Volume 220, number 1, 159-162 FEB 04982 August 1987

Binding of low-density lipoprotein to monolayer cultures of rat hepatocytes is increased by insulin and decreased by dexamethasone

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Received 18 June 1987

Rat hepatocytes were maintained in monolayer culture for 20 h in the presence of 10% (v/v) new-born calf serum and then for a further 1–24 h in serum-free medium containing 2 g bovine serum albumin/l. The specific binding of human ^{125}I-LDL to two distinct sites was then measured at 4°C. Binding to site 1 was displaced by dextran sulphate while that to site 2 was not. The presence of 1–100 nM insulin for 24 h in the second incubation significantly increased binding to site 1. Significant increases were also seen when cells were incubated with 10 nM insulin for 1 h. No significant effects of insulin on binding to site 2 were observed. In contrast, 10 nM–1 μM dexamethasone decreased binding to both sites. The effects of these hormones were mutually antagonistic.

Cholesterol metabolism; Glucocorticoid; Insulin; LDL; (Liver)

1. INTRODUCTION

The liver has an important function in removing LDL from the circulation. The cholesterol contained in these particles can then be re-secreted in VLDL or HDL components. Alternatively, the cholesterol can be secreted into the bile directly, or after its conversion to bile salts. These latter processes provide the major route of cholesterol excretion since the reabsorption of cholesterol and bile acids from the intestine is incomplete.

The uptake of LDL by the liver is mainly by endocytosis via a specific receptor similar to that which has been described in a variety of cultured cell types [1]. As such, the binding is specific for apolipoprotein (apo) B and apoE; it can be inhibited by the modification of lysine and arginine residues of the apolipoprotein; it is Ca^{2+}-dependent and LDL can be displaced by heparin or dextran sulphate. Specific binding sites other than this 'classic' LDL receptor have been described on isolated liver membranes, and for hepatocytes in culture. This site (or sites) generally do not have an absolute requirement for Ca^{2+} [2–10]. Binding of LDL can also be detected in livers of patients suffering from homozygous familial hypercholesterolaemia where the classic receptor should be absent [7,8,11].

There is considerable interest in the factors that may regulate LDL uptake by the liver since this is a major route whereby LDL is eliminated from the circulation. This is important because of the association of high plasma LDL levels with increased risk of developing atherosclerosis. However, our present knowledge of the mechanisms that control the binding, uptake and degradation of LDL by the liver is relatively limited. It is known that incubating a human hepatoma cell line (Hep G2) with 'heavy' HDL

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

(ϱ = 1.16–1.20 g/ml) increased the binding of human ^{125}I-LDL [12]. Similarly, preincubating rat hepatocytes with human HDL_3, but not HDL_2, increased the binding and degradation of human LDL by what appeared to be the classic LDL receptor [9,10,13]. Porcine HDL (ϱ = 1.12–1.16 g/ml) has also been shown to increase the degradation, but apparently not the uptake, of homologous LDL by porcine hepatocytes [6]. In fibroblasts preincubation with LDL has been shown to markedly decrease subsequent binding and uptake of LDL [14]. However, in both Hep G2 cells [12] and hepatocytes [10,13] this effect is much less pronounced. This difference probably relates to the role of the liver in actively removing LDL cholesterol from the circulation.

In terms of hormonal control, it is known that pharmacological doses of 17α-ethinyloestradiol cause a marked decrease in plasma cholesterol, resulting from an up-regulation of LDL receptors in the liver [15–17]. Insulin enhances the degradation of LDL by fibroblasts by increasing the number of LDL receptors [18]. In contrast, cortisol decreased the uptake and degradation of ^{125}I-LDL by human fibroblasts and smooth muscle cells without altering the binding of LDL to the cells [19].

The present work was undertaken to determine the effects of pre-incubation of rat hepatocytes with insulin and a glucocorticoid (dexamethasone) on the ability of the cells to bind human ^{125}I-LDL to two distinct sites. Site 1 was the classic LDL site for which binding was Ca^{2+}-dependent and it was displaced by dextran sulphate. Site 2 was defined as the residual specific binding that was not displaced by dextran sulphate. This binding showed relatively little dependency on Ca^{2+} [9].

2. MATERIALS AND METHODS

Hepatocytes from male Wistar rats were prepared and cultured on collagen-coated tissue culture dishes [9,10,20]. The techniques used for preparing human ^{125}I-LDL, measuring specific binding at 4°C to sites 1 and 2 and determination of protein have been described [9].

3. RESULTS

The hepatocyte cultures were incubated for an initial 20 h in medium containing 10% (v/v) newborn calf serum in order to stabilize the cells and to provide a suitable model for measuring the effects on LDL binding [10]. The medium was then changed and the serum was replaced by 2 g fatty acid-poor bovine serum albumin/l. After a further 1 h incubation cells were transferred to fresh albumin containing medium in the presence or absence of the indicated hormones. Hepatocytes have previously been shown to be responsive to insulin and dexamethasone under such conditions [20,21].

The incubation of hepatocytes for 24 h in serum-free medium in this work caused a significant increase in specific binding to site 1, whereas that to site 2 remained relatively constant (fig.1). The presence of 1–100 nM insulin for 24 h significantly increased the binding of LDL to site

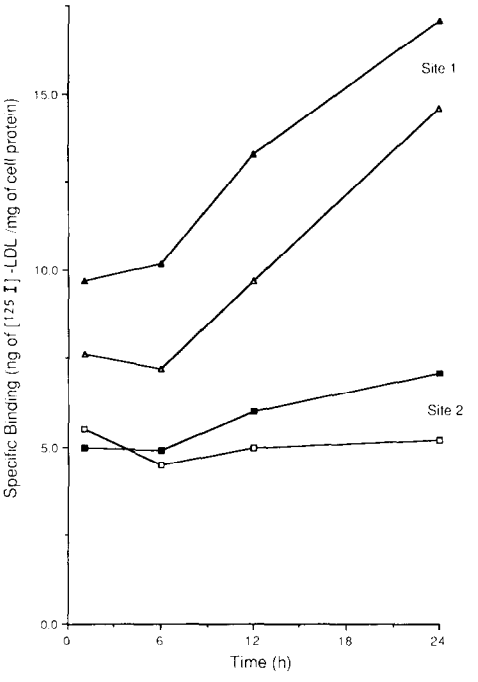

Fig.1. Effect of time in culture on the specific binding of LDL to rat hepatocytes. Rat hepatocytes were cultured for 20 h in the presence of 10% (v/v) newborn calf serum and then up to a further 24 h in serum-free medium containing 2 g bovine serum albumin/l. The specific binding of ^{125}I-LDL to sites 1 (△, ▲) and 2 (□, ■) in the absence (△, □) and presence (▲, ■) of 10 nM insulin is shown.

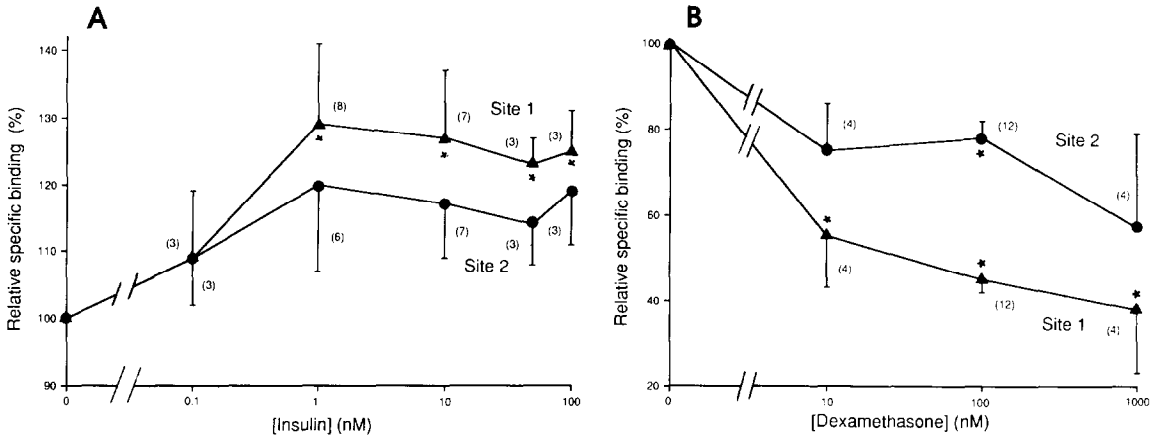

Fig.2. Effects of insulin and dexamethasone on the specific binding of LDL to rat hepatocytes. Rat hepatocytes were cultured for 20 h in the presence of 10% (v/v) newborn calf serum and then for a further 24 h in serum-free medium containing 2 g bovine serum albumin/l. The specific binding of ^{125}I-LDL to sites 1 (▲) and 2 (●) was measured in the absence and presence of different concentrations of (A) insulin and (B) dexamethasone. Results are means ± SE with the number of independent experiments shown in parentheses and they are expressed relative to binding in the absence of added hormone. (*) Values statistically different from control.

1 (fig.2A). This effect with 10 nM insulin was also observed after 1 h on site 1 (fig.1) and in four independent experiments binding was 21 ± 6% higher ($p < 0.05$) than for controls. Although there appeared to be an increase in binding to site 2 in the presence of insulin this did not reach statistical significance at any time point or concentration probably because of the variability of the effect. In two experiments where cells were incubated with 1 nM insulin for 24 h increases in binding to site 2 of 441 and 545% were observed. The reason for the discrepancy between these and the other 6 experiments is unknown and they have been omitted from fig.2A.

Dexamethasone (10 nM–1 μM) decreased LDL binding to sites 1 and 2 and the effect seemed greater on site 1 in all experiments. These effects of dexamethasone did not become apparent until 12–24 h of incubation (not shown). Insulin was able to antagonise the effects of dexamethasone on site 1 such that relative binding in the presence of 100 nM dexamethasone of 42 ± 3% was increased to 57 ± 5% when 1 nM insulin was also present ($n = 8$, $p < 0.05$). Under similar conditions, binding to site 2 appeared to increase from 76 ± 5 to 88 ± 8%, although this was not statistically significant.

4. DISCUSSION

The results indicate for the first time that insulin and a glucocorticoid have direct effects on the binding of LDL to hepatocytes. Insulin increases whereas dexamethasone decreases binding. The mechanisms which produce these changes have not yet been elucidated. However, the insulin effect was seen between 1 and 24 h of incubation, whereas the dexamethasone effect only became apparent after about 12 h. The changes are most consistent for site 1, which has the properties of the classic LDL receptor, and the effects of the two hormones were mutually antagonistic. Our findings on the effects of insulin are in contrast to those of Jensen et al. [22] who found no effect on the uptake of human LDL by rat hepatocytes. However, our experimental system was significantly different from theirs in that they preincubated hepatocytes in the presence of 10% lipoprotein-deficient foetal calf serum and this was also present during the incubation with insulin. Furthermore, unlike these workers, we found no significant effect of insulin on cell density as reflected by the amount of cell protein recovered from each tissue culture dish.

Whether the changes in binding to the

hepatocytes that are produced are paralleled by changes in the uptake and degradation remains to be firmly established however, preliminary evidence suggests that this is so (Brown, Salter and Brindley, unpublished).

The reported effects of insulin and dexamethasone on LDL binding could have important implications for understanding the changes in lipoprotein metabolism that occur in various diseases. For example, diabetes and stress are often considered to be risk factors for the development of premature atherosclerosis. The present results indicate that the decreased binding of LDL by the liver, which would result from an increased action of glucocorticoids relative to insulin, might also decrease the removal of LDL from the blood. The consequent increase in circulating LDL could account for part of the increased atherosclerotic risk associated with these conditions.

ACKNOWLEDGEMENTS

This work was supported by a project grant from the British Heart Foundation and an equipment grant from the Humane Research Trust. We also thank the Boots Company, Nottingham for supplying specially purified insulin.

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