Apolipoproteins AI, AII, and CI stimulate placental lactogen release from human placental tissue. A novel action of high density lipoprotein apolipoproteins.

S Handwerger, … , J Barrett, I Harman

J Clin Invest. 1987;79(2):625-628. https://doi.org/10.1172/JCI112857.

Research Article

High density lipoproteins (HDL) stimulated a dose-dependent increase in the release of placental lactogen (hPL) from human placental explants. The stimulation was not prevented by delipidation of HDL but was completely blocked by tryptic digestion. Delipidated apolipoproteins (Apo) AI, AII, and CI also stimulated hPL release but other apolipoproteins were without effect. HDL and Apo CI had no effects on the release of luteinizing hormone and follicle-stimulating hormone from rat pituitary cells or the release of prolactin from human decidual cells. Because placental cells have specific HDL receptors and plasma HDL concentrations increase during pregnancy, these results strongly suggest a role for HDL in the regulation of hPL release during pregnancy possibly independent of their usual role in plasma lipid transport.

Apolipoproteins AI, AII, and CI Stimulate Placental Lactogen Release from Human Placental Tissue

A Novel Action of High Density Lipoprotein Apolipoproteins

S. Handwerger, S. Quarfordt, J. Barrett, and I. Harman
Departments of Pediatrics, Physiology, and Medicine, Duke University Medical Center, Durham, North Carolina 27710

Abstract

High density lipoproteins (HDL) stimulated a dose-dependent increase in the release of placental lactogen (hPL) from human placental explants. The stimulation was not prevented by delipidation of HDL but was completely blocked by tryptic digestion. Delipidated apolipoproteins (Apo) AI, AII, and CI also stimulated hPL release but other apolipoproteins were without effect. HDL and Apo CI had no effects on the release of luteinizing hormone and follicle-stimulating hormone from rat pituitary cells or the release of prolactin from human decidual cells. Because placental cells have specific HDL receptors and plasma HDL concentrations increase during pregnancy, these results strongly suggest a role for HDL in the regulation of hPL release during pregnancy possibly independent of their usual role in plasma lipid transport.

Introduction

Human trophoblast cells, which synthesize and secrete both steroid and protein hormones, have specific membrane receptors for high density lipoproteins (HDL)[1] (1). The effects of HDL on the synthesis and secretion of steroid and protein hormones and on other aspects of placental physiology, however, are unknown. A major role for HDL in placental steroidogenesis appears unlikely because low density lipoproteins (LDL) appear to be the primary source of cholesterol for placental progesterone synthesis (2).

During pregnancy, human trophoplast cells synthesize and secrete large amounts of human placental lactogen (hPL), a protein hormone with striking chemical and biological similarities to human growth hormone and human prolactin (3). First detected in maternal plasma at ~6 wk of gestation (4), hPL affects maternal lipid, carbohydrate and protein metabolism (5), and stimulates fetal somatomedin production (6). However, despite numerous investigations, the factors regulating the release of hPL are poorly understood. Because both plasma HDL (7–9) and hPL (4) concentrations increase progressively during pregnancy, we have examined whether HDL stimulates the release of hPL from human placental explants.

Methods

Human placental explants were prepared and incubated as previously described (10). After a 24-h preincubation in medium RPMI 1640, the explants were incubated for up to 6 h in either fresh control medium or fresh medium containing HDL, LDL, or different apolipoproteins. Aliquots of the medium after 1, 3, or 6 h of culture were assayed for hPL by specific homologous radioimmunoassay (11). HDL (density 1.10–1.21 g/ml) and LDL (density 1.012–1.063 g/ml) were prepared from pooled normal human nonpregnant female sera by the method of Havel (12). Apoproteins A, C, and E were prepared from human very (V)LDL or HDL by the methods of Edelstein (13), Shore (14), and Shelburne (15), respectively, using molecular sieve DEAE or heparin affinity chromatography. Analysis of the individual apoproteins by overload (100 μg) SDS (16), and urea (17) polyacrylamide gel electrophoresis indicated that each of the apoproteins was >98% purified.

For selected experiments, HDL was delipidated at 4°C with ethanol-ether by the method of Brown (18). Analysis of the delipidated HDL for phospholipid (19), cholesterol (20), and triglyceride (21) indicated that the delipidation procedure removed >98% of the phospholipids and neutral lipids. In other experiments, HDL (600 μg/ml) was treated with trypsin (Sigma Chemical Co., St. Louis, MO) at a ratio of 1 μg trypsin/150 μg HDL for 16 h at 37°C. At the end of the 16 h, trypsin soybean inhibitor (3 μg) was added to the reaction vessels to stop the proteolytic reactions. Explants incubated in control medium that had been exposed to trypsin and soybean inhibitor at the same concentration used for the digestion of HDL released the same amounts of hPL as control explants. Statistical differences between sample means were tested by analysis of variance. Treatment contrasts were performed using Dunnett's test or the Newman-Kuels test.

Results

Human placental explants exposed for 6 h to HDL released significantly more hPL than control explants (Fig. 1). The stimulation was greatest during the first hour of exposure but persisted throughout the exposure period. The effect of HDL was dose-dependent with maximal stimulation of hPL release of ~250% at HDL concentrations equal to or greater than 4.0 mg/ml (Fig. 2). The half-maximal effective dose of HDL was 0.35–0.40 mg/ml, which is below the normal mean female plasma HDL protein concentrations (1.6 mg/ml) (22). LDL also stimulated hPL release, but the stimulatory effect was not dose-dependent and the maximal stimulation was only 16.1% that of HDL (Fig. 2).

Delipidation of lyophilized HDL with ethanol-ether at 4°C

This work was presented in part at the 68th Annual Meeting of the Endocrine Society, Anaheim, CA, June, 1986. Address correspondence to Dr. Handwerger, Duke Medical Center, Box 3080, Durham, NC 27710.

Received for publication 25 September 1986.

1. *Abbreviations used in this paper:* HDL, high density lipoproteins; hPL, human placental lactogen; LDL, low density lipoproteins; TPA, 12-*O*-tetradecanoyl phorbol 13-acetate; VLDL, very low density lipoproteins.

J. Clin. Invest.

0021-9738/87/02/0625/04 $1.00
Volume 79, February 1987, 625–628

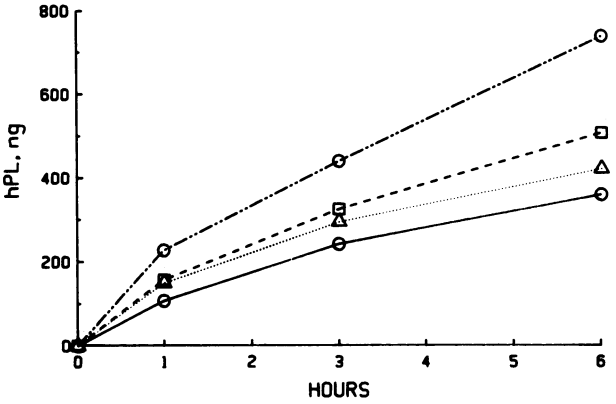

Figure 1. The effects of HDL on the release of hPL from human placental explants. Human placental explants were incubated for 6 h in medium containing 0 (*open circle, solid lines*), 66 (*open triangles*), 200 (*open squares*) or 600 μg (*open circles, dashed lines*) HDL/ml (Bradford protein). The amounts of hPL released at 1, 3 and 6 h after exposure to each of the concentrations of HDL were statistically different from that of control explants ($P < 0.05$ for 66 and 200 μg HDL/ml at each time point; $P < 0.01$ for 600 μg HDL/ml).

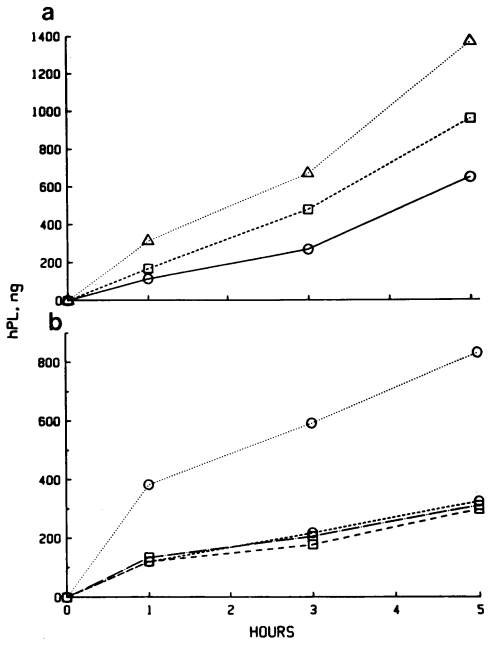

Figure 3. The effects of delipidation (*a*) and tryptic digestion (*b*) on the stimulation of hPL release by HDL. (*a*) Placental explants were incubated for 1 h in control medium (*open circles, dashed lines*) or medium containing intact HDL (300 μg/ml) (*open boxes*) or HDL (300 μg/ml) which had been delipidated at 4°C with ethanol-ether (*open triangles*). (*b*) Placental explants were exposed for 1 h to control medium (*open circles, dashed lines*) or to medium containing either HDL (600 μg/ml) (*open circles, dotted lines*), HDL (600 μg/ml) treated with trypsin (*open boxes, dashed lines*), or control medium treated with trypsin (*open boxes, dot dashed lines*).

to produce nearly complete removal of lipids (>98%) caused no loss of stimulatory activity (Fig. 3 *a*). In fact, the amount of hPL released by delipidated HDL (0.6 mg/ml) was 154.6% greater than that released by an equal amount of native HDL. Proteolytic digestion of HDL with trypsin, however, completely destroyed the stimulatory activity (Fig. 3 *b*). Exposure of placental explants for 1 h to the isolated delipidated apoproteins apo AI and AII (major components of human HDL [23]) and apo CI (a minor component of human HDL) significantly stimulated hPL release, but exposure to other minor components (apo CII, CIII, and E) had no effect (Table I). Over the range of concentrations tested, the relative potencies of the active apolipoproteins were apo CI > AII > AI. VLDL, which contain apo CI (14), also stimulated hPL release (data not shown). However, the potency of HDL, normalized to total protein mass, was four- to fivefold greater than that of VLDL.

Although HDL and apo CI markedly stimulated hPL release, neither HDL nor apo CI stimulated the release of luteinizing hormone from rat anterior pituitary cells (24) or the release of

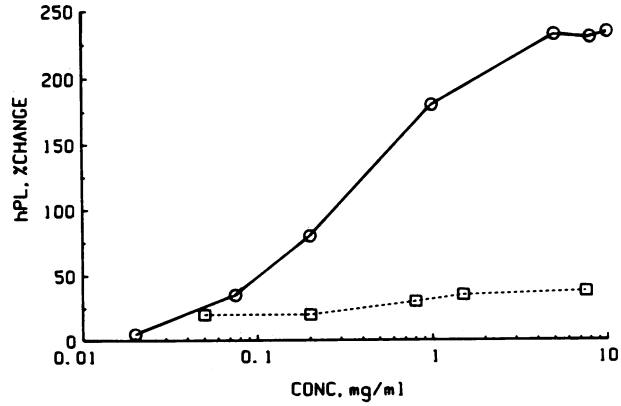

Figure 2. The dose dependency of HDL- and LDL-stimulated hPL release. Human placental explants were exposed for 1 h to HDL (*open circles*) or LDL (*open squares*) as described in Methods.

Table I. Effects of Apolipoproteins on hPL Release from Placental Explants

Treatment		hPL, mean + SEM ($n = 3$)
	μM	*ng*
Control	—	94.5±2.8
AI	0.8	108.8±5.2*
	2.4	129.8±4.1*
	7.2	140.4±2.6‡
	21.5	187.5±3.7‡
AII	0.9	97.5±1.2
	2.6	123.8±2.4*
	8.0	166.5±4.1‡
	23.5	199.8±4.0‡
CI	1.1	111.0±2.1*
	3.4	123.8±3.7*
	10.2	217.5±6.9‡
CII	9.1	96.2±2.5
	18.2	98.1±3.8
CIII	2.2	90.2±5.1
	9.1	94.7±4.2
E	12.1	88.2±4.1
	20.6	90.7±2.1

Human placental explants were exposed for 1 h to different concentrations of apo AI, AII, CI, CII, CIII, or E as described in Methods. Statistical differences from control are indicated by footnotes; *$P < 0.05$, ‡$P < 0.01$.

prolactin from human decidual cells (25). Triplicate cultures of pituitary cells exposed for 1 h to control medium or medium containing HDL (0.1, 0.25, or 1.0 mg/ml) or apo CI (1.6, or 8.0 μM) released 5.3±0.3 (mean±SEM) to 5.6±0.5 ng LH/106 cells, while pituitary cells exposed to gonadotropin-releasing hormone (0.01 μM) released 68.9±2.6 ng luteinizing hormone/10^6 cells. Triplicate cultures of human decidual cells exposed for 1 h to control medium, HDL, and Apo CI at the same concentrations used in the pituitary experiments released 11.9±1.8 to 13.2±1.7 ng prolactin/10^6 cells. HDL also had no effect on the release of TCA-precipitable [^{35}S]methionyl proteins from placental explants preincubated in medium containing [^{35}S]methionine (data not shown).

Discussion

These studies indicate that the stimulation of hPL release by HDL is primarily due to its apoprotein constituents. Proteolytic digestion of HDL by trypsin completely destroyed the stimulatory activity of the lipoprotein, while delipidation to remove >98% of the phospholipids and neutral fats did not. In fact, the stimulatory response to a given apoprotein mass was greater for the delipidated than the lipidated protein, possibly due to differences in protein structure in the nonpolar and polar environments. Although the data indicates that the effect of HDL is primarily due to apoproteins, it is possible that HDL lipid constituents modulate the effect.

The stimulation of hPL release by HDL was not due to a generalized effect on hormone release because maximal stimulatory concentrations of HDL had no effect on the release of luteinizing hormone from rat pituitary cells or the release of prolactin from human decidual cells. The increase in hPL release was also not due to a generalized stimulation of placental protein release because HDL did not affect the release of total [^{35}S]methionyl proteins from placental explants prelabeled for 24 h with [^{35}S]methionine. Likewise, apo CI had no effects on LH and prolactin release or the release of [^{35}S]methionyl placental proteins.

To date, studies of the physiology of the plasma apolipoproteins have focused on the effects of the proteins on lipid transport. The interactions of the apoproteins with cell receptors, along with activation and inhibition of enzymatic and transfer activities of plasma lipids, have been studied extensively (26, 27). However, the physiologic effects of the apoproteins in areas other than plasma lipid transport have not been well characterized. Although the mechanisms by which HDL apoproteins stimulate hPL release is unknown, that delipidated apoproteins stimulate release suggests that the stimulation is not due to the transport of lipids to the placenta. Because each of the stimulatory apolipoproteins are amphipathic (28), having polar and nonpolar domains, the stimulation of hPL release may be related to the detergent effect of the apolipoproteins on plasma membrane lipids. However, other amphipathic apolipoproteins (CI, CIII, and E) were without effect, suggesting a more specific interaction. The absence of a common metabolic function of the three active apoproteins in other systems (26, 27) further suggests that the stimulation of hPL release may be unrelated to an effect of the HDL proteins on lipid structure or metabolism.

In a recent report, Darbon and co-workers (29) demonstrated that HDL stimulate a dose-dependent and rapid phosphorylation of a 27-kD protein in quiescent bovine endothelial cells, an effect mimicked by both 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) and 1-oleoyl-2-acetylglycerol. Total HDL apoproteins but not HDL lipids also mimicked the effect of HDL. Because TPA and 1-deoyl-2-acetylglycerol are known to stimulate protein kinase C (30), this study strongly supports a role for protein kinase C in HDL action on endothelial cells. Investigations from our laboratory have recently demonstrated that TPA and sn 1,2 diacylglycerol stimulate both placental protein kinase C and hPL release from enriched hPL-producing trophoblast cells (31). Because HDL and HDL apoproteins activate protein kinase C in endothelial cells, the stimulation of hPL release may also be secondary to protein kinase C activation.

The demonstration that apo AI, AII, and CI stimulate hPL release provides the first evidence that lipoprotein apoproteins affect protein hormone release. Because human trophoblast cells contain plasma membrane receptors for HDL and the concentration of HDL increases during pregnancy, the observation that physiologic concentrations of HDL stimulate hPL release in vitro suggests an in vivo role for HDL in the release of hPL release during pregnancy. Because immunoaffinity chromatography studies of plasma HDL using antibodies to the different apoproteins indicates that HDL is a very heterogeneous group of lipoproteins (32, 33), it may be possible to identify an HDL particle with an optimum stoichiometry of the three active apoproteins that maximally stimulates hPL release. The demonstration of the novel action of HDL apoproteins on hPL release raises the possibility that apolipoproteins may influence the secretion of other protein hormones and may have other biologic effects unrelated to lipid transport.

Acknowledgments

We thank P. M. Conn for performing the pituitary studies and J. Gwynne for suggestions and criticisms, L. Pearce for technical assistance, and G. Kerr for secretarial assistance.

This work was supported by National Institutes of Health grant HD-07447.

References

1. Cummings, S. W., W. Hartley, E. R. Simpson, and M. Ohaski. 1982. The binding of high and low density lipoproteins to human placental membrane fractions. *J. Clin. Endocrinol. & Metab.* 54:903–908.

2. Winkel, C. A., J. M. Snyder, P. C. MacDonald, and E. R. Simpson. 1980. Regulation of cholesterol and progesterone synthesis in human placental cells in cultures by serum lipoproteins. *Endocrinology.* 106: 1054–1060.

3. Josimovich, J. B., and J. A. McLaren. 1962. Presence in the human placenta and term serum of a highly lactogenic substance immunologically related to pituitary growth hormone. *Endocrinology.* 71:209–220.

4. Grumbach, M. M., and S. L. Kaplan. 1964. On the placental origin and purification of chorionic "growth hormone-prolactin" and its immunoassay in pregnancy. *Trans. NY Acad. Sci.* 27:167–188.

5. Handwerger, S., T. W. Hurley, and A. Golander. 1981. Placental and decidual polypeptide hormones. *In* Principles and Practice of Obstetrics and Perinatology. L. Iffy and H. D. Kaminetzky, editors. John Wiley & Sons, Inc., New York. 243–260.

6. Hill, D. J., C. J. Crace, and R. D. G. Milner. 1985. Incorporation of [^{3}H] thymidine by isolated fetal myoblasts and fibroblasts in response to human placental lactogen (hPL): possible mediations of hPL action by release of somatomedin SM-C. *J. Cell. Physiol.* 125:337–344.

7. Gofman, J. W., O. De Lalla, F. Glazier, N. K. Freeman, F. T. Lingren, A. V. Nichols, B. Strisower, and A. R. Tamplin. 1954. The serum lipoprotein transport system in health, metabolic disorder, arteriosclerosis, and coronary heart disease. *Plasma.* 2:413–420.

8. Fahraeus, L., V. Larson-Cohn, and L. Wallentin. 1985. Plasma lipoproteins including high density lipoprotein sulfractions during normal pregnancy. *Obstet. Gynecol.* 66:468–472.

9. Schonfeld, G., and B. Pfleger. 1974. The structure of human high density lipoprotein and the levels of apolipoprotein A-I in plasma as determined by radioimmunoassay. *J. Clin. Invest.* 54:236–246.

10. Golander, A., J. B. Barrett, L. Tyrey, W. H. Fletcher, and S. Handwerger. 1978. Differential synthesis of human placental lactogen and human chorionic gonadotropin *in vitro*. *Endocrinology.* 102:597–605.

11. Handwerger, S., and L. M. Sherwood. 1974. Human placental lactogen. *In* Methods of Hormone Radioimmunoassay. B. M. Jaffe and H. R. Behrman, editors. Academic Press, Inc., New York. 417–426.

12. Havel, R. J., H. A. Elder, and J. H. Brogdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* 34:1345–1353.

13. Edelstein, C., C. T. Lim, and A. Scanu. 1972. On the subunit structure of the protein of human serum high density lipoprotein. *J. Biol. Chem.* 247:5842–5849.

14. Shore, V. G., and B. Shore. 1973. Heterogeneity of human plasma very low density lipoproteins. Separation of species differing in protein components. *Biochem. J.* 12:502–507.

15. Shelborne, F. A., and S. H. Quarfordt. 1977. The interaction of heparin with an apoprotein of human very low density lipoprotein. *J. Clin. Invest.* 60:944–950.

16. Laemmli, U. K. 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.

17. Kane, J. P. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* 53:350–364.

18. Brown, W., R. Levy, and D. Frederickson. 1969. Studies of the proteins in human plasma very low density lipoproteins. *J. Biol Chem.* 244:5687–5694.

19. Ames, B. N., and D. T. Dubin. 1960. The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. *J. Biol Chem.* 235:769–779.

20. Abell, L. C., B. B. Levy, B. B. Brodie, and F. E. Kendall. 1952. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. Chem.* 195:357–366.

21. Kessler, G., and H. Lederer. 1966. Fluorometric measurement of triglycerides. *In* Automation in Analytical Chemistry. Technician Symposium. L. Skegg, editor. Mediad, New York, 341–344.

22. Eisenberg, S., and R. I. Levy. 1975. Lipoprotein metabolism. *Adv. Lipid Res.* 13:1–89.

23. Morrisett, J. D., R. C. Jackson, and A. M. Gotto. 1975. Lipoproteins: structure and function. *Annu. Rev. Biochem.* 44:183–207.

24. Conn, P. M., J. Marian, M. McMillian, J. Stern, D. Rogers, M. Hamby, A. Penna, and E. Grant. 1981. Gonadotropin-releasing hormone action in the pituitary: a three step mechanism. *Endocrinol. Rev.* 2:174–185.

25. Golander, A., J. Barrett, T. Hurley, S. Barry, and S. Handwerger. 1979. Failure of bromocriptine, dopamine and thyrotropin-releasing hormone to affect prolactin secretion by decidual tissue *in vitro*. *J. Clin. Endocrinol. & Metab.* 49:787–789.

26. Zannis, V. I., and J. L. Breslow. 1985. Genetic mutations affecting human lipoprotein metabolism. *Adv. Hum. Genet.* 14:125–215.

27. Eisenberg, S. 1984. High density lipoprotein metabolism. *J. Lipid Res.* 25:1017–1058.

28. Jackson, R. L., J. D. Morrisett, and A. M. Gotto. 1976. Lipoprotein structure and metabolism. *Physiol. Rev.* 56:259–316.

29. Darbon, J.-M., J.-F. Tournier, J.-P. Tauber, and F. Bayard. 1986. Possible role of protein phosphoylation in the mitogenic effect of high density lipoproteins on cultured vascular endothelial cells. *J. Biol. Chem.* 261:8002–8008.

30. Martin, T., and J. Kowalchyk. 1984. Evidence for the role of calcium and diacylglycerol as dual second messengers in thyrotropin-releasing hormone action: involvement of Ca^{+2}. *Endocrinology.* 115: 1527–1536.

31. Harman, I., P. Zeitler, B. Ganong, R. M. Bell, and S. Handwerger. 1986. Sn 1,2 diacylglycerols and phorbol esters stimulate the synthesis and release of human placental lactogen from placental cells: a role for protein kinase C. *Endocrinology.* 119:1239–1244.

32. Kostner, G., and P. Alaupovic. 1972. Studies of the composition and structure of plasma lipoproteins. Separation and quantification of the lipoprotein families occurring in the high density lipoproteins of human plasma. *Biochemistry.* 11:3419–3428.

33. Kostner, G., and A. Holaski. 1980. Isolation of human serum low-density lipoproteins with the aid of an immune-specific adsorber. *Lipids.* 5:501–508.