Journal of Steroid Biochemistry, Vol. 9, pp. 731–738

0022-4731/78/0801-0731$02.00/0

EFFECTS OF LYSOSOMOTROPIC AGENTS ON PROGESTIN SECRETION BY RAT OVARIAN CELLS*

J. F. STRAUSS III, T. KIRSCH and G. L. FLICKINGER
Departments of Obstetrics and Gynecology, and of Physiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, U.S.A.

(*Received* 2 *November* 1977)

SUMMARY

To study the role of lysosomes in ovarian function, we examined effects of lysosomotropic drugs on progesterone secretion by suspensions of cells prepared from superovulated immature rat ovaries. 50 μM chloroquine (CQ) produced a 45% inhibition of progestin secretion by LH-treated cell suspensions. The effect of CQ was dose-dependent with 30% and 60% inhibition of the secretory response to LH being caused by CQ concentrations of 25 μM and 200 μM, respectively. CQ (50 μM) also partially blocked the steroidogenic response of ovarian cells to dibutyryl cAMP. Incubation of dispersed ovarian cells in the presence of neutral red (0.345 mM), NH_4Cl (10 mM) or Triton WR-1339 (0.75%) also resulted in partial inhibition of progesterone secretion in response to LH. CQ (50 μM) did not alter incorporation of [4,5-^{3}H]-leucine into protein, [U-^{14}C]-glucose oxidation, or lactate production by the ovarian cell suspensions. Furthermore, CQ did not inhibit formation of [^{14}C]-pregnenolone and [^{14}C]-progesterone by ovarian mitochondria incubated in the presence of 5 mM succinate and [4-^{14}C]-cholesterol. Many of the cells exposed to CQ developed large vacuoles during a 1 h incubation. These membrane-enclosed vacuoles contained amorphous material and membrane whorls. Although one must be cautious in assigning specific loci of action to inhibitors, our observations on the effects of several lysosomotropic drugs on progesterone secretion by LH-stimulated ovarian cells suggest that lysosomes are involved in the process of steroidogenesis.

INTRODUCTION

Lysosomes are associated with events occurring during cell demise and it is not surprising that these organelles are thought to play an important role in the process of luteolysis, especially in species like the sheep in which the corpora undergo rapid structural dissolution [1, 2]. However, it is clear that lysosomes have importance beyond mediating degenerative changes and they appear to carry out essential functions in rapidly growing and actively secreting cells [3]. There is also evidence suggesting that lysosomes mediate the action of tropic hormones on certain target tissues [4].

Prompted by these observations, we investigated the function of lysosomes in steroidogenically active luteal tissue. Initially, we measured acid hydrolase levels in ovaries of superovulated immature rats and observed a progressive rise in hydrolase activities following induction of luteinization [5]. The rise in enzymic activities correlated with the increasing blood progesterone titres, suggesting a relationship between increasing lysosomal activity and steroidogenesis in the corpus luteum. To explore this relationship, we examined effects of substances which localize in lysosomes and alter their function on progestin secretion by dispersed ovarian cells exposed to LH. The agents tested included chloroquine (CQ), neutral red and ammonium ion (NH_4Cl), which are known to be concentrated in lysosomes, and Triton WR-1339, a non-ionic detergent, which binds to lipoproteins and is taken into lysosomes [6]. All these substances partially inhibited progestin secretion by cells stimulated with LH.

MATERIALS AND METHODS

Highly luteinized ovaries were produced in Sprague–Dawley rats, 21–23 days old, by subcutaneous injections of 50 IU of pregnant mare's serum (Gestyl, Organon) followed 56 h later by 25 IU of human choriogonadotropin (hCG, Ayerst). Animals were killed by cervical dislocation, and the ovaries were quickly removed, weighed and placed in cold isotonic saline.

Dispersed cells were prepared by incubating minced ovaries in Hank's salt solution containing 2% (w/v) bovine serum albumin (BSA) and collagenase as previously described [7]. Cells were suspended in Hank's or Medium-199 (Grand Island Biological Co.) containing 2% BSA, and 1 ml aliquots (10–20 μg DNA) were pipetted into siliconized tubes. Chloroquine diphosphate (Sigma), neutral red (Harleco), Triton WR-1339 (Ruger Chemical Co.) or NH_4Cl in Hank's salt solution adjusted to neutral pH was added to some tubes while other tubes received vehi-

* This work was supported by NIH grant HD 10451-01 and a grant from the Rockefeller Foundation.

cle. Cells were incubated at 37°C for 30 min in the experiments with CQ, neutral red and NH_4Cl and for 1 h in experiments with Triton WR-1339 and then 1 μg ovine LH (NIH-LH-S19), kindly supplied by N.I.A.M.D.D., a dose which maximally stimulated progesterone secretion ~4-fold above basal levels, or 500 nmol of dibutyryl cAMP (Sigma) was added. The cells were incubated for an additional 30 min, and incubations were terminated by centrifugation at 4°C. In each experiment, every treatment group contained 3–4 tubes and at least 2 separate experiments were performed with each lysosomotropic agent. Aliquots of cells were reserved for DNA determinations [8] and examination under phase contrast optics.

The quantitation of progesterone in incubation media and cell pellets by radioimmunoassay [9] was not affected by the presence of CQ, neutral red or Triton WR-1339. For ultrastructural analysis, cells were fixed in 3% glutaraldehyde buffered with 0.1 M cacodylate, pH 7.4, for 1 h, embedded in Epon 812 and sections were stained with 4% uranyl acetate and lead citrate.

The effect of CQ on protein synthesis was examined by incubating cells in Hank's salt solution or Medium-199 containing 2% BSA and 5 μCi of [4,5-^{3}H]-leucine and trichloroacetic acid precipitable radioactivity of cell pellets was determined [10]. [U-^{14}C]-Glucose oxidation was studied in cells incubated in Krebs–Ringer bicarbonate buffer, pH 7.4, containing 2% BSA and 5.5 mM [U-^{14}C]-glucose (S.A. 1 mCi/mmol). $^{14}CO_2$ was collected in Hyamine after injection of perchloric acid into the incubates [11]. Lactate released into the incubation medium was determined by an enzymatic method as previously noted [11].

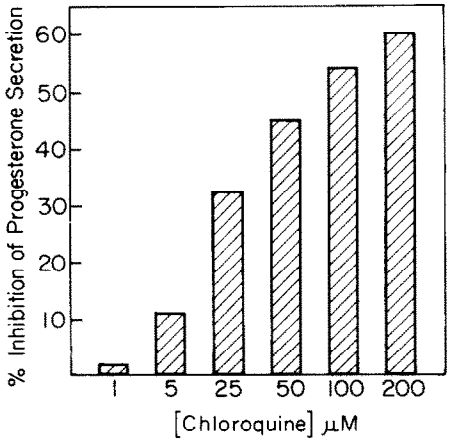

Fig. 1. Cell suspensions were incubated in the presence of various doses of CQ for 30 min and then exposed to LH (1 μg/ml) for a further 30 min. Control tubes contained no CQ. Results are expressed as % inhibition of progesterone secretion relative to the control level of 5.57 ng progesterone released/μg DNA. Each point represents the mean value of 4–8 replicates. The S.E.M. for the values are 3.5, 4.5, 4.9, 1.9, 3.6 and 2.8 for the 1 μM through 200 μM CQ concentrations, respectively.

Mitochondria-rich fractions prepared from homogenates of luteinized ovaries [12] were incubated for 2 h in the presence of [4-^{14}C]-cholesterol and 5 mM succinate as described by Robinson and Stevenson [13]. Conversion of the labeled cholesterol to [^{14}C]-pregnenolone and [^{14}C]-progesterone was measured in the presence and absence of 50 μM CQ.

RESULTS

CQ inhibited progesterone secretion by ovarian cells exposed to LH and the effects of the drug were dose-related (Fig. 1). Since 50 μM CQ is in the range of doses used successfully to study lysosomal function in other cells [14], we routinely used this dose in our studies. Cells required exposure to CQ for at least 10 min prior to stimulation with LH to obtain a consistent suppression of progestin secretion. Cells which were exposed to CQ for 30 min, washed three times, and then stimulated by LH for an additional 30 min in medium without CQ showed a reduction (25–30%) of progesterone secretion, although it was less than that found (~ 45%) when CQ was present throughout the experiment. Progesterone secretion by unstimulated cells was also reduced by CQ (see Table 5) and the progesterone content of CQ-treated cells was 30–50% lower than that of untreated cells. The effect of CQ depended on the age of the luteinized tissue; progesterone secretion from LH-stimulated cells was reduced 43% when ovaries were obtained 3–4 days after hCG treatment while a 25% suppression was found with cells from day 7 ovaries (Table 1). CQ also inhibited the steroidogenic response of ovarian cells to dibutyryl cAMP (Table 2), suggesting that it acts at a point beyond the binding of LH to its membrane receptor. The lysosomotropic agents, neutral red (Table 3), NH_4Cl and Triton WR-1339 (Table 4), also inhibited progesterone secretion by LH-stimulated ovarian cells.

Exposure of ovarian cells to 50 μM CQ for 1 h did not affect their ability to exclude trypan blue. Incorporation of [4,5-^{3}H]-leucine into protein was not influenced by LH and CQ did not alter this process in LH-stimulated cells. In experiment A (Table 5), CQ in the absence of LH appeared to increase [4,5-^{3}H]-leucine incorporation, but this trend was not observed in other studies. Lactate production (LH: 1.20 ± 0.05; LH + 50 μM CQ: 1.28 ± 0.06 nmol lactate formed/μg DNA, mean ± S.E.M., 4 replicates) and oxidation of [U-^{14}C]-glucose to $^{14}CO_2$ (LH: 240 ± 4; LH + 50 μM CQ: 250 ± 14 pmol glucose oxidized/μg DNA, mean ± S.E.M., 4 replicates) by cells was not altered by CQ.

CQ did not interfere with succinate-supported cholesterol side-chain cleavage by mitochondria from luteinized ovaries (Table 6). Failure of CQ to block formation of pregnenolone and progesterone suggests that the drug does not inhibit either Δ5-ene-3β-hydroxysteroid dehydrogenase-isomerase or cholesterol side-chain cleaving enzyme complexes.

Table 1. Effect of chloroquine on progestin secretion by cells prepared from ovaries 3–4 or 7 days post-hCG treatment

Treatment	Progesterone secretion	
	Ovaries removed 3–4 days Post-hCG (N = 8)	Ovaries removed 7 days Post-hCG (N = 6)
None	100	100
LH	463 ± 66*	438 ± 71‡
LH + 50 μM CQ	264 ± 37†	320 ± 38§
% Inhibition of LH-induced progestin secretion by CQ	42.9 ± 1.2‖	24.5 ± 3.7

* Significantly different from no treatment group, $P < 0.001$ by paired t-test.

† Significantly different from LH group, $P < 0.001$ by paired t-test.

‡ Significantly different from no treatment group, $P < 0.01$ by paired t-test.

§ Significantly different from LH group, $P < 0.025$ by paired t-test.

‖ Significantly different from mean obtained with cells from day 7 ovaries, $P < 0.001$ by Student's t-test for unpaired samples.

Dispersed cells were prepared from ovaries removed either 3–4 days or 7 days post-hCG treatment and incubations were carried out in the presence or absence of 50 μM CQ as described in the text. Due to variations in basal progestin secretion and stimulatory response to LH between experiments, data have been presented as percent progestin secretion relative to the no treatment group. Values are means ± S.E.M. of N experiments.

Table 2. Effect of chloroquine on dibutyryl cAMP stimulated progesterone secretion by ovarian cells

Treatment	Progesterone secretion (ng/μg DNA)	
	Experiment A	Experiment B
None	1.75 ± 0.22	2.18 ± 0.08
500 μM db cAMP	3.08 ± 0.15*	3.97 ± 0.32*
500 μM db cAMP + 50 μM CQ	2.00 ± 0.13†	1.97 ± 0.07†

* Significantly different from no treatment group, $P < 0.005$.

† Significantly different from db cAMP group, $P < 0.005$.

Two separate experiments were performed with dispersed cells from ovaries removed 3 days post-hCG. Cells were incubated for 30 min in the presence and absence of chloroquine. Incubations were continued for another 30 min after addition of db cAMP to some tubes. Each value represents mean ± S.E.M. of the 4 replicates in each treatment group. Statistical analyses were carried out using Student's t-test for unpaired samples.

Table 3. Effect of neutral red on progesterone secretion by ovarian cells

Treatment	Progesterone secretion
None	100
Neutral red (100 μg/ml)	92 ± 7.8
LH	407 ± 65*
LH + neutral red (100 μg/ml)	226 ± 38†

* Significantly different from no treatment group, $P < 0.01$ by paired t-test.

† Significantly different from LH group, $P < 0.02$ by paired t-test.

Dispersed cells from ovaries removed 3–4 days post-hCG were incubated in the presence or absence of neutral red for 30 min and then LH was added to some tubes and the incubations continued for a further 30 min. Progesterone released into the incubation fluid was quantitated. Due to variations in the basal progestin secretion and response of cells to LH, data is presented as percent progesterone secretion relative to the no treatment group. Values are means ± S.E.M. of 5 experiments.

Table 4. Effect of NH_4Cl and Triton WR-1339 on progesterone secretion by ovarian cells

Treatment	Progesterone secretion (ng/μg DNA) Experiment A	Experiment B
None	0.30 ± 0.01	0.39 ± 0.02
NH_4Cl (10 mM)	0.27 ± 0.02	0.37 ± 0.02
LH	1.11 ± 0.02*	0.91 ± 0.05*
§ LH + NH_4Cl (10 mM)	0.88 ± 0.05‡	0.61 ± 0.05‡
None	0.82 ± 0.03	0.72 ± 0.08
Triton WR-1339 (0.75%; v/v)	0.27 ± 0.04*	0.21 ± 0.05*
LH	3.65 ± 0.12*	5.29 ± 0.20*
LH + Triton WR-1339 (0.75%; v/v)	1.84 ± 0.12†	2.00 ± 0.11†

* Significantly different from no treatment group, $P < 0.001$ by Student's t-test for unpaired samples.

† Significantly different from LH group, $P < 0.001$.

‡ Significantly different from LH group, $P < 0.005$.

§ Choline chloride (10 mM) did not significantly alter LH-induced progesterone secretion.

Dispersed cells from ovaries 3–4 days post-hCG were incubated in the presence or absence of either NH_4Cl (30 min) or Triton WR-1339 (1 h). In experiments to study the effects of Triton WR-1339 the cells were washed 3 times with Hank's salt solution before addition of LH or vehicle. In all experiments, incubations were continued for 30 min after addition of tropic hormone. Values presented are means ± S.E.M. of the 4 replicates in each treatment group.

Table 5. Effect of chloroquine on incorporation of [^{3}H]-leucine into protein by ovarian cells

Treatment	^{3}H in protein (c.p.m./μg DNA) Experiment A	Experiment B	Progesterone secretion (ng/μg DNA) Experiment A	Experiment B
None	5830 ± 450	144 ± 24	0.43 ± 0.04	0.58 ± 0.08
50 μM CQ	6675 ± 207*	96 ± 3.9	0.28 ± 0.04*	0.32 ± 0.02*
LH	5657 ± 654	113 ± 4.7	1.86 ± 0.11	1.81 ± 0.07
LH + 50 μM CQ	5488 ± 334	105 ± 3.1	0.89 ± 0.05†	1.05 ± 0.07†

* Significantly different from no treatment group, $P < 0.05$.

† Significantly different from LH group, $P < 0.001$.

Dispersed cells from ovaries 3–4 days post-hCG were incubated in Hank's salt solution (Experiment A) containing 2% BSA and 5 μCi [4,5-^{3}H]-leucine (S.A. 60 Ci/mmol) or in Medium 199 containing 2% BSA and 5 μCi [^{3}H]-leucine (S.A. 5.7 μCi/mmol). Trichloroacetic acid precipitable radioactivity was measured in cell pellets and progesterone was assayed in incubation media as described in text. In each experiment, treatment groups contained 4 replicates and values presented are means ± S.E.M.

Table 6. Effect of chloroquine on cholesterol side-chain cleavage by ovarian mitochondria

Treatment	c.p.m. In steroid/mg protein/2 h [^{14}C]-pregnenolone	[^{14}C]-progesterone
Control	6473 ± 267	1966 ± 76
50 μM CQ	6221 ± 257	1963 ± 162

Mitochondria, prepared from ovarian homogenates by differential centrifugation, were incubated for 2 h as described by Robinson and Stevenson [13]. Flasks contained 5 mM succinate and 150,000 c.p.m. [4-^{14}C]-cholesterol (S.A. 55 mCi/mmol) with a final incubation vol. of 1 ml. Extracts were analyzed for steroid radioactivity by thin-layer chromatography. Recovery of total radioactivity from reaction mixtures was 80% ± 3.3. Each value represents mean ± S.E.M. for 3 replicates per treatment group.

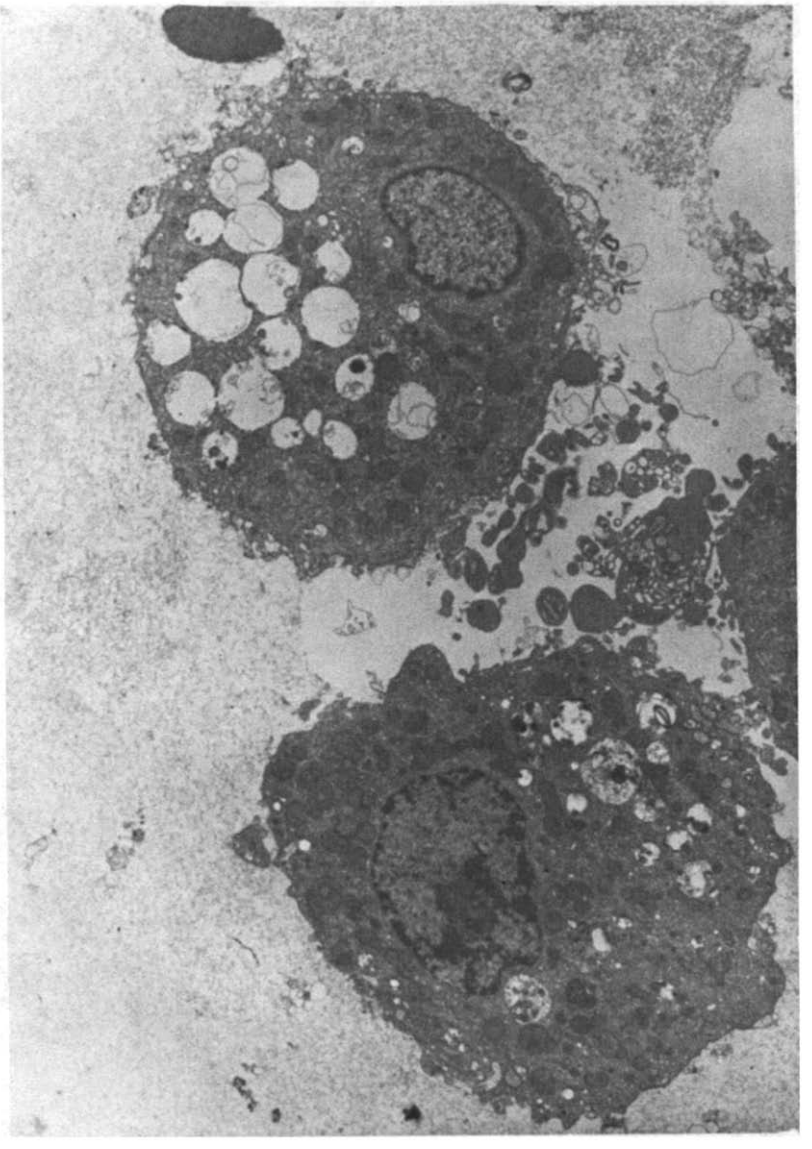

Fig. 2. Luteal cells exposed to 50 μM CQ for 1 h. Large cytoplasmic vacuoles are abundant. × 4950.

Ovarian cells exposed to 50 μM CQ for 1 h developed large cytoplasmic vacuoles (Figs 2 and 3) which were seen infrequently in untreated cells (Fig. 4). These membrane-enclosed vacuoles contained amorphous material, membrane whorls and occasionally, lipid droplets. Lipid droplets were often found in close association with CQ-induced vacuoles.

DISCUSSION

An increase in ovarian lysosomal hydrolase activities, which accompanied the rise in progesterone secretion following induction of luteinization, first suggested to us that there is a relationship between lysosome function and steroidogenesis [5]. Our observations that lysosomotropic substances reduced progesterone production by ovarian cells adds support to the concept that lysosomes play a role in steroidogenesis. However, one must be cautious in assigning a specific locus of action to pharmacologic agents. Indeed, each of the drugs studied in the present work could act at several sites to inhibit steroid secretion. Yet, several experiments indicated that the effect of at least one of the lysosomotropic drugs (CQ) on steroid production was not related to actions on organelles other than lysosomes. CQ did not alter glucose oxidation or lactate production by luteal cells. CQ also did not directly influence the conversion of cholesterol to steroids by ovarian mitochondria-rich subcellular fractions.

Inhibition of protein synthesis can block the steroidogenic response of gonadal tissues to LH [15]. It was important to evaluate the effect of CQ on this process because CQ in high concentrations inhibits synthesis of macromolecules through its interaction with DNA and inhibition of DNA-dependent DNA

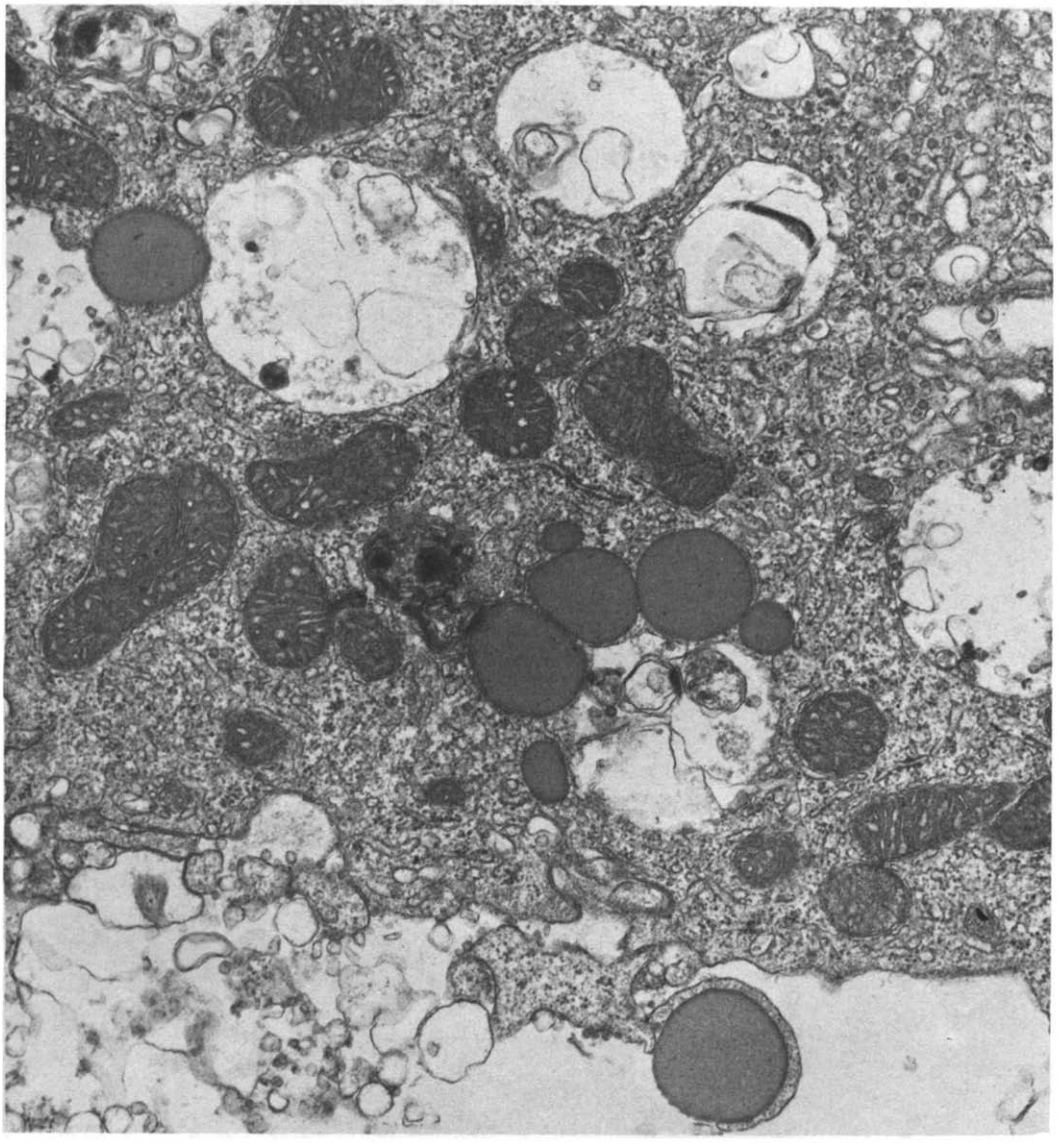

Fig. 3. Higher magnification of luteal cell exposed to CQ shows vacuoles containing amorphous material and membrane whorls. Note the close association between lipid droplets and vacuoles. Mitochondrial structure is well preserved and typical for steroid secreting cells. × 29,700.

and RNA polymerases [16]. Since 50 μM CQ did not alter incorporation of [4,5-^{3}H]-leucine into proteins, this dose apparently did not affect either amino acid uptake or protein synthesis in LH-stimulated ovarian cells.

The abundant cytoplasmic vacuoles which we observed in CQ-treated ovarian cells are similar to those which develop in other cell types exposed to the drug [14, 17, 18]. These structures are clearly members of the lysosomal-vacuolar system since they have been shown to contain acid phosphatase activity [14]. Preliminary studies on the subcellular localization of CQ taken into ovarian cells revealed that the majority of the drug was associated with particulate fractions which contained lysosomal enzyme activities. These findings are similar to those of Wibo and Poole [18] who studied CQ-treated fibroblasts.

The mechanism by which CQ interferes with lysosomal function is not completely understood. CQ may modify the permeability of lysosome membranes [19], as well as have direct effects on specific hydrolases [18]. CQ action may also be related to changes in intralysosomal pH which occurs when the base is protonated within lysosomes [6]. Other weak bases including neutral red and ammonia may affect lysosomal function by similar mechanisms.

The lysosomal functions which are required for steroidogenesis in ovarian cells remain to be elucidated. We postulate that a portion of luteal cell cholesterol must by processed by lysosomes before it can

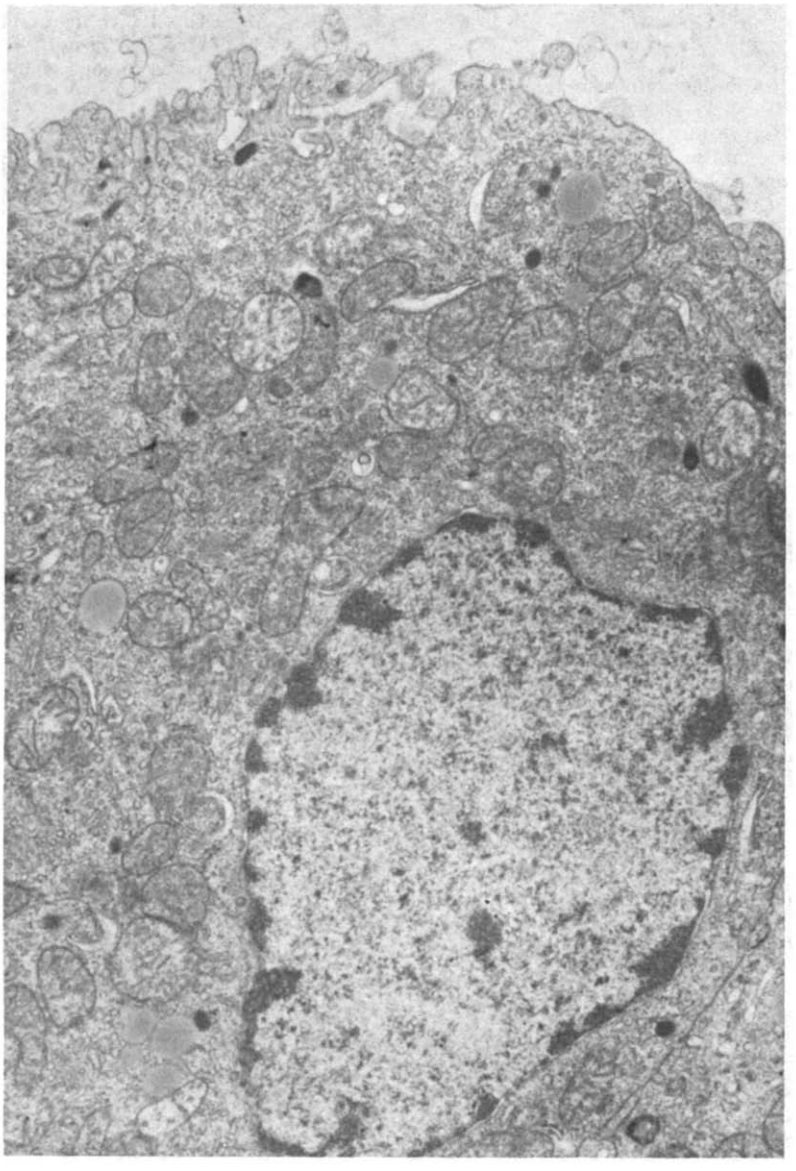

Fig. 4. A section of a control cell × 19,800.

enter the steroidogenic pool. Lysosomal cholesteryl esterase is involved in the metabolism of lipoproteins taken into cells by endocytosis [20]. Faust *et al.* [21] recently reported that adrenal cells form a substantial amount of steroid hormone *in vitro* from lipoprotein-bound cholesterol which has been taken into the cells and processed by lysosomes.

Since depletion of cholesteryl esters is known to be associated with increased steroidogenesis in LH-stimulated corpora lutea [22], lysosomes could also be involved in the metabolism of these stored sterol esters. Lysosomotropic drugs, by interfering with lysosomal function, might reduce the availability of cholesterol for progesterone formation.

The finding that CQ and other lysosomotropic agents only partially inhibited progesterone secretion from LH-stimulated cells may be attributed to the presence of free cholesterol which is immediately used for steroidogenesis. There is an increase in ovarian lipids, including cholesterol, following the induction of luteinization [23] and a larger pool of readily available cholesterol may explain why older luteal cells were less sensitive to the effects of CQ.

In summary, it appears that the role of lysosomes in the corpus luteum can no longer be restricted to the process of luteolysis; these organelles also appear to be important for the steroidogenic function of this tissue.

Acknowledgements—The technical assistance of Mrs. Chi Cho Kao is greatly appreciated. Electron microscopic studies were kindly performed by Dr. Santo Nicosia with support from NIH grant HD 06274-06.

REFERENCES

1. Dingle J. T., Hay M. F. and Moor R. M.: Lysosomal function in the corpus luteum of the sheep. *J. Endocr.* **40** (1968) 325–336.
2. McClellan M. C., Abel J. H. and Niswender G. D.: Function of lysosomes during luteal regression in normally cycling and $PGF_{2\alpha}$-treated ewes. *Biol. Reprod.* **16** (1977) 499–512.
3. Dean R. T. and Barrett A. J.: Lysosomes. *Essays in Biochemistry* **12** (1976) 1–40.
4. Szego C. M.: The lysosome as a mediator of hormone action. *Recent Prog. Horm. Res.* **30** (1974) 171–233.
5. Flickinger G. L. and Strauss, III J. F.: Lysosomes and corpus luteum function. *Gynec. Invest.* 7 (1977) 26.
6. de Duve C., de Barsy R., Poole B., Trouet A., Tulkens P. and Van Hoof F.: Lysosomotropic agents. *Biochem. Pharm.* **23** (1974) 2495–2531.
7. Strauss, III J. F. and Flickinger G. L.: Phospholipid metabolism in cells from highly luteinized rat ovaries. *Endocrinology* **101** (1977) 883–889.
8. Hinegardner R. T.: An improved fluorometric assay for DNA. *Analyt. Biochem.* **39** (1971) 197–201.
9. de Villa G. O., Roberts K. Wiest W. G., Mikhail G. and Flickinger G. L.: A specific radioimmunoassay of plasma progesterone. *J. clin. Endocr. Metab.* **35** (1972) 458–460.
10. Gelehrter T. D. and Emanuel J. R.: Hormonal regulation of protein degradation in hepatoma cells in tissue culture. *Endocrinology* **94** (1974) 676–684.
11. Strauss, III J. F., Seifter E., Lien E. L., Goodman D. B. P. and Stambaugh R. L.: Lipid metabolism in regressing rat corpora lutea of pregnancy. *J. Lipid Res.* **18** (1977) 246–258.
12. Okazaki T., Strauss, III J. F. and Flickinger G. L.: Lysosomal phospholipase A activities of rat ovarian tissue. *Biochim. biophys. Acta* **487** (1977) 343–353.
13. Robinson J. and Stevenson P. M.: Electron flow and cholesterol-side-chain cleavage in ovarian mitochondria. *Eur. J. Biochem.* **24** (1971) 18–30.
14. Fedorko M. E., Hirsch J. G. and Cohn Z. A.: Autophagic vacuoles produced *in vitro*. I. Studies on cultured macrophages exposed to chloroquine. *J. cell Biol.* **38** (1968) 377–391.
15. Marsh J. M., Butcher R. W., Savard K. and Sutherland E. W.: The stimulatory effect of luteinizing hormone on adenosine 3',5'-monophosphate accumulation in corpus luteum slices. *J. biol. Chem.* **241** (1966) 5436–5440.
16. Hahn F. E.: Chloroquine (Resochin) *In Antibiotics, Vol. III* (Edited by J. W. Corcoran and F. E. Hahn). Springer-Verlag, New York (1975) p. 58.
17. Fedorko M. E., Hirsch J. G. and Cohn Z. A.: Autophagic vacuoles produced *in vitro*. II. Studies on the mechanism of formation of autophagic vacuoles produced by chloroquine. *J. cell Biol.* **38** (1968) 392–402.
18. Wibo M. and Poole B.: Protein degradation in cultured cells. II. The uptake of chloroquine by rat fibroblasts and the inhibition of cellular protein degradation by cathepsin B_1. *J. cell Biol.* **63** (1974) 430–440.
19. Filkins J. P.: Comparison of *in vivo* and *in vitro* effects of chloroquine on hepatic lysosomes. *Biochem. Pharm.* **18** (1969) 2655–2600.
20. Goldstein J. L., Dana S. E., Faust J. R., Beaudet A. and Brown M. S.: Role of lysosomal acid lipase in the metabolism of plasma low density lipoprotein. *J. biol. Chem.* **250** (1975) 8487–8495.
21. Faust J. R., Goldstein J. L. and Brown M. S.: Receptor-mediated uptake of low density lipoprotein and utilization of its cholesterol for steroid synthesis in cultured mouse adrenal cells. *J. biol. Chem.* **252** (1977) 4861–4871.
22. Armstrong D. T.: Gonadotropins, ovarian metabolism, and steroid biosynthesis. *Recent Prog. Horm. Res.* **24** (1968) 255–319.
23. Herbst A. L.: Response of rat ovarian cholesterol to gonadotropins and anterior pituitary hormones. *Endocrinology* **81** (1967) 54–60.