Effect of serum lipoproteins on the adenylate cyclase activity of rat liver plasma membranes

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Four rat lipoprotein classes [lymph chylomicrons, VLD (very-low-density), LD (low-density) and HD (high-density) lipoproteins] were tested for their ability to affect basal adenylate cyclase (EC 4.6.1.1) activity of rat liver plasma membranes. All the lipoproteins, with the exception of lymph chylomicrons, effectively increase the enzyme activity. VLD lipoproteins are the most active class (67% maximal increase), followed by HD lipoproteins (33%) and LD lipoproteins (23%). The effect of VLD lipoproteins is additive to that elicited by GTP or GTP plus glucagon (at least within a certain concentration range). VLD lipoproteins affect only the $V_{\max}$ of the enzyme, not the K_m .

The interaction of different plasma lipoproteins with cell membranes has been the object of detailed investigations in recent years (Goldstein & Brown, 1977). The major advance has been the recognition of a surface-receptor-mediated uptake system, responsible for the regulation of cellular sterol biosynthesis (Brown & Goldstein, 1974). The physiological and clinical significance of this finding, as well as the observed ineffective interaction in cell systems lacking receptor sites (Goldstein & Brown, 1973) have wide-reaching implications (Willingham & Pastan, 1980).

The existence of other types of lipoprotein–cell-surface interactions, i.e. their effect on cell-membrane enzymes, has received only limited attention (Shore & Shore, 1975). An increase of adenylate cyclase activity in plasma membranes of human and rat adipocytes has been shown by Pairault *et al.* (1977). The human plasma VLD lipoproteins are the most effective stimulators. More recently, Taylor *et al.* (1979) reported increased cyclic AMP concentrations in avian thrombocytes exposed to different concentrations of LD lipoproteins.

In order to test the general validity of the above-reported observations *in vitro*, different rat lipoprotein fractions (lymph chylomicrons, VLD, LD and HD lipoproteins) were incubated with rat liver plasma membranes and their modulation of adenylate cyclase activity examined. The effects of plasma

lipoproteins on the glucagon- and GTP-stimulated adenylate cyclase were also tested.

Materials and methods

Male Sprague–Dawley rats (250–300 g) were used throughout the experiments, for both blood sampling and hepatic plasma-membrane preparations. Animals were killed by decapitation and blood collected in ice-cooled tubes containing EDTA/Na₃ [final concns. 0.3 and 0.03% (w/v) respectively]. Lipoproteins were separated from plasma by ultracentrifugation, the medium density being sequentially adjusted to 1.019 mg/ml for VLD lipoproteins, 1.063 mg/ml for LD lipoproteins and 1.215 mg/ml for HD lipoproteins as previously described (Rodriguez *et al.*, 1976).

Lymph chylomicrons were collected by cannulation of the rat lymph duct (Redgrave, 1970) over a period of 3 h. To increase lymph flow and chylomicron production, rats were given 2 ml of olive oil by stomach intubation 2 h before cannulation. Lipoproteins with Svedberg flotation rates exceeding $S_f > 400$ were obtained by ultracentrifugation of lymph in a Beckman SW 41 rotor (Beckman, Palo Alto, CA, U.S.A.) as described by Lindgren *et al.* (1972). Each lipoprotein fraction, from either plasma or lymph, was concentrated and repurified by a second ultracentrifugal run at the same medium salt (KBr) density used for separation. Before use in the adenylate cyclase assay, lipoprotein fractions were dialysed against 5000 vol. of 0.15 M-NaCl/30 mM-Tris/HCl buffer, pH 7.4, for 24 h at 4°C to remove the excess KBr. Chemical

Abbreviations used: VLD, LD and HD lipoproteins, very-low-density, low-density and high-density lipoproteins.

analyses of lipoproteins were performed as previously described (Rodriguez *et al.*, 1976) on pooled plasma samples (10–15 rats).

Partially purified plasma membranes from rat liver were prepared by a modification of the procedure of Neville (1968), as described by Pohl *et al.* (1971). Membrane preparations were completed within 4 h of killing the animals. The plasma-membrane protein content was quantified by the Lowry procedure (Lowry *et al.*, 1951), with bovine serum albumin as standard.

The adenylate cyclase assay was performed as described by Salomon *et al.* (1974). All the reagents were from Sigma. The standard assay medium contained: MgATP²⁻, 0.15 mM; MgCl₂, 5 mM; dithiothreitol, 1 mM; bovine serum albumin, 1% (w/v); phosphocreatine, 5 mM; creatine phosphokinase, 28 units/ml; and Tris/HCl, 30 mM, pH 7.4. The final volume was 100 μ l. Lipoproteins, GTP and glucagon (porcine; Eli Lilly and Co.) were added in 0.15 M-NaCl/30 mM-Tris/HCl, pH 7.4, at the concentrations indicated in the Figures. Membranes were added (approx. 100 μ g in 10 μ l) at the beginning of the preincubation period (Pairault *et al.*, 1977). After 15 min at 30°C, [8-¹⁴C]ATP (750 000 d.p.m.; 55 mCi/mmol) and cyclic [2,8-³H]-AMP (30 000 d.p.m., 50 Ci/mmol) (The Radiochemical Centre, Amersham, Bucks, U.K.) were added, the incubation continued for 10 min at 30°C and finally stopped by the addition of 0.1 ml of a solution containing 2% (w/v) sodium dodecyl sulphate, 40 mM-ATP and 1.4 mM-cyclic AMP.

Results and discussion

The chemical composition of plasma and lymph lipoproteins used in the present study is given in Table 1. All the lipoproteins tested, with the exception of chylomicrons, significantly increased the liver plasma-membrane adenylate cyclase activity above basal values. However, the various fractions exhibited a different activity; VLD lipoproteins, the most active fraction (Fig. 1), elicited a 67% increase in adenylate cyclase activity. By contrast, HD and LD lipoproteins only induced 33 and 23% rises respectively in enzyme activity. Chylomicrons

were ineffective. The ED₅₀ (concentration giving half-maximal stimulation) was 48 μ g of apoprotein/ml for VLD lipoproteins, 32 μ g/ml for LD lipoproteins and 240 μ g/ml for HD lipoproteins. It should be noted, however, that the ED₅₀ obtained for the different fractions were lower than the actual plasma lipoprotein concentrations in rats (170, 50 and 300 μ g/ml for VLD, LD and HD lipoproteins respectively).

The effect of lipoproteins appeared to be independent of total protein mass and type of lipid in the incubation mixture (see Table 1). In particular, the triacylglycerol content of VLD lipoproteins and of the other triacylglycerol-rich lipoprotein classes did not seem to influence enzyme activation. Chylomicrons were inactive, and, moreover, a triacylglycerol/phospholipid emulsion (Intralipid; Vitrum AB, Stockholm, Sweden), tested in other experiments, was similarly ineffective (results not shown).

The activity of VLD lipoproteins and lymph chylomicrons was tested at increasing concentrations (0.1–12 mg/ml as triacylglycerols) in the presence of three different concentrations of HD lipoprotein (50, 300 and 1000 μ g/ml as protein). The effects were purely additive. Similarly, a lack of synergism was observed between the stimulation induced by plasma lipoproteins and that effected by GTP. In Fig. 2 the experiment with VLD lipoproteins is shown (LD and HD lipoproteins behaved similarly). Addition of VLD lipoproteins to the adenylate cyclase stimulated by GTP plus glucagon showed a different pattern (Fig. 2a): at low concentration, VLD lipoproteins slightly but consistently inhibited the adenylate cyclase activity; at present it is difficult to assess the meaning or the importance of such a modest inhibition (approx. 8%). On the other hand, the dose-response curve at higher concentration was parallel with those obtained for the basal and GTP-stimulated adenylate cyclase activities.

The present data, although clearly demonstrating a lipoprotein effect on the adenylate cyclase activity in liver plasma membranes, do not provide an immediate explanation of the underlying mechanism. Lipid membrane composition has been shown by

Table 1. Percentage composition of rat plasma lipoproteins and lymph chylomicrons

Results are means $\pm$ s.d. ($n = 6$).

	Composition (% w/w)			
	Total cholesterol	Triacylglycerol	Phospholipid	Protein
Chylomicrons	2.7 $\pm$ 0.5	93.8 $\pm$ 7.3	2.4 $\pm$ 1.2	1.1 $\pm$ 0.2
VLD lipoprotein ($d = 1.019$)	17.8 $\pm$ 2.1	52.3 $\pm$ 7.1	16.2 $\pm$ 2.1	13.7 $\pm$ 1.4
LDL lipoprotein ($d = 1.019$ –1.063)	35.0 $\pm$ 3.3	15.7 $\pm$ 2.3	21.6 $\pm$ 2.5	27.7 $\pm$ 2.3
HD lipoprotein ($d = 1.063$ –1.215)	39.8 $\pm$ 4.7	2.4 $\pm$ 0.3	24.2 $\pm$ 3.1	33.6 $\pm$ 2.3

Fig. 1. Effect of different rat plasma lipoproteins (▲, VLD; ●, LD; ■, HD) and lymph chylomicrons (×) on the basal adenylate cyclase activity (□) of rat liver plasma membranes

Lipoproteins and chylomicrons (30 μl) were added at the indicated protein concentrations to the incubation mixture. Values are means ± S.D. of three determinations.

Fig. 2. (a) Effect of VLD lipoproteins on the basal (■), 1 mM-GTP (▲)- and 1 mM-GTP-plus-0.3 mM-glucagon (●)-stimulated adenylate cyclase activity, and (b) stimulation of adenylate cyclase by GTP without (■) and with (●) VLD lipoproteins (152 μg/ml as protein)

Values are means ± S.D. of three determinations.

different authors markedly to affect adenylate cyclase activity (Sinha *et al.*, 1977; Houslay *et al.*, 1976). Moreover, exchange of cholesterol and phospholipids between plasma lipoproteins and cell membranes may take place (Basford *et al.*, 1964). It seems, however, unlikely that a simple addition of lipids could account for the observed enzyme activation, since the final effect was unrelated to a particular lipid component in the assay mixture. On the other hand, one could hypothesize a role for apoproteins in the activation mechanism. At first glance, the effect of LD and HD lipoproteins, when compared with that elicited by VLD lipoproteins at similar protein concentrations, may seem to exclude a significant role for the apoprotein moiety. Nevertheless, apoproteins could be differently exposed at the lipoprotein surface, as indicated by immunological studies (Schonfeld *et al.*, 1979). The stimu-

latory effect of lipoproteins might be, therefore, linked to their whole structure, rather than to a particular component.

The independence of the effects elicited by lipoproteins and hormones on the adenylate cyclase needs some comment. The presence of GTP is necessary for the activity of many hormonal mediators at the plasma-membrane level, through a mechanism coupling the hormonal receptor with the adenylate cyclase enzyme (Rodbell, 1978). The lack of interaction with GTP (Figs. 2a and 2b) may indicate that the lipoprotein effect involves a mechanism by-passing the guanine-nucleotide-binding site. On the other hand, the parallelism between VLD-lipoprotein dose-response curves in the presence and absence of glucagon (at least at concentrations higher than 20 μg/ml) (Fig. 2a) is suggestive of a selective activity on the catalytic

moiety of the adenylate cyclase system, without interaction with the hormone receptor or the coupling process.

Fig. 3 demonstrates that the stimulation of adenylate cyclase by VLD lipoproteins is accounted for by an increase in the $V_{\max}$ (from 1612 to 2380 pmol/10 min per mg of protein), without change in the apparent affinity for the substrate; on the basis of this experiment, a facilitated transition from the inactive state (E) to the active state (E') of the enzyme (Rodbell, 1978), effected by VLD lipoproteins, may be suggested.

The relative potency of the tested lipoproteins was in the same order as that indicated by Pairault *et al.* (1977) with human or rat adipocyte plasma membranes. In both cases, VLD lipoproteins were the most potent fraction. The present studies extend the observations to the liver, i.e. a major site for lipid and lipoprotein metabolism. In the liver, a strict dependency of many other cellular metabolic functions on cyclic AMP concentrations has been indi-

cated; in particular, the relationship between cyclic AMP concentration in liver cell and the lipid-biosynthetic pattern has been described by several authors (Heimberg *et al.*, 1969; George & Ramasarma, 1977). From our studies it appears that plasma lipoproteins could modulate biosynthetic activities in the liver by a mechanism not involving cellular lipoprotein internalization (Goldstein & Brown, 1977), but rather by affecting cyclic-nucleotide metabolism at the membrane level.

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Fig. 3. Lineweaver-Burk plot of the stimulatory action of VLD lipoproteins (147 μ g/ml as protein, $\blacktriangle$) on the basal adenylate cyclase activity ($\blacksquare$)

The concentration of MgATP^{2-} was varied while the concentration of free Mg^{2+} (5 mM) was kept constant. For $\blacktriangle$, $K_m = 0.46 \text{ mM}^{-1}$; $V_{\max} = 2380 \text{ pmol/10 min per mg of protein}$; for $\blacksquare$, $K_m = 0.45 \text{ mM}^{-1}$; $V_{\max} = 1612 \text{ pmol/10 min per mg of protein}$. Values are means $\pm$ S.D. of three determinations. The experiment is representative of two other similar ones.