Density lipoprotein; WHHL, Watanabe heritable hyperlipidaemic.

Normann, P. T. Schwartz.


Received 30 October 1986

Receptor-mediated endocytosis in steroid hormone-producing tissue

KEITH E. SUCKLING* and BEGONA OCHOA†

*Smith Kline and French Research Ltd., The Frythe, Welwyn, Herts., AL6 9AR, U.K., and †Department of Physiological Biochemistry, University of the Basque Country, Leioa (Vizcaya), Spain

Steroid hormone-producing tissue has been important in the study of receptor-mediated endocytosis of lipoproteins from a very early stage. Although relatively little of the total body turnover of cholesterol is accounted for by its conversion into steroid hormones, it is generally agreed that most steroid hormone-producing cells, especially those of the adrenal cortex, are dependent on plasma lipoproteins for their supply of cholesterol for steroidogenesis (Gwynne & Strauss, 1982). Many lines of evidence support the concept that receptor-mediated endocytosis is important in the supply of cholesterol to these tissues. Perhaps one of the clearest is in WHHL rabbits, in which the low-density lipoprotein (LDL) receptor is not functional. Here the adrenal gland shows a five-fold increase in cholesterol synthesis over normal rabbits as a response to maintain the cholesterol supply for synthesis of adrenal cortical hormones (Dietschy et al., 1983).

In the rat LDL is not the main carrier of cholesterol in the blood and much evidence suggests that cholesterol is delivered by high-density lipoprotein (HDL) (reviewed in Gwynne & Strauss, 1982). Thus in steroid hormone-producing tissues, within a broad general pattern of cholesterol metabolism, several sources of cholesterol are available and different mechanisms of uptake of cholesterol from the blood may operate. In addition to the sources mentioned already, plasma lipoproteins and intracellular synthesis, steroid hormone-producing tissues frequently contain a store of cholesteryl ester that can be mobilized by the action of the appropriate tropic hormone (Boyd et al., 1983). The effect of these hormones is to initiate a dramatic change in the intracellular organization of cholesterol metabolism. When considering the function of steroid hormone-producing tissue and the control of its cholesterol metabolism, we have to understand the role of these three sources of cholesterol and how they relate to the overall fluxes of cholesterol within the cell.

In order to determine the importance of cholesterol synthesis and receptor-mediated uptake of lipoproteins in the adrenal cortex, Spady & Dietschy (1985) have made an extensive series of studies in vivo in the rat, hamster and rabbit. In all three species receptor-mediated uptake accounted for over 93% of the total uptake of cholesterol from the blood, but the extent to which cholesterol synthesis and uptake of lipoprotein was dominant depended on the species. The hamster derived 10 times more cholesterol from intracellular synthesis than from LDL uptake. The opposite was the case in the rabbit. Spady & Dietschy consider that man is most closely modelled by the situation in the hamster. The importance of receptor-mediated uptake is as great in the adrenal as in any tissue. These studies were performed by continuous infusion of lipoproteins into the animals over a period of several hours. It would be interesting to know what effect the injection of a tropic hormone would have on the degree of endocytosis. 

Abbreviations used: LDL, low-density lipoprotein; HDL, high-density lipoprotein; WHHL, Watanabe heritable hyperlipidaemic.

Sodhi, H. S., Orchard, R. C., Agniesz, N. D., Varughese, P. V. & Kudchadkar, B. J. (1973) Atherosclerosis 17, 197-210


have on lipoprotein uptake. There is evidence in some experimental systems that a tropic hormone can increase the binding and uptake of lipoproteins, in some cases very rapidly (Watanuki & Hall, 1979, see also below). In other cases there seems to be little effect (Verschoor-Klootwyk et al., 1982; Bisgaier et al., 1985). Rat HDL receptor activity appears to be hormonally regulated (Gwynne et al., 1985) and the regulation of the LDL receptor in the guinea-pig adrenal appears to differ between the two major zones of that tissue (Nonomura & Strauss, 1986). Other hormones have been shown to affect the binding of LDL to membranes of steroid hormone-producing cells. Oestrogen increases the high-affinity saturable binding of LDL by porcine granulosa cells (Veldhuis & Gwynne, 1985). In the same system insulin has been shown to promote LDL binding and steroid hormone synthesis (Veldhuis et al., 1986). In human granulosa cells human chorionic gonadotropin has been shown to increase the synthesis of LDL receptors by a mechanism in part independent of cholesterol balance (Golos & Strauss, 1985).

The differences between the mechanisms of uptake of cholesterol from LDL and from HDL are intriguing and are especially important in the rat. Uptake of LDL follows the classic pathway. The cellular architecture is important here since inhibitors of microfilaments and microtubules can inhibit uptake of lipoproteins (Azhar & Menon, 1981; Rajan & Menon, 1985). HDL-derived free and esterified cholesterol can support synthesis of steroid hormones (Gwynne et al., 1982), and although this particle can be internalized by cells (Chen & Abel, 1986) it does not have to enter the cell in order to supply cholesterol (Nestler et al., 1985). It is clear that uptake of cholesteryl ester from HDL in the adrenal cortex occurs independently of the apoproteins (Glass et al., 1985). Hepatic lipase may play a role here (Phillips & Rothblat, 1985).

At present we do not completely understand how the steroid hormone-producing cell uses the cholesterol derived from the cholesteryl ester in the lipoproteins. In considering organization of this we can envisage an acute and a long-term effect of the tropic hormone. In the longer term changes in the synthesis of important enzymes occur (Boggar et al., 1985) and the cells reach a new internal balance of cholesterol metabolism. Cholesterol itself may regulate this process (Boggar et al., 1985). In the acute phase of stimulation more rapid changes in cholesterol flux have been envisaged (Gwynne et al., 1983; Suckling, 1985). Corticotropin should bear in mind that other regulatory systems may participate. There is some evidence that Ca2+ and protein kinase C can affect the activity of steroidogenic enzymes (Culty et al., 1984). Since very rapid changes in cholesterol flux occur in adrenal cortical cells on stimulation with corticotropin, and others (Bisgaier et al., 1985; B. Ochoa & K. E. Suckling, unpublished work) have attempted to define the nature of the changes in metabolism of cholesterol derived from LDL. Cholesterol taken up as cholesteryl ester in LDL and released from the lysosomes as free cholesterol can, in principle, have two intracellular fates. Either it can travel to the mitochondria for hormone synthesis or it can be esterified in the endoplasmic reticulum by acyl-CoA:cholesterol acyltransferase (Suckling, 1985). Studies in cultured bovine adrenal cortical cells with the acyl-CoA: cholesterol acyltransferase inhibitor, Sandoz compound 58-035, showed that in the absence of corticotropin at least 90% of the cholesterol released from LDL would normally be esterified. In cells stimulated with corticotropin for 18 h most of the incoming cholesterol appeared to be directed towards steroidogenesis (Jamal et al., 1985).

We have recently examined this system in more detail over times from 30 min to 8 h after stimulation with cortico-

tropin using bovine LDL labelled with [14C]cholesteryl oleate as a source of cholesterol. The synthesis of cholesterol in the cells was inhibited by the incubation of the inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase, mevinolin. Thus the cells had either the stored cholesteryl ester or lipoprotein-derived cholesterol as potential sources of substrate for hormone synthesis. We found that corticotropin stimulated the uptake of the labelled LDL within a very short time. After 30 min both labelled free cholesterol and cholesteryl ester were detectable in the cells (Fig 1; B. Ochoa & K. E. Suckling, unpublished work). Within the first 2 h a greater increase in radioactivity in the cytosolic and microsomal fraction of the cells was observed in the cholesteryl ester fraction, but by 8 h the increase due to corticotropin was greater in the free cholesterol fraction and the effect on the cholesteryl ester was not significant. Thus after the initial 2 h the effect of the corticotropin was to switch the balance of cholesterol towards free cholesterol to make it available for hormone synthesis.

It appears that the cells in our cultured system take some time to accommodate themselves to the new state caused by corticotropin. This is hidden by the rapid increase in the mass of cortisol secreted by the cells when they are stimulated. However, within the cell we can discern changes in the source of cholesterol being used for steroidogenesis. One of the earliest changes that has been described, taking place

---

**Fig. 1. Change in accumulation of radioactivity from [14C]cholesteryl ester in bovine LDL in the free and esterified cholesterol in the cytosol and microsomal fraction of bovine adrenal cortical cells due to corticotropin**

- Free cholesterol
- Cholesteryl ester

---

**Fig. 2. Change in accumulation of free cholesterol in the mitochondrial fraction of the cells in the experiment shown in Fig. 1.**

The broken line is a reference line showing the control level (1).
within 10 min, is an increase in delivery of free cholesterol to the mitochondria (Crivello & Jefcoate, 1980). We also observed this in our studies (Fig. 2). Presumably the mitochondrial transport system required for the delivery of cholesterol to the cytochrome P-450 takes some time to activate so that an initial accumulation of LDL-derived cholesterol occurs.

By measuring the specific activity of the cortisol secreted by the cells we were able to observe the competition between corticotropin and cholesterol ester hydrolase. Under the action of the activated cholesterol ester hydrolase, a switch in cholesterol flux occurs which is only fully apparent after 2 h (Fig. 3). Thus during the first 2 h in the stimulated cells some of the incoming cholesterol is re-esterified but once the cells are fully accommodated to steroid hormone synthesis the flux of cholesterol is largely diverted to the mitochondria.

Little is known about how this control is achieved. Sterol carrier proteins such as SCF (Scallen & Vahouny, 1985) may have a role to play here: their activity could be altered as the metabolic state of the cell is changed so that they recognize a specific source of cholesterol and its destination in the cell. Alternatively, the architecture of the cell may permit certain fluxes but not others. For example, this may be the way in which the intestinal epithelial cell segregates absorbed cholesterol from that derived from lipoprotein and from newly synthesized cholesterol (Stange et al., 1983).

Because of the control that the adrenal cortical cell exerts over the uptake of cholesterol from lipoproteins and its subsequent metabolism, this system will remain an important model in studies of the intracellular sequel to receptor-mediated endocytosis.

Work in the authors' laboratory in the University of Edinburgh was supported in part by the Medical Research Council and a grant from the Spanish Ministry of Education and Science (to B.O.)

---