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LIPOPROTEIN RECEPTORS AND STEROIDOGENESIS IN ADRENOCORTICAL CELLS

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Summary—Steroid-producing tissues require a continuous supply of cholesterol for hormone synthesis In the majority of the steroidogenic tissues the cholesterol is imported via the receptor-mediated uptake of lipoproteins, and therefore the influence on the lipoprotein receptors provides an additional level for the regulation of hormone synthesis Hormones regulating the andrenocortical activity exert both short- and long-term action, and thus they may control the interactions of the major cholesterol delivery particles—low- (LDLs) and high-density lipoproteins (HDLs)—and their receptors in short- and long-term action, possibly modulating the signal transduction in the former case and the number and distribution in the latter The LDL and HDL pathway and the signal transduction mechanism is briefly reviewed Data are discussed concerning short- and long-term action of hormones (α-MSH and ACTH, respectively) on the HDL_3 receptors of isolated adrenocortical cells Short-term treatment with α-MSH and long-term treatment with ACTH increased the binding of HDL_3 to zona glomerulosa and fasciculata cells, respectively, while both treatments increased the hormone production in the presence of HDL The lipoprotein receptors were frequently found on the microvilli of adrenocortical cell membranes

INTRODUCTION

Corticoids are synthesized within minutes in response to stimuli [1,2] The precursor, cholesterol, is stored in esterified form in the intracellular lipid droplets The cholesterol may originate from local synthesis, which under basal conditions is very low [3], but under stimulated conditions when the activity of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) is enhanced, can serve as a supply for hormone synthesis [4] Most of the adrenal cholesterol comes from the serum lipoproteins [5] All mammalian adrenocortical cells thus far examined have the potential to express the low-density lipoprotein (LDL) pathway (importing the cholesterol by receptor-mediated endocytosis), but in rodents the high-density lipoprotein (HDL) pathway for cholesterol delivery is superior [5]

The hormones regulating the adrenocortical function exert their effects in short-term action or increase the long-term capacity for steroidogenesis [1] In the short-term action the main tropic hormone of the adrenal cortex, ACTH, increases the availability of cholesterol for the synthesis of corticoids by increasing the activity of cholesterol esterase [1] and by increasing the delivery of cholesterol to the inner mitochondrial membrane Once the rate-limiting conversion of cholesterol to pregnenolone is fully activated, the supply of cholesterol through the import of lipoproteins or local synthesis may become rate-limiting In the long-term action, tropic hormones boost the plasma level of corticoids by increasing the activities of steroidogenic enzymes and of the cholesterol side-chain cleavage system or by raising the number of lipoprotein receptors [4, 6]

LDL RECEPTORS AND SIGNAL TRANSDUCTION

LDL pathway

Brown and Goldstein identified those plasma membrane receptors which initiate the uptake of plasma LDL particles into the cell via endocytosis [7] Similarly to many receptors involved in the pathways of endocytosis (either constitutive or ligand-induced), these receptors then cluster in clathrin-coated pits, invaginate, enter the cell via clathrin-coated vesicles and, after discharging their ligands in the endosome, return to the surface while the ligands are degraded in the lysosomes The factors regulating the initiation

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and subsequent route of internalization are not fully understood

Signal transduction

Some of the events following ligand–receptor interaction are known The internalization of LDLs and subsequent lysosomal release of free cholesterol down-regulates the number of LDL receptors, suppresses the activity of HMG-CoA reductase (the key regulatory enzyme of cholesterol biosynthesis), and stimulates the activity of acyl CoA–cholesterol acyltransferase (ACAT, the enzyme esterifying free cholesterol) thus coordinately regulating the cell's requirement for free cholesterol [8] Other events that may be involved in cellular regulation of the cholesterol metabolism have also been described The soluble protein kinase which phosphorylates the last serine residue in the cytoplasmic domain of the LDL receptor has been purified from adrenal cytosol [9] The interaction of LDLs and receptors stimulates the intracellular phosphoinositide metabolism, elevates $[Ca^{2+}]_i$ and enhances the phosphorylation of cellular proteins in various cell types [10–13] The LDLs induce early growth-related events, i e they transcribe the expression of c-*fos* and c-*myc* in vascular smooth muscle cells [14, 15] Events directly related to the intracellular cholesterol metabolism have been observed in adrenocortical cells, while the others have not been studied

Regulation of LDL receptor activity, biochemical properties

Growth factors (platelet-derived growth factor, endothelial cell-derived growth factor and the postulated macrophage-derived growth factor), hormones (estrogens, thyroid hormones and insulin) [8] and the constituents of the dietary fat [16, 17] could stimulate the activity of the LDL receptors of various cells Some of these mechanisms are predictably active in the adrenocortical cells we observed increased numbers of macrophages in lipid-laden cells of the adrenal cortex of rabbits fed with a high cholesterol-containing diet [18], the phenomenon may indicate the active role of macrophages in the increased uptake of LDL The stimulatory effect of ACTH on the expression of lipoprotein receptors in adrenocortical cells has been described [5]. The adrenal gland is the organ and the highest density of LDL receptors [4] and these receptors have been characterized [19]

Localization of LDL receptors on the adrenocortical cells

The LDL receptors have been localized on non-steroidogenic tissues by different methods [20, 22] In our studies the receptors were localized by using gold probes conjugated to an anti-receptor IgG [20] or a ligand [23] Both immuno- and affinity-cytochemistry localized the LDL receptors at the sites of microvilli and in coated pits on the surface of Y-1 adrenocortical cells, and in vesicles and endosomes inside the cells (Figs 1–3)

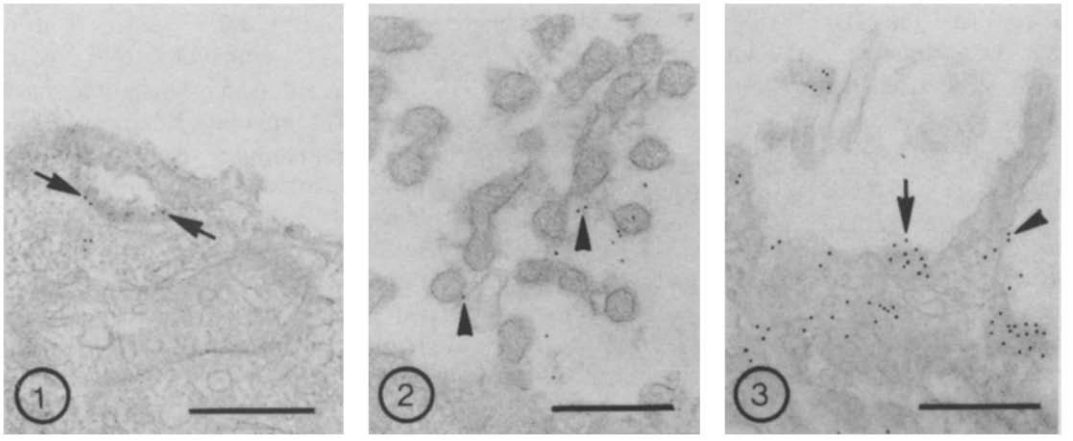
HDL RECEPTORS AND SIGNAL TRANSDUCTION

HDL pathway

HDLs are thought to be promoters of cholesterol efflux from extrahepatic, non-steroidogenic cells, while in steroidogenic tissues they provide cholesterol for corticoidogenesis [5] HDL binding proteins have been identified [24–27] Their physiological role may be the regulation of cholesterol synthesis and esterification [28], the translocation of cholesterol from intracellular membranes to the cell surface [29] or the catabolism of HDL [30], since the binding sites are not necessary for cholesterol transport between cells and HDLs [29, 31] The HDLs seem to interact with steroidogenic cells in two ways [5, 32] (i) through apoB/E receptors [33] and (ii) HDL_3 (devoid of apoE) through apolipoprotein A-I [34, 35] With the exception of macrophages, the binding of HDLs to specific receptors on the cell surface does not result in endocytosis of a great amount of receptor–HDL complex [36, 37]

Signal transduction

The action of HDLs at a cellular level is less well established than that of LDLs In addition to providing cholesterol as a substrate, HDLs may influence the membrane composition and in this way increase corticoid production It has been reported that an increased cholesterol content of the plasma membrane increases the binding of ACTH to its receptor in mouse adrenocortical Y-1 cells [38] There are data indicating that the mechanism by which HDLs alter the steroidogenic response involves the calcium metabolism Cholesterol and HDLs alter the free calcium level in erythrocytes [39] HDLs may act through cell surface receptors to activate intracellular second messenger pathways the cyclic AMP pathways or the phosphoinosi-



Figs 1–3 Electron micrographs of parts of the Y-1 cells in culture LDL receptors were localized by the dinitrophenol-IgG immunogold technique [20] LDL receptors on the microvilli (arrows), and at the base of the microvilli (arrowheads) Fig 1 Receptors in a coated pit Fig 2 Receptors on microvilli Fig 3 Many LDL receptors on the microvilli after application of 25 μmol/l compactin (inhibitor of HMG-CoA reductase) for 24 h Bars 0 5 μm

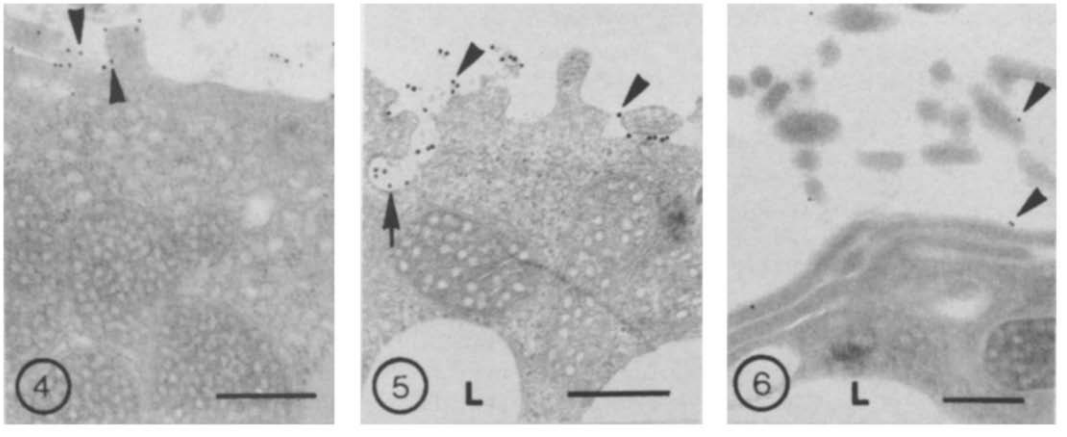
tide pathway [40] Exposure of cells to HDLs generates an intracellular signal (release of calcium from intracellular stores) induced by a lipid component of HDL_3 and possibly mediated by IP_3, and it is suggested that the HDL-induced Ca^{2+} release may be a signal for mitogenesis [41] Although there are many similarities between the LDL and HDL cholesterol metabolisms, there are also important differences with regard to the hydrolysis of lipoprotein cholesteryl ester [42]

Regulation of HDL receptor activity, biochemical properties

Stimuli have been shown to affect HDL binding to a variety of cells after loading with cholesterol [37, 43], or after treatment with growth factors [44] and hormones [24, 45] ACTH induces the expression of HDL receptors on undifferentiated glomerulosa-like cells in culture [46] The HDL receptor is insensitive to pronase or phospholipase treatment [47], but it is calcium ion dependent [48] Lysine and arginine residues seem not to be involved in HDL binding, but tetranitromethane modification of HDLs has been shown to prevent their binding [49] The binding is more enhanced at 37 than at 4°C [52]

Distribution of HDL receptors on adrenocortical cells

The distribution of HDL receptors on the plasma membrane of steroidogenic tissue has been investigated by several methods [51, 52]



Figs 4–6 Electron micrographs of parts of zona fasciculata cells The rats were treated with ACTH for 14 days (30 μg/kg/day) HDL receptors on the microvilli (arrows), and at the base of the microvilli (arrowheads) The isolated zona fasciculata cells from the untreated (Fig 4) and the treated (Fig 5) rats were incubated with colloidal gold-labelled HDL_3 (0 1 ml, protein content <70 μg) for 5 h at 37°C, and processed for electron microscopy Fig 6 Immunogold localization of apoA-I on ultrathin frozen section of ACTH-treated rats The method of Tokuyashu [61] was used Bars 0 5 μm

We localized the HDL binding by an indirect method in different functional states of rat adrenocortical cells (Figs 4–6)

EFFECT OF SHORT-TERM HORMONE STIMULATION ON THE LIPOPROTEIN RECEPTORS OF ADRENOCORTICAL CELLS

In acute, *in vitro* studies, it is assumed that abundant stores of cholesterol within cytoplasmic lipid droplets provide sufficient substrate for corticoidogenesis However, there are circumstances (treatment with serum cholesterol-lowering drugs) when the reduced concentrations of plasma and adrenal cholesterol limit both basal and stimulated steroid output [53–55] After isolation, the cells are devoid of extracellular cholesterol, which in turn could influence their steroidogenic capacity

Freshly isolated adrenocortical zona glomerulosa cells increase their angiotensin-II, K^+- and ACTH-stimulated hormone production upon reincubation with HDL [56] These studies showed that, in short-term incubations of fresh tissue, the supply of cholesterol may be a limiting factor in aldosterone synthesis

The rodents may be the only mammalian species whose adrenocortical cells use the cholesterol from the HDLs more than that from the LDLs as a source of steroid precursor [5, 32] Hammami *et al* [35, 37] showed that the addition of whole plasma HDLs to ACTH-stimulated adrenocortical cells in culture resulted in a saturable concentration-dependent enhancement of corticoid production, and that the HDLs are partly internalized and take phospholipids and apolipoproteins into the cells The mechanism of corticoid induction is not known We studied the effect of an increasing concentration of HDL_3 on the α-MSH-stimulated corticoid production of zona glomerulosa cells in short-term (5 h) experiments [58, 59] The binding and uptake of HDL_3 was detected via the colloidal gold labelling of the HDL_3 at electron microscopic level The majority of the gold particles could be seen on the plasma membrane, frequently on the surface of microvilli, and rarely in coated pits and vesicles Besides lysosomes, endosomes and multivesicular bodies contained gold particles After short-term α-MSH treatment the qualitative distribution of the gold particles was unchanged, but the surface-bound HDL–gold particles were increased significantly (Fig 7) The α-MSH-stimulated aldosterone production was further increased by the addition of HDL_3 (Fig 8)

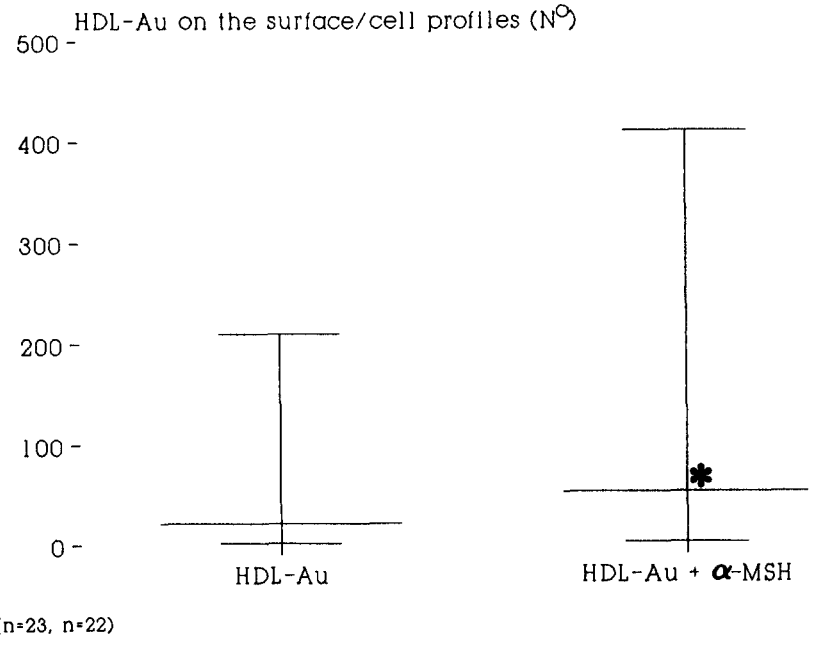


Fig 7 Distribution of colloidal gold-labelled HDL_3 on the surface of isolated zona glomerulosa cells of rats The cells were derived from untreated rats Aliquots of cell suspension were incubated with labelled HDL_3 in the absence and presence of 1 3 × 10E-7 mol/l α-MSH At the end of incubation, 5 parallel tubes from each experiment were combined and processed for electron microscopy The cells were photographed at their entirety and the gold particles were counted on the cell surface, then expressed as particles on cell surface/cellular profile Numbers of gold particles were compared by Kruskal–Wallis non-parametric analysis [62] The data (median, minimum and maximum) obtained in this way were used to express the HDL_3 binding sites The median values of the surface-bound HDL_3 differs significantly ($^*P < 0\,05$)

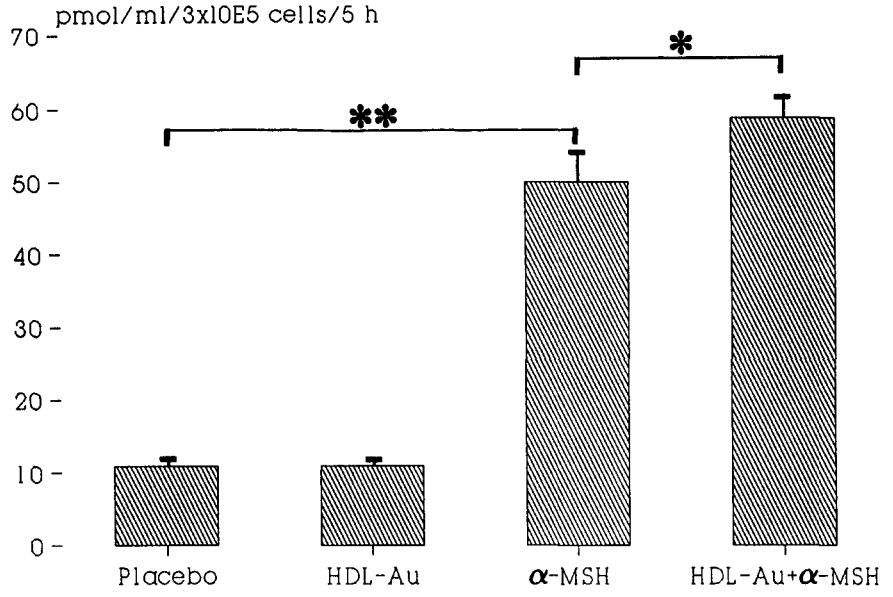


Fig 8 Aldosterone production of zona glomerulosa cells of rats The isolated cells were incubated with or without HDL_3-Au in the absence or presence of 1 3 × 10E-7 mol/l α-MSH (The same experiments as described in Fig 7) At the end of incubation, 3–4 parallel tubes from each experiment were combined and used for determination of aldosterone by RIA [63] The data were compared by analysis of variance and Dunnet contrasts [64] (*$P < 0\,05$, **$P < 0\,01$)

EFFECT OF LONG-TERM HORMONE STIMULATION ON THE LIPOPROTEIN RECEPTORS OF ADRENOCORTICAL CELLS

Simultaneous increase in the number of LDL receptors and aldosterone production has been described in cultured bovine zona glomerulosa cells in response to angiotensin II stimulation suggesting that in analogy to ACTH, angiotensin II can influence receptor-mediated uptake of LDL [60]

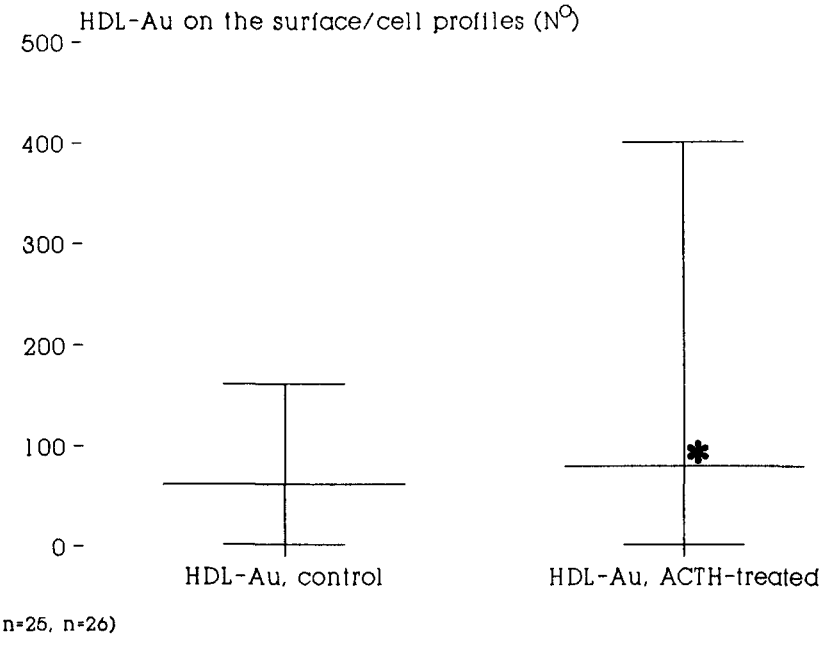


Fig 9 Distribution of colloidal gold-labelled HDL_3 on the surface of zona fasciculata cells derived from untreated and ACTH-treated rats (the same experiment as described in Figs 4–6) The cells were photographed in their entirety and the gold particles were counted on the cell surfaces, then expressed as particles on cell surface/cellular profile Numbers of gold particles were compared by Kruskal–Wallis non-parametric analysis [62] The data (median, minimum and maximum) obtained in this way were used to express the HDL_3 binding sites The median values of the surface-bound HDL_3 differs significantly (*$P < 0\,05$)

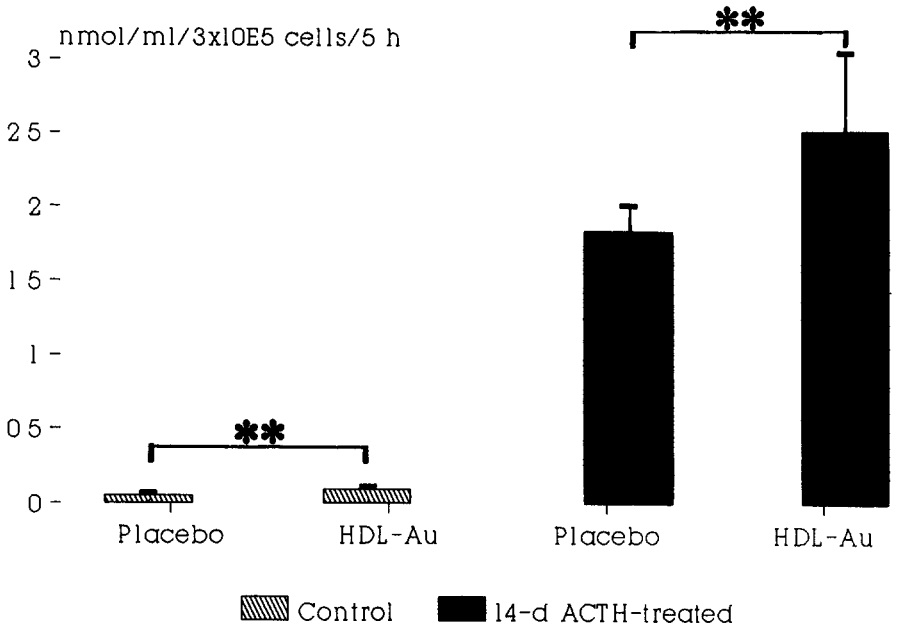


Fig 10 The resting corticosterone production of zona fasciculata cells derived from placebo- and ACTH-treated rats (14 days, the same experiment as described in Figs 4–6) The cell suspensions were incubated with colloidal gold-labelled HDL for 5 h at 37°C, and processed for determination of the corticosterone production by RIA [63] The values were compared by analysis of variance and Dunnet contrasts [64] (**$P < 0\ 01$)

In biochemical studies the HDL_3 receptor has been shown to be up-regulated by ACTH [24, 25, 32] We studied the binding and internalization of colloidal gold-labelled HDL_3 by isolated zona fasciculata cells derived from long-term (14 days) ACTH-treated rats The HDL_3–gold particles were bound to the cell surface, mainly at the sites of microvilli, and were accumulated in the lysosomes, both in non-treated and in long-term treated rats (Figs 4–6) Quantitative analysis of the distribution of the gold particles revealed a significant increase in the binding of the HDL_3 to the cell surface as a result of the chronic ACTH treatment (Fig 9) The corticosterone production of the isolated zona fasciculata cells increased in ACTH-treated rats (Fig 10) Long-term ACTH treatment resulted in an increase in the intracellular cholesterol through an enhanced uptake of the plasma HDL_3 HDL_3 can stimulate hormone production, possibly by providing more accessible cholesterol, or by providing an optimal lipid environment for the association of cholesterol and cytochrome $P\text{-}450_{scc}$ The steroidogenic effect of HDL is most striking on the basal hormone-producing activity in long-term ACTH-treated rats

CONCLUSIONS

In steroid hormone-producing tissues of many species, cholesterol is the major source of steroidogenic substrate The lipoprotein cholesterol is hormonally regulated and coordinated with intracellular cholesterol synthesis and mobilization of cholesteryl esters from lipid droplets Study of the interaction of lipoproteins with cells is important from several aspects the components of lipoproteins function as sources of lipid constituents and steroid hormones, and lipoproteins convey signal transduction The post-ligand binding events need more detailed molecular biological studies

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