Stimulation of progesterone synthesis, in corpus luteum cells isolated from 4-amino-3,4-d-pyrazolopyrimidine-treated rats, by cholesterol presented in non-lipoprotein form

Christos Petrou*, Roger Booth+ and David A. Stansfield

Department of Biochemistry, Medical Sciences Institute, University of Dundee, Dundee DD1 4HN, Scotland

Received 26 June 1986

We have previously shown that the administration of 4-amino-3,4-d-pyrazolopyrimidine (4-APP) to rats reduces progesterone synthesis by cells subsequently isolated from their corpora lutea, and that plasma high density lipoprotein (HDL) can restore this progesterone synthesis to normal. In this paper we demonstrate that a dispersion of phospholipid and cholesterol, but not other sterols, can enhance this 4-APP-disabled progesterone synthesis to the same level as can HDL, thus providing the first direct evidence that cholesterol is the component of HDL upon which rat corpus luteum depends for its ability to synthesize progesterone.

Progesterone synthesis (Rat corpus luteum) 4-Amino-3,4-d-pyrazolopyrimidine HDL Phospholipid/cholesterol dispersion

1. INTRODUCTION

The administration of the plasma lipoprotein-lowering agent 4-APP to rats is known to reduce progesterone synthesis as estimated in vivo [1,2] or in vitro [3] and HDL administered in vivo [2,4] or presented to isolated corpus luteum cells in vitro [3] can restore this progesterone synthesis to normal. From this evidence we have suggested that rat corpus luteum depends upon access to circulating HDL for its ability to synthesize progesterone [3]. However, it seems clear from a recent review [5] that the same evidence has been interpreted by others as indicating that the rat corpus luteum depends upon circulating HDL cholesterol. Without direct experimental evidence it would seem unwise to assume that cholesterol must be the component of HDL that is involved in the mechanism by which HDL supports luteal progesterone synthesis. Clearly, 4-APP does not cause selective lowering of plasma cholesterol levels, but rather a general lowering of plasma lipoprotein levels such that tissues are deprived of access to all of the components of the lipoproteins, not to cholesterol alone. Similarly, infusion of HDL in vivo or presentation of HDL to isolated cells exposes the cells to the entire HDL species and not just to cholesterol selectively. In theory, any component of HDL could be involved in supporting cellular steroid synthesis.

Other evidence linking plasma cholesterol with progesterone synthesis is also indirect. The virtual equilibration of specific radioactivity between circulating progesterone, ovarian cholesterol and plasma cholesterol after administration of radioac-

+ To whom reprint requests should be addressed
* Present address: Department of General Biology, Faculty of Medicine, Aristotelian University, Thessaloniki, Greece

Abbreviations: 4-APP, 4-amino-3,4-d-pyrazolopyrimidine; HLD, high density lipoprotein (d = 1.065–1.209 g/ml)

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

tively labelled cholesterol or acetate [6–8] has been cited to support the view that steroid hormones are synthesized from cholesterol taken from the plasma [5] but because of the evidence that cholesterol exchange occurs between plasma lipoproteins and cells (reviewed in [9]), this cannot be taken to indicate the net uptake of cholesterol from plasma by tissues.

A fall in the luteal content of cholesteryl ester is known to result from 4-APP treatment [1,3], but 4-APP treatment does not change the amount of unesterified cholesterol in these cells which, even when depleted of cholesteryl esters, still contain unesterified cholesterol equivalent to over 1000-times the amount of progesterone that they can synthesize when fully stimulated with lutropin and HDL [3]. Virtually nothing is known about the metabolic compartmentation of cholesterol or cholesteryl esters within the luteal cell, and the intracellular pool of sterol from which progesterone is synthesized has not been identified. Clearly, it would be wrong to argue that, because 4-APP treatment lowers luteal cell cholesteryl ester content and also impairs luteal cellular progesterone synthesis, the impairment in progesterone synthesis must be due to the lowering of cellular cholesteryl esters.

The mechanism by which HDL interacts with the luteal cell, although apparently different from the LDL-pathway, has not yet been defined [10]. Fundamental to the investigation and understanding of this mechanism is the question of which of its components HDL contributes to luteal progesterone synthesis. The present work was undertaken because of the indirectness of the evidence, presented above, on this point. By demonstrating that the low rate of progesterone synthesis in luteal cells derived from rats treated with 4-APP can be restored to normal by supplying them with cholesterol in non-lipoprotein form, this work provides the first direct evidence that the cholesterol component of HDL is involved in the mechanism by which HDL can support luteal progesterone synthesis.

2. MATERIALS AND METHODS

The source of rats, methods for inducing superovulation, treatment with 4-APP, preparation and incubation of isolated luteal cells and assay of progesterone were as described [11]. L-α-Phosphatidylcholine (from egg yolk), ergosterol, β-sitosterol and dihydrocholesterol were purchased from Sigma, Poole, England, and heparin-agarose was purchased from Pharmacia, Milton Keynes, England. Cholesterol was purified as described in [12]; this, and all other sterols used, were recrystallised before use [13]. All other chemicals were as previously listed [11]. HDL was prepared as in [14], except that apo-E-containing HDL was removed by chromatography on heparin-agarose [15]. Lipid dispersions were prepared by sonicating 3 mg L-α-phosphatidylcholine and 2 mg sterol in 1 ml buffer (2 mM sodium phosphate; 50 mM sodium chloride; pH 7.4) at low power and amplitude setting no.1, in a 150 W ultrasonic disintegrator (type PG 100; MSE, Crawley, England) for 20 min. Delipidated HDL apoproteins were prepared as in [16]. When present in the incubations, lutropin was at 200 ng/ml and HDL at 0.8 mg HDL protein/ml, unless otherwise stated in the text, or in the legends to tables and figures. When phospholipid/sterol dispersions were present during incubations their concentrations were such as to provide 0.4 mg sterol/ml, unless otherwise indicated.

3. RESULTS AND DISCUSSION

The data in table 1 show that, under the conditions used, the phospholipid/cholesterol dispersion stimulated luteal cellular progesterone formation to virtually the same extent as did HDL. This effect of the phospholipid/cholesterol dispersion was saturable (fig.1). A double-reciprocal plot of the data shown in fig.1 (not shown) gave 0.07 mg/ml as the concentration of cholesterol necessary for half-maximal stimulation of progesterone synthesis. In experiments similar to those described in fig.1, but using native rat HDL instead of the phospholipid/cholesterol dispersion, it was found that half-maximal stimulation of progesterone synthesis occurred in the presence of 0.13 mg HDL protein/ml [17]. From the known composition of rat HDL [18] this was equivalent to 0.02 mg HDL cholesterol/ml or to 0.1 mg HDL total cholesterol (unesterified cholesterol + cholesteryl ester)/ml. In view of the uncertainty over whether or not the luteal cells might be able to utilise HDL cholesteryl esters or HDL

Table 1

Effect of a phospholipid/cholesterol dispersion on progesterone synthesis by luteal cells isolated from rats treated with 4-APP

Present during incubation	Progesterone formed (ng/10^6 cells per 2 h)	
	(a)	(b)
Lutropin	457 (36.1)	578 (19.1)
Lutropin + phospholipid/ cholesterol	1191 (94.2)	2793 (92.3)
Lutropin + HDL	1264 (100)	3024 (100)

Rats were treated with 4-APP and cells were prepared and incubated as described in section 2, where the procedures for assaying progesterone and for preparing HDL and phospholipid/cholesterol dispersions are also given. Lutropin was present at 200 ng/ml, HDL at 0.8 mg/ml and phospholipid/cholesterol dispersion at 0.4 mg cholesterol/ml. (a) and (b) refer to two separate experiments using different preparations of luteal cells. Numbers in parentheses are the % yields of progesterone in each experiment, relative to that produced in the presence of lutropin + HDL (100%)

unesterified cholesterol or both, there appears to be reasonable agreement between the amounts of cholesterol required for half-maximal stimulation of luteal progesterone synthesis, whether presented as phospholipid/cholesterol dispersion or as HDL (0.07 mg/ml, cf. 0.02 mg/ml).

A control experiment was needed to determine whether or not the stimulation of progesterone synthesis by phospholipid/cholesterol dispersions (table 1) was due to the cholesterol or to the phospholipid component of the dispersions. Rather than use a dispersion of phospholipid alone, which on available evidence [19] might have been expected to extract cholesterol from the luteal cells and consequently make the cells fragile to the point of lysis, we prepared dispersions from phospholipid and sterols other than cholesterol, on the grounds that these other sterols would largely fulfil any relevant structural function performed by cholesterol in the lipid dispersions, but would be unacceptable to the luteal cells as substrates for progesterone synthesis. Table 2 demonstrates that none of the dispersions tested, other than the one containing cholesterol as its sterol component, could enhance luteal cell progesterone synthesis. It is also apparent from the data of table 2 that the presence of delipidated HDL-apoproteins in the sterol/phospholipid dispersions had no appreciable effect upon the efficiency of exogenous sterol utilization.

It is necessary to comment upon an apparent discrepancy between the results described above and those reported in [20] which indicated that a phospholipid/cholesterol dispersion was ineffective in stimulating luteal cell progesterone formation. The experimental conditions reported above are not comparable with those of the study described in [20] in which the rats were not treated with 4-APP and lutropin was absent from the cell incubation medium. We have shown previously that, whereas HDL was able to stimulate progesterone synthesis by luteal cells from rats treated with 4-APP, HDL did not significantly affect progesterone synthesis by luteal cells from rats which had not been treated with 4-APP [3]. We also

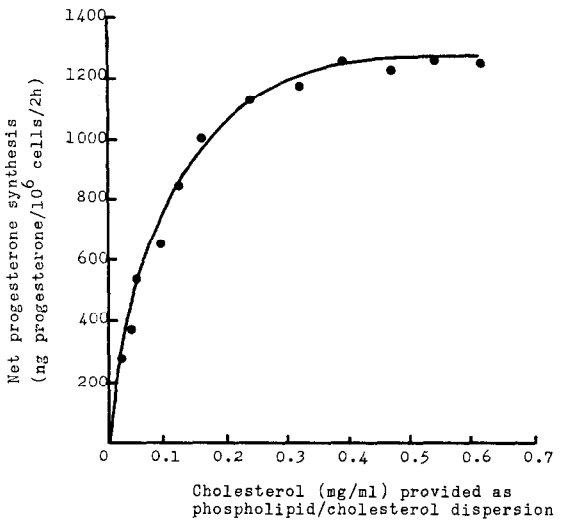

Fig.1. The effect of increasing concentrations of cholesterol, presented as phospholipid/cholesterol dispersion, upon progesterone synthesis by luteal cells from rats treated with 4-APP. Experimental conditions were as described in the legend to table 1 except that the amount of phospholipid/cholesterol dispersion was varied so as to give the concentrations shown on the graph. Lutropin was present in all incubations at 200 ng/ml. The amount of progesterone formed in the presence of lutropin but in the absence of phospholipid/cholesterol dispersion was subtracted from that formed in all other incubations.

Table 2

Effect of various sterols on progesterone synthesis by luteal cells isolated from rats treated with 4-APP

Present during incubation	Progesterone formed ($ng/10^6$ cells per 2 h)	
	(a)	(b)
Lutropin	447 (14.1)	368 (17.1)
Lutropin + phospholipid/ dihydrocholesterol	450 (14.7)	360 (16.7)
Lutropin + phospholipid/ β-sitosterol	461 (15.1)	383 (17.8)
Lutropin + phospholipid/ ergosterol	437 (14.3)	346 (16.1)
Lutropin + phospholipid/ cholesterol	2794 (91.5)	1891 (87.9)
Lutropin + HDL	3053 (100)	2151 (100)

Experimental conditions were as described in the legend to table 1. The phospholipid/sterol dispersions were prepared as described in section 2. (a) and (b) refer to two separate experiments using different cell preparations. In (b), lipid-free HDL apoproteins (prepared as described in section 2) were incorporated into the lipid dispersions at a concentration of 5 mg/ml, by adding the HDL-apoproteins to the dispersions and sonicating for a further 2 min. Numbers in parentheses are the % yields of progesterone in each experiment, relative to that produced in the presence of lutropin + HDL (100%)

showed that added lipoprotein had no significant effect on luteal cell progesterone synthesis unless lutropin was also present during the incubation of the cells [3]. It is also well known that luteal cells, incubated in the absence of lutropin, synthesize only about one-tenth of the progesterone which they can make in its presence [11]. A significant stimulation of progesterone synthesis by lipoproteins or phospholipid/cholesterol dispersions would not, therefore, be expected under the experimental conditions reported in [20].

In view of recent reports that (i) the association of HDL with aortic endothelial cells and muscle cells and with skin fibroblasts can occur via the interaction of cell surface- and HDL surface-lipids [21] and (ii) the delivery of cholesterol from HDL to rat ovary cells is not strictly dependent upon the presence of a specific apolipoprotein [10] it is interesting that we have shown a protein-free dispersion of cholesterol and phospholipid to be able to increase lutropin-stimulated luteal cell progesterone synthesis to the same level as that given by HDL (tables 1,2) and that HDL-apoproteins, when incorporated into such dispersions, have no demonstrable effect upon the yield of progesterone (table 2). It is not possible at present, however, to know whether or not the lipid dispersion acts via the same mechanism as that by which HDL functions in progesterone synthesis.

The data that we have presented provide the only direct evidence to support the view that it is the cholesterol component of HDL upon which the rat corpus luteum depends for its ability to synthesize progesterone.

ACKNOWLEDGEMENT

This work was supported by a grant from the Medical Research Council.

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