 to 1.063 g/ml) and HDL (density 1.125 to 1.215 g/ml) were fractionated by differential ultracentrifugation (11) as previously described (10). Each lipoprotein fraction migrated as a homogenous peak on agarose gel electrophoresis at pH 8.3. The mass ratio of total cholesterol to total protein was 1.5:1 for human LDL and HDL, respectively. Unless otherwise indicated, the concentrations of LDL and HDL are expressed in terms of their protein content.

**Intravenous Injection of $^{125}$I-Lipoproteins and Measurement of Tissue Content of $^{125}$I Radioactivity**—After treatment with 4-APP and ACTH for 48 h as indicated in the legends, mice were injected intravenously with either $^{125}$I-HDL or $^{125}$I-LDL. Each $^{125}$I-lipoprotein solution was prepared in 0.15 M sodium chloride containing 2 mg/ml of bovine albumin and was administered as a bolus injection into a tail vein while the animal was kept under light ether anesthesia for 2 to 3 min. In a given experiment, all mice received the same injection volume, either 100 or 200 $\mu$L. The animals were then returned to their cages without restraint and decapitated at various intervals after injection of the $^{125}$I-lipoprotein. Blood was collected by drainage from the neck into a tube containing EDTA (final concentration, 10 mM), after which the plasma fraction was obtained by centrifugation and an aliquot (10 $\mu$L) was counted for its $^{125}$I radioactivity. The adrenal glands were removed immediately, placed into ice cold 0.15 M sodium chloride, and stripped of adherent fatty and fibrous tissue. The two adrenal glands from each mouse were then placed in a tube containing 10% trichloroacetic acid and less than 2% was extractable into chloroform/methanol (2:1). For experiments, each $^{125}$I-protein was diluted with the corresponding unlabeled protein to give the final concentration and specific activity as indicated in the legends.

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**TABLE 1**

| Treatment | Plasma cholesterol level | Content of $^{125}$I radioactivity in tissues | Wet weight of tissues |
|-----------|--------------------------|---------------------------------------------|----------------------|
|           | mg/dl                    | Adrenal gland | Kidney | Lung | Adrenal gland | Kidney | Lung |
| Control   | 66 ± 3                   | 1700 ± 61 | 0.83 ± 0.08 | 28 ± 1 | 10.0 ± 1.4 | 3.9 ± 0.2 | 220 ± 11 | 140 ± 5 |
| 4-APP     | 4 ± 1                    | 800 ± 22 | 2.0 ± 0.0 | 79 ± 3.8 | 8.6 ± 0.61 | 5.4 ± 0.1 | 220 ± 9 | 130 ± 5 |
| 4-APP + ACTH | 3 ± 1                | 690 ± 31 | 3.0 ± 0.4 | 84 ± 3.5 | 7.4 ± 0.98 | 5.5 ± 0.3 | 220 ± 16 | 120 ± 6 |
| 4-APP + ACTH + Dex | 3 ± 1             | 680 ± 32 | 1.8 ± 0.9 | 75 ± 5.6 | 5.1 ± 0.59 | 5.2 ± 0.2 | 250 ± 13 | 120 ± 5 |
| 4-APP + Dex | 1 ± 2                   | 690 ± 23 | 4.7 ± 0.4 | 62 ± 2.2 | 7.4 ± 0.96 | 4.6 ± 0.5 | 240 ± 8 | 120 ± 7 |

**Experiment A:** $^{125}$I-HDL

| Treatment | Plasma cholesterol level | Content of $^{125}$I radioactivity in tissues | Wet weight of tissues |
|-----------|--------------------------|---------------------------------------------|----------------------|
|           | mg/dl                    | Adrenal gland | Kidney | Lung | Adrenal gland | Kidney | Lung |
| Control   | 64 ± 3                   | 1600 ± 55 | 0.79 ± 0.07 | 18 ± 1 | 14 ± 1.7 | 3.5 ± 0.3 | 240 ± 14 | 130 ± 5 |
| 4-APP     | 4 ± 1                    | 1400 ± 55 | 0.17 ± 0.04 | 27 ± 1.7 | 20 ± 5.7 | 3.7 ± 0.2 | 220 ± 6 | 130 ± 7 |
| 4-APP + ACTH | 3 ± 1                | 1600 ± 55 | 0.19 ± 0.0 | 24 ± 0.0 | 15 ± 1.1 | 5.4 ± 0.1 | 220 ± 13 | 120 ± 2 |
| 4-APP + ACTH + Dex | 3 ± 1             | 1600 ± 55 | 0.25 ± 0.03 | 29 ± 2.3 | 13 ± 2.8 | 5.3 ± 0.2 | 220 ± 4 | 110 ± 3 |
| 4-APP + Dex | 3 ± 1                   | 1400 ± 38 | 0.14 ± 0.02 | 29 ± 6.7 | 8 ± 0.8 | 4.8 ± 0.1 | 230 ± 9 | 97 ± 2 |

a. Dex = Dexamethasone acetate.
were perfused in vivo as follows. Sixty minutes after the injection of the 125I-HDL, 131I-LDL, or 125I-albumin, each mouse was placed under ether anesthesia. Blood was obtained from the inferior vena cava.

The inferior vena cava was then perfused with 5 ml of 0.15 M sodium chloride containing 2 mg/ml of bovine albumin at a hydrostatic pressure of 1 m. The perfusion was carried out for 90 s with the thoracic aorta opened and for an additional 1 min with the aorta opened. After completion of the perfusion, both kidneys, both lungs, and both adrenal glands were removed, placed into ice cold 0.15 M sodium chloride, and dissected free of adherent fatty and fibrous tissue. The tissues were then counted for their content of 125I-radioactivity, weighed, and homogenized for determination of the percent-age of 125I-radioactivity that was precipitable by 10% trichloroacetic acid. Perfusion was not carried out in 8 experiments in which only adrenal radioactivity was measured since such perfusion did not lower the number of counts per minute in the gland.

Preparation of Adrenal Microsomes and Assay of Microsomal HMG-CoA Reductase—Mice were killed by decapitation, and the adrenal glands were removed immediately and placed into ice cold medium containing 0.3 M sucrose 25 mM 2-mercaptoethanol, and 10 mM EDTA, pH 7. A microsomal fraction was prepared and the activity of HMG-CoA reductase was assayed as previously described for rat adrenal microsomes (7). The incubations were carried out in the presence of 87 mEq DL-[3-14C]HMG-CoA (11,000 cpm/nmol) at 37°C for 30 min. For each assay, parallel tubes containing three different amounts of microsomal protein (4 to 40 pg) were incubated to assure that the rate of the reaction was linear with respect to microsomal protein concentration. HMG-CoA reductase activity is expressed as nanomoles of ["Clmevalonate formed/min/mg of microsomal protein.

SDS Polyacrylamide Gel Electrophoresis—Samples were subjected to electrophoresis in SDS-polyacrylamide gels using the system described by Laemmli (13) with minor modifications. A 15% acrylamide slab gel (15 × 15 × 0.15 cm) was overlaid with a 5% acrylamide stacking gel (2 × 15 × 0.15 cm) that contained 30% glycerol. All buffers contained 2 mM sodium EDTA. Samples for electrophoresis were prepared as follows. Lipid was extracted with chloroform/methanol by the method of Bligh and Dyer (14). The upper aqueous phase, which contained the precipitated protein and greater than 98% of the HMG-CoA reductase activity, was subjected to electrophoresis at a constant current of 40 mA per slab for 3 h at room temperature. The electrophoresis buffer contained 0.05 M Tris, 0.384 M glycine, 2 mM sodium EDTA, and 0.1% SDS at pH 8.3. The gels were fixed and stained with Coomassie Blue G (15), dried to paper, and exposed for 1 h to Kodak X-Omat film. Following autoradiography, the labeled bands were cut out and counted for radioactivity in a well-type scintillation counter. Gels were calibrated with the following molecular weight (M,) standards: phosphorylase B (M, = 94,000); bovine serum albumin (M, = 68,000); ovalbumin (M, = 45,000); carbonic anhydrase (M, = 30,000); soybean trypsin inhibitor (M, = 21,000); and lysozyme (M, = 14,300).

Assays—The total cholesterol content in plasma was assayed by the cholesterol oxidase method (Boehringer Mannheim Cholesterol Test Combination). The content of free and esterified cholesterol in adrenal gland homogenates and in plasma lipoproteins was determined by a previously described method in which the steroids were extracted with chloroform/methanol, the free and esterified cholesterol fractions were separated on silicic acid/Celite columns, the cholesterol esters were hydrolyzed, and the cholesterol content in each fraction was measured by gas-liquid chromatography (16). Correction for procedural losses (which averaged 20%) was made by utilizing [2-14CHDL, [1-14C]cholesterol, [14C]C20:0 fatty acids, and stigmasterol as internal standards (10). The cellular content of cholesterol is expressed as micrograms of sterol/mg of total cell protein. The cellular content of homogenates, microsomal preparations, and lipoproteins was determined by the method of Lowry et al. (17) with bovine serum albumin as a standard.

RESULTS

In untreated DBA/2J mice, the mean plasma cholesterol level was approximately 60 mg/dl. The amounts of free and esterified cholesterol in the adrenal gland were in the range of 25 and 80 µg of sterol/mg of protein, respectively, and the specific activity of HMG-CoA reductase in adrenal microsomes averaged 0.15 nmol·min⁻¹·mg of protein⁻¹. Treatment of the mice with 4-APP for 48 h produced a 50% fall in plasma cholesterol to the range of 5 mg/dl, a 95% fall in the adrenal content of esterified cholesterol to about 5 µg of sterol/mg of protein, no change in the adrenal content of free cholesterol, and a 20-fold increase in the activity of adrenal HMG-CoA reductase to the range of 3.5 nmol·min⁻¹·mg of protein⁻¹. All of these changes were virtually identical to those previously observed in the adrenal gland of 4-APP-treated rats (7). The addition of ACTH to the 4-APP treatment regimen did not significantly affect the magnitude of the changes observed, but did lessen variability between animals. Thus, in all experiments a standard protocol was used in which the mice were treated with 4-APP plus ACTH for 48 h prior to the experiment.

Fig. 1 shows an experiment in which mice were treated with 4-APP plus ACTH for 48 h. The mice were then given various amounts of human HDL as a bolus intravenously and were killed 12 h later. The HDL infusion raised the plasma cholesterol level (Fig. 1A), suppressed adrenal HMG-CoA reductase activity (Fig. 1B), and restored the adrenal content of esterified cholesterol (Fig. 1C). Fig. 2 shows a similar experiment in which mice were infused with various amounts of human LDL instead of HDL. As with the HDL infusion, the infusion of LDL raised the plasma cholesterol level (Fig. 2A), suppressed adrenal HMG-CoA reductase activity (Fig. 2B), and restored the adrenal content of esterified cholesterol (Fig. 2C). The LDL-mediated cholesterol uptake process in the adrenal gland was less efficient than the HDL-mediated uptake process in the injection of larger amounts of cholesterol and the attainment of higher plasma cholesterol levels were necessary to achieve a given effect on the adrenal gland when LDL was used as opposed to HDL.

The data in Figs. 1 and 2 raised the possibility that the adrenal gland of the 4-APP-treated mouse possessed mechanisms for taking up the cholesterol of HDL and LDL. To study these uptake processes in more detail, we turned to the use of lipoproteins labeled with 125I in the protein component. Figure 3 shows an experiment in which mice were treated for 48 h with 4-APP plus ACTH and were then given an intravenous bolus of 2.6 × 10⁹ cpm of human 125I-HDL or 125I-LDL. Groups of mice were killed at various times after the injection and the adrenal content of 125I-radioactivity was measured. After the infusion of either lipoprotein, adrenal 125I-radioactivity rose rapidly and reached a steady state by 15 min. During this steady state, the two adrenal glands of a single mouse contained 2.3% of the administered 125I-HDL radioactivity and 1% of the administered 125I-LDL radioactivity. In both cases, more than 95% of the adrenal 125I-radioactivity was precipitable by 10% trichloroacetic acid.

To test the specificity of the adrenal uptake process, we measured the adrenal content of 125I-radioactivity after infusion of human 125I-LDL, to which mice were treated with various combinations of 4-APP, ACTH, and desamethasone acetate (Fig. 4). For comparative purposes, we also measured the content of radioactivity in two other extrahepatic tissues, kidney and lung. In the adrenal gland, treatment with 4-APP enhanced the uptake of 125I-HDL by 20-fold (Fig. 4A). The addition of ACTH gave a small additional stimulation. In the presence of exogenous ACTH, suppression of endogenous ACTH secretion with dexamethasone had little effect on 125I-HDL uptake. However, in the absence of exogenous ACTH, the administration of desamethasone decreased the uptake of 125I-HDL by 80% (Fig. 4A). In other experiments not shown, we observed that ACTH itself in the
Fig. 1 (left). Effect of varying amounts of human HDL on the plasma cholesterol level (A), adrenal HMG-CoA reductase activity (B), and adrenal cholesterol content (C) of mice previously treated with 4-APP. Five groups of mice (three mice per group) received three doses of 4-APP (40 μg/g of body weight) and ACTH (4 units) at 24-h intervals. The mice then received the indicated amount of human LDL intravenously as a bolus in 200 μl of 0.15 M NaCl containing 2 mg/ml of bovine serum albumin. All of the animals were killed 12 h after the injection, and the six adrenal glands from the three mice in each group were pooled. All measurements were made as described under “Experimental Procedures.” Each point represents the average of duplicate determinations from each pool of six adrenal glands.

Fig. 2 (right). Effect of varying doses of human LDL on the plasma cholesterol level (A), adrenal HMG-CoA reductase activity (B), and adrenal cholesterol content (C) of mice previously treated with 4-APP. Five groups of mice (three mice per group) received three doses of 4-APP (40 μg/g of body weight) (4 units) at 24-h intervals. The mice then received the indicated amount of human LDL intravenously as a bolus in 200 μl of 0.15 M NaCl containing 2 mg/ml of bovine serum albumin. All of the animals were killed 12 h after the injection, and the six adrenal glands from the three mice in each group were pooled. All measurements were made as described under “Experimental Procedures.” Each point represents the average of duplicate determinations from each pool of six adrenal glands.

Absence of 4-APP did not significantly stimulate 125I-HDL uptake. These data indicate that the increase in adrenal 125I-HDL uptake observed in Fig. 4 requires both the lowering of endogenous plasma lipoprotein levels with 4-APP and the presence of endogenous ACTH. The uptake of 125I-LDL in the adrenal gland followed a pattern similar to that of 125I-HDL, except that the maximal uptake of 125I-LDL radioactivity was about half as great as with 125I-HDL (Fig. 4B). In untreated animals, the uptake of 125I-albumin was significantly lower than that for either 125I-LDL or 125I-HDL (45 ± 9 cpm/mg for albumin as compared to 229 ± 18 and 215 ± 35 cpm/mg for LDL and HDL, respectively) and it failed to rise with 4-APP or ACTH treatment (Fig. 4C). Similarly, in untreated animals the uptake values for 125I-LDL and 125I-HDL in the kidney and lung were much lower than for the adrenal gland (Fig. 4, A to C). Administration of 4-APP produced a slight increase in 125I radioactivity in the kidneys after injection of 125I-HDL (Fig. 4A). However, 30% of this radioactivity was not precipitable with 10% trichloroacetic acid (see legend to Table I), suggesting that it represented degradation products of 125I-HDL that may have been formed in other organs. With this exception there were no other effects of 4-APP on ACTH on the uptake of any of the radioactive proteins in kidney or lung.

Table I presents the data of Fig. 4 expressed as total cpm/organ pair. Again, in untreated animals the data for the adrenal gland show a 5-fold higher amount of 125I-HDL or 125I-LDL uptake as compared with 125I-albumin. No such difference existed for kidney or lung, in which the contents of the 125I-lipoproteins and 125I-albumin were comparable.

The data of Fig. 4 and Table I demonstrate that the adrenal gland of the 4-APP-treated mouse has selective uptake systems for 125I-LDL and 125I-HDL. To determine whether these uptake systems were saturable and to determine whether there was cross-competition between the two lipoproteins, we injected mice with 125I-LDL or 125I-HDL in the presence of increasing concentrations of unlabeled LDL or HDL. Fig. 5A shows that the uptake of 10 μg of protein of 125I-LDL was inhibited by excess unlabeled LDL, 50% inhibition occurring with the injection of 1 mg of protein of LDL, which produced a plasma cholesterol level of 125 mg/dl. Unlabeled HDL was much less effective than unlabeled LDL in inhibiting 125I-LDL uptake; 50% inhibition of 125I-LDL uptake was not achieved at the highest level of HDL injected, 10 μg of protein, which produced a plasma cholesterol level of 240 mg/dl.

A converse set of results was obtained when 10 μg of protein of 125I-HDL was injected (Fig. 5B). In this case, unlabeled HDL was a more effective inhibitor of 125I-HDL uptake than LDL, 80% inhibition occurring with the injection of 1 mg of HDL protein, which produced a plasma cholesterol level of 24 mg/dl. In contrast, the injection of 5 mg of protein of LDL, which produced a plasma cholesterol level of 570 mg/dl, was required to produce a 80% inhibition of 125I-HDL uptake.

Fig. 6 shows an experiment in which we tested the effect of unlabeled LDL and HDL on the turnover of 125I-HDL in plasma and adrenal glands. Injection of unlabeled HDL together with 125I-HDL markedly retarded the rate at which the 125I-HDL disappeared from the plasma (Fig. 6A) and completely blocked the uptake of 125I-HDL by the adrenal gland (Fig. 6B). On the other hand, unlabeled LDL had only a small effect in blocking the removal of 125I-HDL from plasma (Fig. 6A) and in blocking 125I-HDL uptake into the adrenal gland (Fig. 6B).

Fig. 7 shows an experiment designed to follow in more detail the turnover of 125I-HDL in the adrenal gland and plasma of the 4-APP-treated mouse. After a bolus injection of 125I-HDL, the disappearance of radioactivity from the plasma was quite rapid with a half-time of about 90 min. The disappearance

![Fig. 3](https://doi.org/10.1074/jbc.M202003.2002031)

**Fig. 3.** Time course of uptake of 125I-HDL (○) and 125I-LDL (●) in the adrenal gland of mice previously treated with 4-APP. Mice received 5 doses of 4-APP (20 μg/g of body weight) and ACTH (2 units) at 12-h intervals. Each mouse was then injected with 15 μg of protein (2.6 x 10⁶ cpm) of either 125I-HDL (●) or 125I-LDL (○). The animals were killed at the indicated time after injection, and the content of 125I radioactivity in the adrenal gland was determined as described under "Experimental Procedures." Each point represents the average of values obtained from three mice. The brackets represent ± S.E. The mean plasma cholesterol level in the mice treated with 4-APP and ACTH was 2.0 mg/dl (range, 0 to 4.6).
A. Lipoprotein Uptake by Mouse Adrenal Gland

Experimental Treatment

- None
- 4-APP
- 4-APP + ACTH
- 4-APP + ACTH + Dex
- 4-APP + Dex

Fig. 4. Uptake of $^{125}$I-HDL (A), $^{125}$I-LDL (B), and $^{125}$I-albumin (C) in the adrenal gland, kidney, and lung of mice subjected to various experimental treatments. Mice were injected with one of the following combinations at 24-h intervals for three doses: a, none; b, 4-APP (40 µg/g of body weight); c, 4-APP plus ACTH (4 units); d, 4-APP plus ACTH plus dexamethasone acetate (4 µg/g of body weight); or e, 4-APP plus dexamethasone acetate. Each mouse was then injected intravenously with 15 µg of protein (2.4 X 10^6 cpm) of either $^{125}$I-HDL (A), $^{125}$I-LDL (B), or $^{125}$I-albumin (C). The animals were killed 60 min after injection, the organs were perfused with a solution of sodium chloride and albumin via the inferior vena cava, and the tissue content of $^{125}$I radioactivity was determined as described under “Experimental Procedures.” Each point represents the average of values obtained from five mice (A and B) or three mice (C). The brackets represent 1 S.E. Data on the plasma cholesterol level, the plasma content of $^{125}$I radioactivity, and the weight of the various tissues are shown in Table I. Dex = dexamethasone acetate.

B. Turnover of $^{125}$I-HDL

Fig. 5. Effect of increasing concentrations of unlabeled LDL (O) and unlabeled HDL (C) on the uptake of tracer concentrations of $^{125}$I-LDL (A) and $^{125}$I-HDL (B) in the adrenal gland of mice previously treated with 4-APP. Mice received three doses of 4-APP (40 µg/g of body weight) and ACTH (4 units) at 24-h intervals. Each mouse was then injected intravenously with either $^{125}$I-LDL (10 µg, 3.4 X 10^6 cpm) (A) or $^{125}$I-HDL (10 µg, 1.9 X 10^6 cpm) (B) together with the indicated amount of unlabeled LDL (O) or unlabeled HDL (C). The animals were killed 30 min after injection, and the content of $^{125}$I radioactivity in the adrenal gland was determined as described under “Experimental Procedures.” Each point represents the average of values obtained from either four mice (O) or two mice (O, C). The mean plasma cholesterol level in the mice treated with 4-APP and ACTH was 5.0 mg/dl (range, 0.5 to 7.7).

Fig. 6. Turnover of $^{125}$I-HDL in the plasma (A) and adrenal gland (B) of mice previously treated with 4-APP: effect of simultaneous injection of unlabeled HDL (C) and LDL (O). Mice received three doses of 4-APP (40 µg/g of body weight) and ACTH (4 units) at 24-h intervals. Each mouse was then injected intravenously with $^{125}$I-HDL (20 µg of protein, 1.6 X 10^6 cpm) together with one of the following unlabeled lipoproteins: O, none; C, HDL, 5 mg of protein; or D, LDL, 5 mg of protein. The animals were killed at the indicated time after injection, and the content of $^{125}$I radioactivity in plasma (A) and in the adrenal gland (B) was determined as described under “Experimental Procedures.” Each point represents the average of values obtained from three mice. The brackets represent 1 S.E. The mean plasma cholesterol level in the mice treated with 4-APP and ACTH was 3.1 mg/dl (range, 0 to 8.8).

Fig. 7. Turnover of $^{125}$I-HDL in the plasma (O) and adrenal gland (C) of mice previously treated with 4-APP. Mice received three doses of 4-APP (40 µg/g of body weight) and ACTH (4 units) at 24-h intervals. Each mouse was then injected intravenously with $^{125}$I-HDL (90 µg of protein, 9 X 10^6 cpm). The animals were killed at the indicated time after injection, and the content of $^{125}$I radioactivity in plasma (O) and in the adrenal gland (C) was determined as described under “Experimental Procedures.” Each point represents the average of values obtained from three or four mice. The “100% value” was 1,720 ± 140 cpm/ml for plasma (O) and 6,200 ± 1,100 cpm/gland for the adrenal gland (C). The mean plasma cholesterol level in the mice treated with 4-APP and ACTH was 10 mg/dl (range, 0 to 24).

The kinetic data of Figs. 6 and 7 are compatible with a mechanism in which the adrenal content of $^{125}$I-HDL is in constant and rapid flux, with the $^{125}$I-HDL having a residence time in the adrenal gland of less than 60 min. To test this...
hypothesis in another way, we injected animals with 15 μg protein of [125I]-HDL, allowed it to be taken up by the adrenal gland for 15 min when a near-steady state had been reached, and then injected a bolus of unlabeled HDL (5 mg of protein) (Fig. 8A). By diluting the specific radioactivity of the circulating [125I]-HDL, the unlabeled HDL prevented further entry of [125I]-HDL into the gland. Within 15 min after the infusion of the unlabeled HDL, the adrenal content of [125I] radioactivity had declined to the same level that it assumed when the unlabeled HDL was injected simultaneously with the labeled HDL at zero time (Fig. 8A). A similar rapid decline in the adrenal content of [125I]-LDL occurred when unlabeled LDL was injected 15 min after the injection of [125I]-LDL (Fig. 8B).

The data of Fig. 5 suggested that HDL did not compete effectively with [125I]-LDL for uptake by the adrenal. This lack of cross-competition allowed us to perform an experiment to determine whether prior uptake of unlabeled HDL could metabolically suppress the uptake of [125I]-LDL (Fig. 9). In one part of this experiment, mice were injected with 5 mg of protein of unlabeled LDL. After varying intervals, the mice were injected with 10 μg of protein of [125I]-LDL and killed 30 min later (Fig. 9A). As expected, the unlabeled LDL competed directly with the [125I]-LDL and hence the adrenal uptake of [125I]-LDL was low at each time point. In the second part of this experiment, the mice were injected with 5 mg of protein of unlabeled HDL instead of unlabeled LDL (Fig. 9B). The unlabeled HDL produced only a slight reduction of [125I]-LDL uptake when the [125I]-LDL was injected simultaneously with the unlabeled HDL, at zero time or when the [125I]-LDL was injected 1 h after the injection of unlabeled HDL. However, when the [125I]-LDL was injected 4 or 8 h after the unlabeled HDL, the uptake of [125I]-LDL was reduced by 75%. These data suggest that the uptake of lipoproteins in the adrenal may be regulated by the cholesterol content and that the cholesterol derived from the prior uptake of unlabeled HDL may have suppressed the uptake of [125I]-LDL.

Whereas human LDL contains only one major protein component, human HDL contains several proteins, of which the major ones are apoproteins A-I and A-II (18). To determine whether both of these apoproteins were being taken up by the adrenal gland, we administered 38 μg of [125I]-HDL in the absence or presence of a 170-fold excess of unlabeled HDL to 4-APP-treated animals and killed the animals after 15 min.

The adrenal glands were homogenized and an aliquot of the whole homogenate was subjected to SDS-polyacrylamide gel electrophoresis. For comparative purposes, a sample of the initial [125I]-HDL and a sample of the plasma from one of the animals, which were also reduced with 2-mercaptoethanol and subjected to SDS-polyacrylamide gel electrophoresis. Electrophoresis was also performed on one sample of [125I]-HDL that was not reduced with 2-mercaptoethanol. The slab gel was subjected to autoradiography (Fig. 10) after which the bands corresponding to A-I and A-II were cut out and their content of [125I] radioactivity was determined in a scintillation counter. In the sample of [125I]-HDL that was not reduced with 2-mercaptoethanol, the two major labeled proteins migrated to positions corresponding to molecular weights of 29,000 and 17,000, which are similar to the reported molecular weights of A-I and A-II, respectively (18-20) (Lane 1, Fig. 10). When the [125I]-HDL was reduced with 2-mercaptoethanol, the A-I band disappeared and a new band at a molecular weight of 8,500 appeared (Lanes 2 and 3, Fig. 10). A-I is known to be reduced to a molecular weight of 8,500 in the presence of 2-mercaptoethanol, whereas A-II is unaffected (18-20). By scintillation counting, the ratio of radioactivity in A-I to A-II was 1.3, and these two bands accounted for 75% of the [125I] radioactivity on the gel.

The electrophoretic pattern of the [125I] radioactivity in the plasma of the mice injected with [125I]-HDL was qualitatively (Lane 5, Fig. 10) and quantitatively similar to that of the starting [125I]-HDL preparation. There was no change in the pattern of plasma radioactivity when the animals received unlabeled HDL together with the [125I]-HDL (Lane 4, Fig. 10). In the animal that received only [125I]-HDLs, the two adrenal glands contained a total of 150,000 cpm. The electrophoretic pattern of the adrenal radioactivity (Lane 6, Fig. 10) was similar to that of the infused [125I]-HDL. The A-I to A-II ratio was 1.5 and the sum of the radioactivity in A-I plus A-II

![Figure 9](http://www.jbc.org/)

**Fig. 9.** Uptake of [125I]-LDL by the adrenal gland of mice previously treated with 4-APP: effect of prior injection of unlabeled LDL (A) and unlabeled HDL (B). Mice received three doses of 4-APP (40 μg/g of body weight) and ACTH (4 units) at 24-h intervals. Each mouse was then injected intravenously with a bolus containing either no unlabeled lipoproteins (C in A and B), unlabeled LDL (5 mg of protein) (C in A), or unlabeled HDL (5 mg of protein) (C in B). After the indicated interval, each mouse was injected intravenously with [125I]-LDL (10 μg, 2.4 x 10⁶ cpm). The animals were killed 30 min after the [125I]-LDL injection, and the content of [125I] radioactivity in the adrenal gland was determined as described under "Experimental Procedures." Each point represents the average of values obtained from three or four mice. The brackets represent 1 S.E. The mean plasma cholesterol level in the mice treated with 4-APP and ACTH was 0.5 mg/dl (range, 2.1 to 13.4).
organ of the 4-APP-treated mouse were compared, the adrenal gland was found to accumulate 20-fold more $^{125}$I-LDL and HDL/mg of tissue than kidney or lung. Second, when various proteins were compared, the adrenal gland was found to accumulate much more $^{125}$I-LDL or $^{125}$I-HDL (0.5 to 2% of injected dose) than $^{125}$I-albumin (0.01% of injected dose) (Figs. 3 and 4 and Table I).

The adrenal lipoprotein uptake process was extremely rapid. Selective uptake was demonstrated within 2 min after injection and the content of $^{125}$I-HDL or $^{125}$I-LDL in the gland reached a maximum within 15 to 30 min. The rate-limiting step in the uptake process was saturable as evidenced by the ability of unlabeled lipoproteins to compete with $^{125}$I-lipoproteins for uptake. This saturable step appeared to be different for LDL and HDL. Thus, the uptake of $^{125}$I-HDL was inhibited more effectively by unlabeled HDL than LDL. Conversely, $^{125}$I-LDL uptake was more effectively competed by unlabeled LDL than by HDL. Based on analogy with lipoprotein metabolism in cultured fibroblasts and adrenal cells (1–4), it seems likely that the saturable rate-limiting step involves binding of the $^{125}$I-lipoproteins to cell surface receptors. The cross-competition experiments raise the possibility that two different receptors are involved, one for LDL and one for HDL.

The uptake of $^{125}$I-LDL and of $^{125}$I-HDL appeared to become regulated by metabolic regulation in the adrenal. Thus, uptake of both lipoproteins was suppressed when ACTH secretion was suppressed with dexamethasone, and this suppression of uptake was prevented by exogenous ACTH. A similar enhancement of $^{125}$I-LDL uptake by ACTH occurs in cultured mouse and bovine adrenal cells (1–3).

The uptake systems for both $^{125}$I-LDL and $^{125}$I-HDL appeared to be functional in mouse adrenal even in the absence of 4-APP treatment as indicated by the 5-fold higher uptake of the two lipoproteins as compared with $^{125}$I-albumin in untreated animals (Table I). The adrenal uptake of $^{125}$I-lipoproteins increased markedly when the animals were treated with 4-APP. This enhancement was due in part to the reduction of endogenous unlabeled lipoproteins so that the labeled $^{125}$I-LDL and $^{125}$I-HDL were no longer diluted with unlabeled material. In addition, it is possible that the number of adrenal lipoprotein binding sites also increased in the 4-APP-treated animals in a manner analogous to the increase in LDL receptors that occurs in cultured fibroblasts and adrenal cells that are deprived of exogenous lipoproteins (1–4). The current types of studies cannot evaluate this latter possibility.

Whether the in vivo adrenal uptake processes involve internalization of the intact lipoprotein or whether they represent only binding to surface structures cannot yet be determined. The turnover experiments of Figs. 6 and 7 suggest that, at least for HDL, the bound $^{125}$I-lipoprotein is in rapid flux. Whether the $^{125}$I-LDL leaves the gland as intact protein or whether it is degraded by the cells in a manner similar to that demonstrated for LDL in cultured cells is not yet known. The pulse-chase experiments of Fig. 9 in which the injection of tracer amounts of $^{125}$I-lipoproteins was followed by a large dose of unlabeled lipoproteins suggested that $^{125}$I-LDL and $^{125}$I-HDL could leave the adrenal gland within 15 min. This seems too rapid to be accounted for by degradation and suggests that a large portion of the $^{125}$I-lipoproteins is initially bound to a surface from which it can be displaced by the unlabeled lipoproteins.

Is the LDL uptake process in the adrenal of the 4-APP-treated mouse mediated by the same LDL receptor that is demonstrable in cultured mouse adrenal cells? Several lines of evidence address this point. In experiments to be published elsewhere, we have been able to demonstrate high affinity binding of $^{125}$I-LDL to membranes prepared from homoge-

**DISCUSSION**

In the current experiments, the adrenal gland of the 4-APP-treated mouse was observed to take up $^{125}$I-labeled human LDL and HDL with selectivity, speed, and saturability. The selectivity was evident in two ways. First, when different
nates of normal mouse adrenal glands. This binding site is similar to the one demonstrated in homogenates of fresh bovine adrenal glands (21) and is similar to the functional LDL receptor in cultured mouse and bovine adrenal cells (2, 3). HDL does not compete for $^{125}$I-LDL binding to this membrane binding site in vitro, an observation that correlates with its reduced ability to compete with $^{125}$I-LDL uptake in vivo. Although indirect, these data are suggestive that LDL uptake in the adrenal in vivo is mediated by the same LDL receptor that is demonstrable in vitro.

How then can the in vivo $^{125}$I-HDL uptake process be explained? As discussed above, the in vivo cross-competition experiments suggest that HDL uptake is mediated by a different receptor than the one for LDL. This putative HDL receptor has not yet been detected in any in vitro system. For example, in experiments not shown, we have found that the same $^{125}$I-HDL preparation that is taken up with great avidity by the adrenal gland of the 4-APP-treated mouse is not taken up by cultured mouse Y-1 adrenal cells, cultured bovine adrenal cells, or cultured human fibroblasts. Moreover, the same HDL preparation that raises the cholesterol content of the mouse adrenal in vivo and suppresses its HMG CoA reductase activity has no such effect on any of the cultured cell systems. Finally, although high affinity $^{125}$I-LDL binding can be demonstrated with isolated adrenal membranes, in vitro binding of $^{125}$I-HDL cannot yet be demonstrated.

Several possible mechanisms might explain the differences between the metabolism of HDL in the adrenal in vivo and in vitro. First, the adrenal gland in vivo may express an HDL receptor that is not expressed by isolated adrenal cells in tissue culture. This HDL receptor in vivo need not be present on adrenal cells per se. It might be present on neighboring cells, such as endothelial or sinusoidal lining cells. Such a receptor could supply cholesterol to adrenal cells by a mechanism analogous to the delivery of fatty acids to adipose cells by lipoprotein lipase bound to the capillary endothelium (22). Finally, the metabolism of HDL in vivo appears to be complex in that its apoproteins can exchange with those of other lipoproteins (23). Thus, even though uptake of $^{125}$I-HDL can be demonstrated as early as 2 min after injection into 4-APP-treated mice, it remains possible that immediately upon infusion the injected lipoprotein is modified into a form that can bind to an adrenal receptor. Such binding would not be detected in vitro since the HDL would have no opportunity to be modified. As discussed above, even if it recognized modified HDL, the putative HDL receptor is not likely to be the LDL receptor, since LDL is not an effective competitor for $^{125}$I HDL uptake.

In summary, while the current in vivo data are not yet definitive as to mechanisms, they suggest that the adrenal gland of the 4-APP-treated mouse expresses two types of lipoprotein receptors. One of these is analogous to the LDL receptor previously demonstrated in cultured adrenal cells and other in vitro systems (1-4) and the other is an HDL receptor whose counterpart has not yet been demonstrated in vitro.

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