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SHORT COMMUNICATION

EFFECT OF HIGH AND LOW DENSITY LIPOPROTEINS ON CORTICOTROPIN-MEDIATED CORTISOL SYNTHESIS BY BOVINE ZONA FASCICULATA CELLS

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Summary—The role of bovine HDL and LDL in supporting corticotropin-stimulated steroidogenesis has been investigated, using acutely dispersed zona fasciculata cells. Using a dose of corticotropin sufficient to maximally stimulate steroidogenesis (in the absence of lipoproteins) both HDL and LDL increased steroidogenesis in a dose-dependent manner. At higher concentrations of lipoprotein, HDL caused approx 3-fold greater increase in steroid production than did LDL. Taken with the knowledge that HDL is the major cholesterol carrier in bovine serum, these findings suggest that in cattle HDL is a major source of cholesterol for steroidogenesis.

INTRODUCTION

Lipoproteins are generally recognised as the major source of cholesterol for steroidogenesis by steroid-producing tissues such as adrenal cortex and ovaries [1, 2]. However the class of lipoprotein utilised by the various tissues remains controversial.

Using rats treated with 4-aminopyrazolo [3, 4-d] pyrimidine to reduce serum lipoprotein levels, Andersen and Dietschy[3] found that plasma corticosterone levels were 6- to 12-fold higher following infusion with HDL than following infusion with LDL. In similar experiments with mice it has been shown that both HDL and LDL are taken up and utilised by the adrenal gland [4]. Studies with isolated rat adrenal quarters *in vitro* demonstrated that the rate of transfer of cholesterol into the adrenal was 2- to 3-fold greater from HDL than from LDL [5]. In contrast experiments with cultured adrenal tumour cells from mouse have shown that these cells have a high-affinity, receptor-mediated pathway for the utilisation of cholesterol from LDL but are essentially unable to utilise cholesterol supplied as HDL [6]. Similarly, cultured adrenocortical cells from bovine adrenals utilise LDL cholesterol to support steroidogenesis. HDL, even when used at a 10-fold higher concentration of cholesterol, was unable to act as a source of cholesterol [7].

This inability of cultured bovine cells to utilise cholesterol from HDL is somewhat surprising as approx 80% of bovine serum cholesterol is present in the HDL component. In contrast LDL contains only approx 15% of serum cholesterol [8]. We have therefore investigated in this study the effect of bovine HDL and LDL on basal and corticotropin-stimulated steroidogenesis, using acutely dispersed zona fasciculata cells from bovine adrenals.

EXPERIMENTAL

Zona fasciculata cells were prepared from fresh bovine adrenals using a Percoll gradient [9] and stored at 4°C in alpha-minimum essential medium [Gibco Europe] (adjusted to pH 6.8 at 22°C) containing 2% (w/v) bovine serum albumin. Prior to use, cells were collected by centrifugation and resuspended in fresh alpha-minimum essential medium, containing 0.5% (w/v) bovine serum albumin, in which all incubations were carried out. Where appropriate, corticotropin$_{1-24}$ was present at a concentration of 100 pg/ml. HDL and LDL were purified from fresh citrated bovine blood, using ultracentrifugation, followed by gel filtration and heparin-Sepharose chromatography. The purified HDL and LDL migrated as single bands on agarose gel electrophoresis with α and β mobilities respectively. The mass ratio of total cholesterol to total protein was 1.8:1 and 3.6:1 for HDL and LDL [10]. Total cholesterol in lipoproteins was determined colorometrically using an enzymatic kit supplied by Boehringer Mannheim. The protein content of cells and lipoproteins was determined by the method of Bradford[11].

Cortisol was determined by radioimmunoassay using [1,2,6,7-^{3}H]cortisol (Amersham International) and rabbit anti-cortisol-21-thyroglobulin serum (Miles Laboratories). Corticotropin$_{1-24}$ was from Ciba-Geigy Pharmaceuticals.

RESULTS

As shown in [9], corticotropin (100 pg/ml) causes a 3–5-fold increase in cortisol production by isolated bovine zona fasciculata cells (Fig. 1). Addition of bovine HDL and LDL significantly enhances cortisol production, both in the presence and absence of corticotropin (Fig. 1). The enhancement by the lipoproteins is dose-dependent and saturable, with maximum enhancement being observed in the presence of corticotropin at a lipoprotein cholesterol concentration of approx 130 and 50 μg/ml for HDL and LDL respectively. At optimal cholesterol concentrations HDL is approx 3-fold more effective than LDL in promoting steroidogenesis by the isolated cells but this difference is much less pronounced at lower levels of cholesterol. The values of lipoprotein cholesterol used compare with a total cholesterol concentration in bovine serum of approx 800 μg/ml (S. R. Cordle, unpublished) of which 80% is present in HDL and 15% in LDL [8].

Pre-incubation of the cells with the lipoproteins prior to the addition of corticotropin leads to greater rates of cortisol production than when lipoprotein and corticotropin are supplied simultaneously (Table 1). This indi-

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Abbreviations: HDL, high density lipoprotein. LDL, low density lipoprotein.

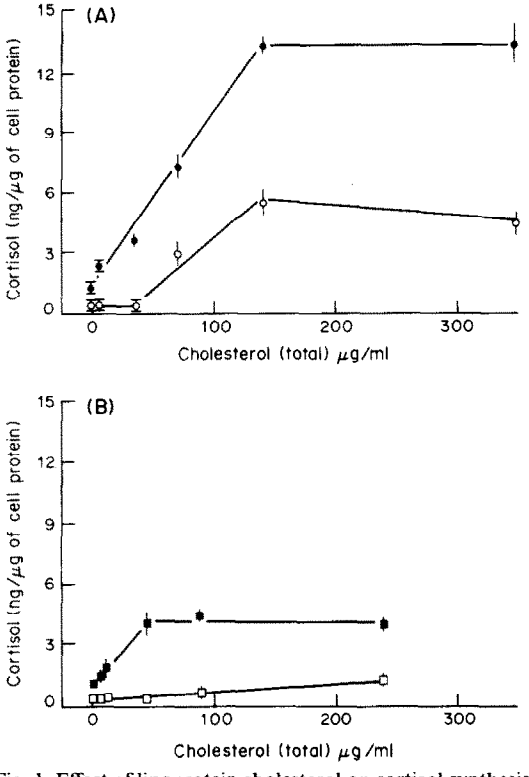

Fig. 1. Effect of lipoprotein cholesterol on cortisol synthesis. Zona fasciculata cells were diluted to a concentration of 4 μg cell protein/ml in alpha-minimum essential medium, and incubated at 37°C in the presence of lipoproteins ± corticotropin$_{1-24}$ (100 pg/ml) as indicated. After 120 min incubations were terminated by addition of methanol (58% final concentration) and cortisol determined. A (○) HDL; (●) HDL + corticotropin; B (□) LDL; (■) LDL + corticotropin. All determinations were carried out in duplicate. For cholesterol values below 50 μg/ml, $n = 4$; for higher values, $n = 3$.

cates that the uptake of cholesterol from lipoprotein is not corticotropin-dependent, as is also suggested by the observation that the lipoproteins increase basal rates of cortisol synthesis (Fig. 1). Previous work [5] using rats has also indicated that uptake of cholesterol from HDL and LDL is not dependent on corticotropin, although uptake from HDL is stimulated by corticotropin. Whether cholesterol uptake is influenced by corticotropin in this system has yet to be investigated. As yet there is not evidence to suggest that *in vivo* changes in the circulating levels of lipoproteins can influence cortisol production.

DISCUSSION

Taken with the knowledge that HDL is the major cholesterol carrier in bovine serum, the findings reported here suggest that HDL is a significant physiological source of the cholesterol used by bovine zona fasciculata cells to support steroidogenesis. This does not of course imply that LDL is unimportant in this regard and the receptor-mediated mechanism for uptake of cholesterol from LDL [12] is well-established. Our results are in accord with observations *in vivo* and *in vitro* in other species including rat [3] and mouse [4], but are at variance with results obtained using cultured bovine adrenal cells [7], which utilise LDL predominantly. The reason for this discrepancy is not clear but, in view of the fact that cultured mouse tumour cells also utilise LDL as the major source of cholesterol, it is possible that cells in culture lose the ability to utilise HDL.

Table 1. The effect of pre-incubation with lipoproteins on cortisol production

	Lipoprotein	Cortisol production* (ng/μg cell protein) ± SD: Control	Cortisol production* (ng/μg cell protein) ± SD: Corticotropin (100 pg/ml)
	—	0.26 ± 0.06	1.89 ± 0.76
A.	LDL	0.60 ± 0.25	3.98 ± 0.47
	HDL	1.76 ± 0.82	9.42 ± 2.08
B.	LDL	1.39 ± 0.25	7.83 ± 1.28
	HDL	0.86 ± 0.40	13.99 ± 1.59

*Represents net cortisol synthesis during 60 min incubation. Each determination was carried out in duplicate. For A, $n = 3$; for B and the incubation minus lipoproteins, $n = 4$.

Where indicated, cells were incubated with lipoproteins containing 35 μg total cholesterol, under conditions as in Fig. 1 except that cortisol production was determined after 60 min incubation. In A, lipoproteins and corticotropin (when appropriate) were added at start of incubation, after pre-incubation of the cells for 60 min at 37°C. In B, cells were pre-incubated with lipoproteins for 60 min at 37°C prior to incubation with corticotropin (when appropriate).

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REFERENCES

1. Brown M. S., Kovanen P. T. and Goldstein J. L.: Receptor-mediated uptake of lipoprotein-cholesterol and its utilization for steroid synthesis in the adrenal cortex. *Recent Prog. Horm. Res.* **35** (1979) 215–257.
2. Strauss J. F. III, Schuler L. A., Rosenblum M. F. and Tamaka T.: Cholesterol metabolism by ovarian tissue. *Adv. Lipid Res.* **18** (1981) 99–157.
3. Andersen J. M. and Dietschy J. M.: Relative importance of high and low density lipoproteins in the regulation of cholesterol synthesis in the adrenal gland, ovary and testis of the rat. *J. biol. Chem.* **253** (1978) 9024–9032.
4. Kovanen P. T., Scheider W. J., Hillman G. M., Goldstein J. L. and Brown M. S.: Separate mechanisms for the uptake of high and low density lipoproteins by mouse adrenal glands *in vivo*. *J. biol. Chem.* **254** (1979) 5498–5505.
5. Gwynne J. T., Mahaffee D., Brewer H. B. Jr and Ney R. L.: Adrenal cholesterol uptake from plasma lipoproteins: Regulation by corticotropin. *Proc. natn. Acad. Sci. U.S.A.* (1976) 4329–4333.
6. Faust J. R., Goldstein J. L. and Brown M. S.: Receptor-mediated uptake of low density lipoprotein and utilization of its cholesterol for steroid biosynthesis in cultured mouse adrenal cells. *J. biol. Chem.* **252** (1977) 4861–4871.
7. Kovanen P. T., Faust J. R., Brown M. S. and Goldstein J. L.: Low density lipoprotein receptors in bovine adrenal cortex. I. Receptor-mediated uptake of low density lipoprotein and utilization of its cholesterol for steroid synthesis in cultured adrenocortical cells. *Endocrinology* **104** (1979) 599–609.
8. Terpstra A. H. M., Sanchez-Muniz F. J., West C. E. and Woodward C. J. H.: The density profile and cholesterol concentration of serum lipoproteins in domestic and laboratory aniamls. *Comp. biochem. Physiol.* **71B** (1982) 669–673.

9. Koper W. J. and Yeaman S. J.: Characterization of a homogeneous preparation of zona fasciculata cells from bovine adrenal cortex. *Biochem. Soc. Trans.* **11** (1983) 705.
10. Cordle S. R., Clegg R. A. and Yeaman S. J.: Purification and characterization of bovine lipoproteins: resolution of HDL and LDL using heparin–Sepharose chromatography. *J. Lipid Res.* (1985). In press.
11. Bradford M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72** (1976) 248–254.
12. Brown M. S. and Goldstein J. L.: Receptor-mediated endocytosis: Insights from the lipoprotein receptor system. *Proc. natn. Acad. Sci. U.S.A.* **76** (1979) 3330–3337.