EVIDENCE FOR AN ESSENTIAL ROLE FOR HIGH-DENSITY LIPOPROTEIN IN PROGESTERONE SYNTHESIS BY RAT CORPUS LUTEUM

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1. Introduction

This work was designed to test the possibility that in vivo the rat corpus luteum might depend upon access to a specific plasma lipoprotein for its ability to synthesize progesterone in response to lutropin. Previous work relevant to this question has yielded inconclusive evidence. For example, in rats treated [1] with 4-APP to lower their plasma lipoprotein levels [2], plasma progesterone levels fell below normal but were restored after infusing HDL into the circulation for 42 h. However, changes in plasma progesterone levels do not necessarily indicate changes in the rate of luteal progesterone synthesis. Furthermore, the infused HDL need not have been acting directly upon the corpus luteum and indeed might have undergone metabolic modification, either in the plasma [3] or following uptake by the liver [4], during the long period of infusion. A lower rate of progesterone synthesis was observed [5] in luteal cells isolated from rats treated with 4-APP than in similar cells isolated from untreated rats, but this observation alone gives no significant information regarding the role of lipoproteins in luteal progesterone synthesis. Clearly instead of affecting progesterone synthesis indirectly, through its effect on plasma lipoprotein levels, the 4-APP could have been acting directly on the corpus luteum in such a way as to damage the mechanism of progesterone synthesis. Without evidence on this latter possibility the observed effect of 4-APP on progesterone synthesis cannot be interpreted unequivocally, but no convincing evidence was presented [5]. Such evidence is, however, described below. We have shown that the low rate of lutropin-dependent progesterone synthesis in luteal cells from 4-APP-treated rats was restored to within the normal range by providing the isolated cells with rat plasma total lipoprotein fraction, and that of the main species of rat plasma lipoproteins, only HDL was able to enhance progesterone synthesis.

Abbreviations: 4-APP, 4-aminopyrazolo 3, 4-d pyrimidine; HDL, high-density lipoprotein (d = 1.065–1.209); LDL, low-density lipoprotein (d = 1.006–1.065); VLDL, very low-density lipoprotein (d < 1.006); TLC, thin-layer chromatography

2. Materials and methods

Sources of animals and chemicals were as listed in [6]. Methods for superovulating rats, treating them with 4-APP, preparing and incubating isolated luteal cells and assaying progesterone were as in [6]. Rat serum total lipoprotein fraction (d < 1.225) was prepared as in [7] and dialysed for 48 h against four 1 litre volumes of 0.15 M NaCl before use. Separated rat plasma lipoproteins, prepared as in [8] were kindly provided by Dr E. R. Skinner, Department of Biochemistry, Marischal College, University of Aberdeen. Cholesterol and cholesteryl ester were extracted from cell suspensions, isolated by TLC and estimated by procedures used for sterol analysis of microsomal suspensions [9].

3. Results and discussion

The rate of lutropin-dependent progesterone synthesis by luteal cells isolated from 4-APP-treated rats was linear for up to 2 h incubation time, but thereafter slowed to a plateau after 4–5 h (not shown);

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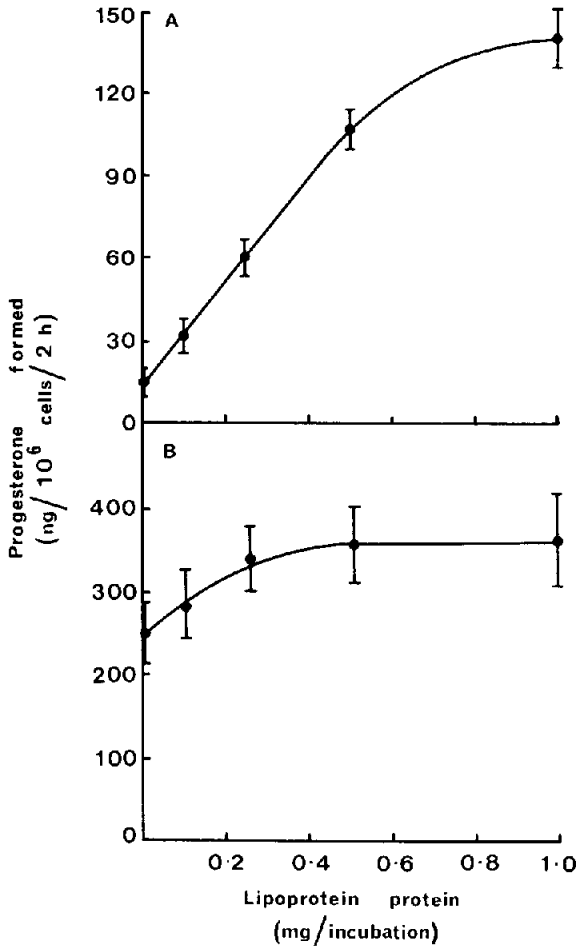

Fig.1. Effect of plasma total lipoprotein fraction on progesterone synthesis by isolated luteal cells. Conditions were as in table 1 except that the amount of plasma total lipoprotein fraction added to each incubation was varied as shown in the graphs; lutropin (100 ng/ml) was present in all incubations: (A) obtained using luteal cells from 4-APP-treated rats; (B) using cells from rats not treated with 4-APP. Each point on the graph is the mean ± SEM of 3 (A) or 5 (B) separate determinations on separate batches of cells.

this rate was also ~1/8th of that given by cells from rats not treated with 4-APP (table 1A). Fig.1A shows however that the rate of progesterone synthesis by these cells was enhanced 9-fold, to values within the range of rates given by cells isolated from rats not treated with 4-APP, by the total lipoprotein fraction of rat plasma. Increasing the concentration of this lipoprotein fraction beyond that shown in fig.1A was found to give no further enhancement of progesterone synthesis (not shown). In contrast, progesterone synthesis by cells isolated from rats not treated with 4-APP was not significantly affected ($P > 0.05$) by plasma lipoproteins (fig.1B); evidently the steroidogenic response to lipoproteins by cells isolated from 4-APP-treated rats (fig.1A) is a consequence of the 4-APP treatment, and is not a general property shared with cells from untreated rats. Plasma lipoproteins had no effect on progesterone synthesis unless lutropin was present (table 1B), demonstrating that the lipoproteins themselves do not stimulate progesterone synthesis, but rather enable the cells to respond to lutropin. The above results (table 1B; fig.1A,B) constitute direct evidence, lacking in [5], that 4-APP treatment does not seriously damage the luteal cellular mechanism for lutropin-dependent progesterone synthesis. This evidence thus validates the conclusion from table 1A that, when deprived of access to circulating lipoproteins, the corpus luteum loses much of its capacity for progesterone synthesis. The results in table 1A,B and fig.1 together constitute the first direct and conclusive evidence that the rat corpus luteum in vivo depends upon plasma lipoproteins for the maintenance of its steroidogenic function. The data given in table 1C demonstrate that HDL is the specific lipoprotein upon which the corpus luteum depends, indicating an essential role for HDL in luteal progesterone synthesis in the rat. It should be noted that although LDL (d = 1.006–1.065) is a major component of human plasma, rat plasma contains hardly any material in this density band [10]. We have been able to obtain enough rat LDL for only a single experiment; this lipoprotein fraction had no effect on progesterone synthesis (not shown).

There is at present no evidence to explain in molecular terms how HDL functions in luteal steroidogenesis but several observations suggest that it would be premature to assume that the only possible role of HDL is to supply cholesterol as a substrate for progesterone synthesis [1,5], especially in view of the report of how specific lipoproteins can modulate adenylate cyclase activity in cell membranes [11].

(i) 4-APP treatment lowers plasma lipoprotein levels but not cholesterol levels selectively; lipoproteins contain several components other than cholesterol and its esters.

(ii) The proposed [1,5] connection between plasma and tissue cholesteryl ester levels and luteal progesterone synthesis is based upon an observed correlation rather than upon direct experimentation.

Table 1
Progesterone synthesis by isolated luteal cells

Present during incubation	Progesterone formed (ng . 10^6 cells^{-1} . 2 h^{-1})	
	Cells from rats not treated with 4-APP	Cells from rats treated with 4-APP
A –	17.4 ± 7.2 (3)	3.0 ± 0.8 (3)
Lutropin (100 ng/ml)	95.8 ± 19.0 (3)	11.5 ± 4.0 (3)
B –	–	2.4 ± 0.8 (3)
Lutropin (100 ng/ml)	–	11.9 ± 4.1 (3)
Plasma total lipoprotein ($d < 1.225$)	–	18.4 ± 4.2 (3)
Lutropin (100 ng/ml) + plasma total lipoprotein ($d < 1.225$)	–	139.1 ± 10.4 (3)
C –		
Lutropin (100 ng/ml)	–	9.2 ± 1.9 (3)
Lutropin + VLDL ($d < 1.006$)	–	13.8 ± 3.3 (3)
Lutropin + HDL (d = 1.065–1.209)	–	47.1 ± 8.8 (3)
Lutropin + plasma total lipoprotein ($d < 1.225$)	–	41.6 ± 8.2 (3)

Methods for superovulating the rats, treating them with 4-APP and isolating the luteal cells are in section 2. Cells were incubated for 2 h at 37°C and, when present, lipoprotein fractions containing 1 mg protein were added to each incubation. Progesterone was extracted and assayed as in section 2. Values are means ± SEM; no. obs. shown in parentheses

Measurements of the effect of 4-APP treatment on luteal content of cholesteryl ester were made using whole ovaries rather than cells isolated from these and used in measuring progesterone synthesis [5]. These isolated cells need not have been typical, in their cholesteryl ester content, of the whole organ from which they were derived. Measurements on our own isolated cells showed that 4-APP treatment lowered luteal cell cholesteryl ester content from 20.0 ± 3.8(3)–4.7 ± 0.8(3) $\mu g/10^6$ cells, but it should be noted that the quantity of cholesteryl ester in these cells still exceeded by ~1000-fold the amount of progesterone which these cells could synthesize. Thus there was no direct quantitative relationship between cellular cholesteryl ester content and cellular capacity to synthesize progesterone. Furthermore, a time-course of progesterone synthesis, by cells isolated from 4-APP-treated rats incubated in the presence of plasma total lipoprotein fraction, was qualitatively identical to that given by similar cells incubated without lipoproteins (not shown). There was no 'lag-period' which might have been expected if progesterone synthesis had had to await the replenishing of cellular cholesteryl ester through uptake from external lipoprotein. There was no significant difference in unesterified cholesterol content between luteal cells from rats not treated with 4-APP (5.2 ± 2.4(3) $\mu g/10^6$ cells) and luteal cells from 4-APP-treated rats (5.8 ± 1.0(3) $\mu g/10^6$ cells).

It would also be premature to suppose that a mechanism analagous to the LDL-pathway [12], but utilising HDL instead of LDL, is involved in luteal progesterone synthesis. Although the component events of the LDL-pathway have been demonstrated in cultured mouse adrenal tumour cells [13], no direct evidence was presented to show that this LDL-pathway constituted an essential part of the overall process of steroid synthesis by these cultured cells. Specific HDL receptors have been observed in rat testis cell membranes [14] but no evidence was given to link these receptors with the process of steroid production. Mechanisms for cell and lipoprotein interaction not involving lipoprotein internalization are known to exist in steroidogenic tissues [15]. Clearly, comprehensive experimentation will be necessary to define the processes whereby the entire HDL complex interacts with the mechanism of progesterone synthesis in our corpus luteum cell preparation. Experiments which focus upon one HDL component alone, e.g., its sterol, or which simply measure the uptake and metabolism

of HDL without attempting to relate these events to the mechanism of progesterone synthesis, will give only a preliminary and incomplete answer to the question of how HDL functions in luteal steroidogenesis.

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