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DIFFERENCES IN HYDROXYSTEROL METABOLISM BETWEEN RAT AND MOUSE LEYDIG CELLS. Patrick G. Quinn*, Markos Georgiou and Anita H. Payne, Depts. Biol. Chem. and Ob.-Gyn., Univ. of Michigan, Ann Arbor, MI 48109

The metabolism of 25-,20α- and 22R-hydroxycholesterols (25C, 20C and 22C), which bypass the cAMP-dependent, cycloheximide(CHX)-sensitive transport to P-450$_{scc}$ required by cholesterol, has been investigated in rat and mouse Leydig cells (LC). Purified LC were incubated with a saturating concentration of hydroxysterol (20 μM) in the presence and absence of a maximal dose of 8-Br-cAMP (cA, 1mM) or CHX (25 μg/ml). Testosterone (T) was measured by RIA. Results are expressed as per cent of cA-stimulated T ($\bar{x}$+SEM).

	CHX	cA	25C	25C+cA	20C	20C+cA	22C	22C+cA
Rat	-	100	235+ 6	247+25	238+15	233+31	374+18	430+45
Rat	+	7+1	212+11	N.D.	238+28	N.D.	380+24	N.D.
Mouse	-	100	24+ 1	85+ 2	116+11	126+ 8	324+28	352+44
Mouse	+	2+1	25+ 1	21+ 2	100+ 3	N.D.	260+33	N.D.

Hydroxysterol metabolism was not stimulated by cA. CHX, which abolished the response to cA, did not reduce T production from hydroxysterols in either species, confirming that hydroxysterols are able to bypass transport to P-450$_{scc}$ in LC. Rat LC produced 2-4 times as much T from hydroxysterols as in response to cA. In comparison to cA-stimulated T, mouse LC produced more T from 22C, equal T from 20C and much less T from 25C. In addition, 25C consistently inhibited cA-stimulated T production in all experiments with mouse LC and this could not be accounted for by inhibition of metabolism of ^{3}H-pregnenolone to ^{3}H-T. The results suggest that the P-450$_{scc}$ of mouse LC has a lower V_{max} *in situ* for 25C than for cholesterol. Supported by NIH Grants HD-08358 and HD-07048.

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ROLE OF MICROTUBULES IN THE UTILIZATION OF LOW DENSITY LIPOPROTEIN BY CULTURED RAT LUTEAL CELLS. V.P. Rajan and K.M.J. Menon, Dept. of Ob/Gyn, University of Michigan Medical School, Ann Arbor, MI 48109

Previous studies have shown that cultured rat luteal cells utilize low density lipoproteins (LDL) by receptor mediated endocytosis followed by lysosomal degradation. The mechanisms involved in the transport of endocytotic vesicles carrying LDL to the lysosomes are not clearly established. In the present study, we report on the possible involvement of microtubules in the endocytotic process. Luteal cell cultures prepared from PMSG-hCG primed immature rats were incubated in McCoy's 5A medium (1ml) with various doses (1-1000μM) of the microtubule inhibitor colchicine for 4h. [^{125}I]LDL (400,000 cpm, specific activity 500 cpm/ng protein) was added to the cultures and incubated for an additional 6h. The medium was collected and precipitated with 10% trichloroacetic acid (TCA). Degradation of [^{125}I]LDL was assessed by determination of the TCA soluble radioactivity. Colchicine inhibited [^{125}I]LDL degradation in a dose dependent manner. Maximum inhibition (~60%) was obtained at a concentration of 100 μM or higher and required at least 4h of preincubation with colchicine. Pretreatment with colchicine had no effect on [^{125}I]LDL binding to luteal cells. To further investigate the role of microtubules on LDL degradation, we determined the effect of dimethyl sulfoxide (DMSO), an agent known to rapidly polymerize tubulin. Addition of DMSO (1-10% v/v) to the incubation medium showed a dose-dependent inhibition of [^{125}I]LDL degradation, with 10% DMSO completely preventing degradation. From this study we conclude that microtubules may be involved in the uptake and utiliation of LDL in the rat corpus luteum. (Supported by NIH HD06656).