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21. Molecular Mechanisms of Steroid Synthesis

ACTH REGULATION OF CHOLESTEROL MOVEMENT IN ISOLATED ADRENAL CELLS

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Summary—Confluent bovine adrenal cell primary cultures respond to stimulation by adrenocorticotropin (ACTH) to produce steroids (initially predominantly cortisol and corticosterone) at about one-tenth of the output of similarly stimulated rat adrenal cells. The early events of steroidogenesis, following ACTH stimulation, have been investigated in primary cultures of bovine adrenal cortical cells. Steroidogenesis was elevated 4–6-fold within 5 min of exposure to 10^{-7} M ACTH and increased linearly for 12 h and declined thereafter. Cholesterol side-chain cleavage (SCC) activity was increased 2.5-fold in mitochondria isolated from cells exposed for 2 h to ACTH and 0.5 mM aminoglutethimide (AMG), even though cytochrome P-450$_{scc}$ only increases after 12 h. Mitochondrial-free cholesterol levels increased during the same time period (16.5–25 μg/mg of protein), but then both cholesterol levels and SCC activity declined in parallel. More prolonged exposure to ACTH prior to addition of AMG caused the elevation in mitochondrial cholesterol to more than double, possibly due to enhanced binding capacity. Early ACTH-induced effects on cellular steroidogenesis result from these changes in mitochondrial-free cholesterol. The maximum rate of cholesterol transport to mitochondria in AMG-blocked cells was consistent with the maximum rate of cellular steroidogenesis. Cycloheximide (0.2 mM) rapidly blocked (< 10 min) cellular steroidogenesis, cholesterol SCC activity, and access of cholesterol to cytochrome P-450$_{scc}$ without affecting mitochondrial-free cholesterol. Exposure of confluent cultures to the potent environmental toxicant, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (10^{-8} M), for 24 h prior to ACTH addition decreased the rates of ACTH- and cAMP-stimulated steroidogenesis but did not affect the basal rate. In both cases, the effectiveness of TCDD increased with time of exposure to the stimulant. Although cholesterol accumulated in the presence of ACTH and AMG (13–28 μg/mg), pretreatment of cells with TCDD caused a decrease in mitochondrial cholesterol (13–8 μg/mg). The effect of TCDD was produced relatively rapidly ($t_{1/2} \sim 4$ h). Since even in the absence of TCDD, the mitochondria of ACTH-stimulated cells also eventually lose cholesterol (after 2 h) TCDD pretreatment may increase the presence of a protein(s) that cause this mitochondrial-cholesterol depletion following stimulation by ACTH or cAMP. Examination of the effects of *in vivo* treatment of rats with ACTH and cycloheximide on isolated rat adrenal mitochondria supports the hypothesis that ACTH stimulates highly labile regulatory protein(s) that modulate transfer of cholesterol between mitochondrial membranes and possibly also the phospholipid content of the outer membrane. Studies from several laboratories suggest that SCP_2 and phosphatidyl inositides may exert additional synergistic effects on cholesterol transfer to P-450$_{scc}$ while fatty acid binding protein and Apo E_1 may be candidates for mitochondrial-cholesterol storage and removal. Bovine adrenal cells offer an excellent model to study these processes further.

INTRODUCTION

Cholesterol side-chain cleavage (SCC) to pregnenolone is the rate-limiting step in the production of adrenal steroids [1]. This step is catalyzed by cytochrome P-450$_{scc}$ [2] located on the matrix side of the inner membrane [3] and is activated by ACTH [1, 4]. Adrenal cholesterol is stored as esters in cytosolic lipid droplets that result from the uptake of lipoproteins from the plasma or external medium [5] (HDL in rat cells, LDL in bovine cells [5, 6]). During the acute phase of ACTH stimulation of the rat adrenal *in vivo*, the cholesterol that is metabolized in the mitochondria is released from lipid droplets rather than entering mitochondria by a more direct route from the serum [7]. Prior to metabolism, cholesterol is released from esters by ACTH activation of cholesterol esterase (a cAMP-mediated phosphorylation) and then transfers to the mitochondria [8]. Cholesterol access to mitochondria, together with steroidogenesis, are inhibited in rats by cytochalasin B (disrupts microfilaments) and by vinblastine or colchicine (disrupts microtubules) [9]. Only the former seems to be involved in cultured Y-1 mouse adrenal tumor cells [10] while disruption of microfilaments has no effect on steroid synthesis in bovine adrenal cells [11]. Steroidogenesis is also rapidly inhibited by protein synthesis inhibitors and this leads to a failure of isolated mitochondria to utilize cholesterol, which then accumulates [12].

Cholesterol can be effectively replaced as a substrate for P-450$_{scc}$ by more water-soluble analogs that also pass more readily between cell membranes. The absence of any effect of either ACTH or cycloheximide on the capacity of cells [13] or mitochondria [14] to metabolize hydroxylated cholesterol derivatives has indicated, first, a special mechanism in the adrenal cell to utilize cholesterol

and, second, that direct modulation of cytochrome P-450$_{scc}$ is not involved in either ACTH stimulation or inhibition by protein synthesis inhibitors.

These and many other experiments have led to the hypothesis that ACTH regulates cholesterol metabolism through control of cholesterol availability to cytochrome P-450$_{scc}$. Recently, interest in this process has heightened through the discovery in the adrenal cell of at least four proteins capable of affecting cholesterol distribution, including one steroid regulatory protein that is very labile and sensitive to ACTH [15–18]. In this communication, we describe studies in cultured bovine adrenal cells that demonstrate new features of this cholesterol regulatory process. We also report experiments that probe the nature of the intramitochondrial transfer of cholesterol in the adrenal cell.

METHODS

Culturing of bovine adrenal cortical (BAC) cells, quantitation of steroids and cholesterol, and mitochondrial isolation from the cells were carried out as previously described [19]. Inner and outer mitochondrial membranes from rat adrenal mitochondria were separated by hypo-osmotic shock and gradient centrifugation [20]. Cholesterol complex formation with cytochrome P-450$_{scc}$ in isolated mitochondria was measured by means of the pregnenolone-induced difference spectrum [21]. The effect of ACTH (10^{-7} M) on mitochondrial-cholesterol accumulation in cultured cells was carried out with 0.5 mM aminoglutethimide (AMG) [19]. For equivalent experiments with rats *in vivo*, AMG (20 mg) was administered 20 min prior to ACTH. Rats were killed and adrenals homogenized at either 10 or 20 min after administration of ACTH [20].

RESULTS AND DISCUSSION

When ACTH (10^{-7} M) is added to primary monolayers of BAC cells, there is a 4-fold stimulation of steroid production. Cortisol and corticosterone are the predominant steroids in the first few hours, along with various steroid intermediates such as a 17α-hydroxyprogesterone [19, 22]. Here we will focus just on total steroid production, which then reflects the rate of cholesterol metabolism. The stimulation of total steroidogenesis is almost immediate since a linear rate of production occurs through the first 12 h, excluding a lag time of <5 min [19] (Fig. 1). The results are, therefore, compatible with those from rat adrenal cell suspensions where a lag of 2–3 min was reported for the ACTH stimulation of corticosterone formation [23]. This immediacy of response evidently excludes new synthesis of steroidogenic enzymes, which is only significant after 6–12 h [24].

In rat adrenals, an ACTH-stimulation of cholesterol metabolism is retained by isolated mitochondria [25]. This is made evident as an increased extent of fast-phase cholesterol metabolism (<5 min). Intact mitochondria have been isolated from BAC cells by a combination of hypo-osmotic swelling and gentle homogenization. Cholesterol metabolism was initiated by isocitrate and, therefore, required intact mitochondria for the generation of NADPH. Mitochondria isolated during the first 8 h after exposure of cells to ACTH showed no increase in cholesterol SCC activity relative to activities from unstimulated cells (Fig. 2A). After about 8 h, a linear increase in pregnenolone formation was observed, consistent with the reported increased synthesis of cytochrome P-450$_{scc}$ [24]. By contrast, when cholesterol metabolism was fully inhibited in the cells by 0.5 mM AMG, SCC activity in the isolated mitochondria was stimulated 3-fold in about 2 h (Fig. 2B). An increase between 12 and 24 h was again observed, but, surprisingly, there was a significant decline in activityn between 2 and 8 h. Measurement of cholesterol levels in mitochondria from AMG-treated cells indicated a 60% ACTH-induced increase in the cholesterol level, peaking at 2 h, exactly parallel to the peak in SCC activity (Fig. 3). This was followed by a dramatic decline in mitochondrial cholesterol, reaching unstimulated levels after 12 h. Again, this parallels the decline in SCC activity.

These experiments demonstrate that ACTH can stimulate an influx of cholesterol into the mitochondria sufficiently rapidly to correspond to the stimulation of steroidogenesis. A net increase in mitochondrial cholesterol is not observed in the absence of inhibition of cholesterol metabolism (Table 1), in-

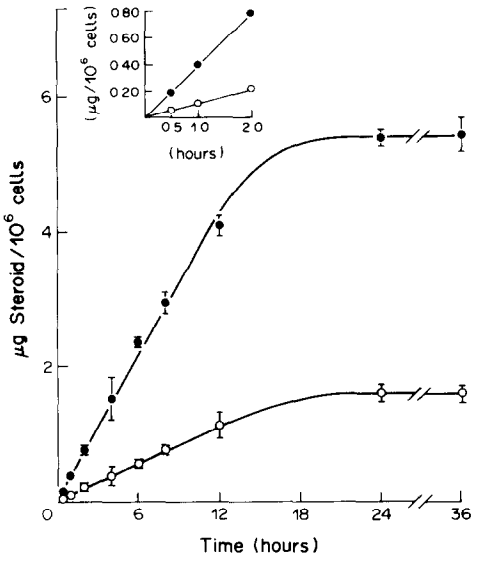

Fig. 1. Time course of total steroidogenesis in bovine adrenal cells. ACTH (10^{-7} M) was added to confluent bovine adrenal cell cultures (1.5×10^6 cells/4-cm diameter plate). One time point was taken from individual plates. Each point represents mean ± SEM for 3 identical experiments. Steroids were extracted and quantitated by HPLC. ○, control cells; ●, +ACTH. (From Ref. [19] with permission.)

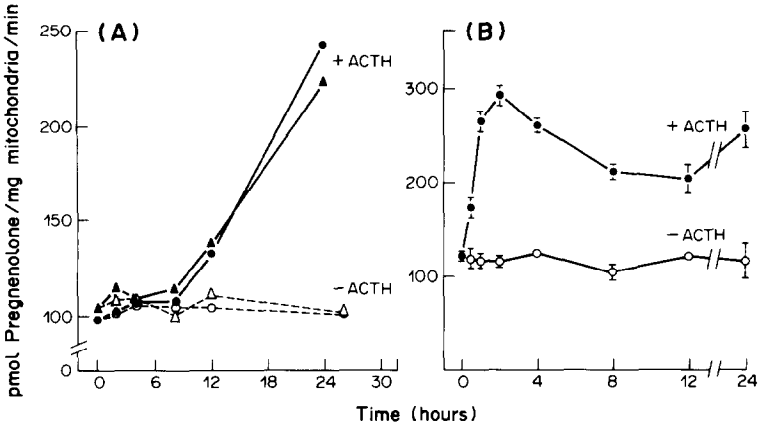

Fig. 2. SCC activity in mitochondria isolated from BAC cell cultures. A, Cells incubated with (●, ▲) or without 10^{-7} M ACTH (○, △). Intact (○, ●) or broken (△, ▲) mitochondria were incubated in the presence of 10 μM cyanoketone with, respectively, either isocitrate or NADPH, adenodoxin reductase, and adrenodoxin for 10 min at 37°C. Pregnenolone synthesis was qμ. B. Cells were incubated in the presence of 0.5 mM AMG.

dicating that normally in the cell the SCC turnover rate is much faster than the rate of transfer of cholesterol into the mitochondria; that is, this transfer is rate-limiting in cellular cholesterol metabolism.

Does this same cholesterol transfer to mitochondria occur in the absence of AMG? In this regard, the ACTH-stimulated rate of cholesterol transfer to mitochondria (8.5 μg/mg mitochondria per h) in the presence of AMG is comparable to the stimulated rate of cellular steroidogenesis (5.5 μg steroids/mg mitochondria per h) under comparable conditions except without AMG. This correlation strongly suggests that the same cholesterol transfer occurs, irrespective of the presence of AMG, and that this process has a central role in the stimulation of steroidogenesis by ACTH.

The loss of mitochondrial cholesterol, even when SCC is fully inhibited, points to an additional mechanism for removal of cholesterol from mitochondria within adrenal cells. This loss of cholesterol beginning after 2 h of ACTH exposure also clearly accounts for the decline in the SCC activity in the isolated mitochondria during this period. This decline could potentially be a response either to prolonged (2-h) exposure to ACTH or to the elevated level of mitochondrial cholesterol. In order to resolve this question, cells were exposed to ACTH for varying periods prior to the introduction of AMG. In Table 2, it can be seen that prolonged exposure to ACTH prior to the addition of AMG

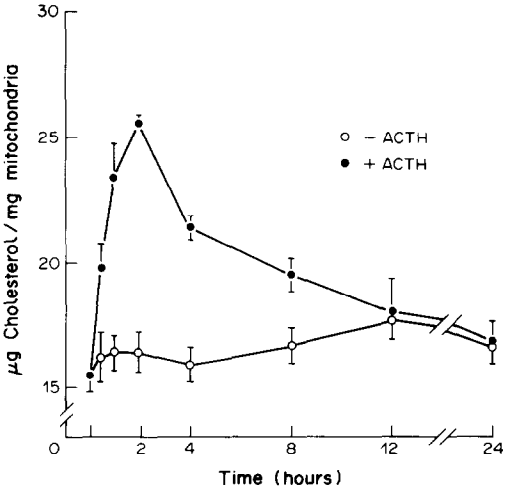

Fig. 3. Free cholesterol levels in mitochondria isolated from primary cultures of BAC cells. Mitochondria were isolated from BAC cell cultures after various times of exposure to 0.5 mM AMG in the presence (●) or absence (○) of 10^{-7} M ACTH. Cholesterol was extracted and quantitated by GC. Each point represents the mean ± SEM for 3 experiments. (From Ref. [19] with permission.)

Table 1. Cholesterol determination in isolated mitochondria from BAC cell cultures

	μg cholesterol/mg protein	
Treatment	−ACTH	+ACTH
Control	12.6 ± 0.6	13.6 ± 0.1
AMG	13.8 ± 0.3	19.0 ± 0.3
CX	13.2 + 0.4	20.1 ± 1.3
CX + AMG	ND*	21.3 ± 1.2

Confluent BAC cell cultures were incubated for 1 h at 37°C in the presence of various combinations of 10^{-7} M ACTH, 0.2 mM cycloheximide (CX) and 0.5 mM aminoglutethimide (AMG). Mitochondria were isolated and free cholesterol levels quantitated as described under Materials and Methods. Each value represents the mean ± SEM ($n = 4$).

*ND: not determined.

Table 2. Effect of ACTH exposure time on the accumulation of mitochondrial-cholesterol in BAC cells inhibited for 2 h by AMG

Exposure times (h)		Final mitochondrial cholesterol level	Increase in cholesterol
ACTH	AMG	(μg/mg mitochondrial protein)	
0–2	None	17.9 ± 1.3	7.8
0–2	0–2	25.7 ± 1.6	
2–4	None	18.8 ± 2.1	11.9
0–4	2–4	30.7 ± 2.1	
0–6	None	15.3 ± 2.4	18.1
0–6	4–6	33.4 ± 0.8	
0–8	None	20.5 ± 1.9	17.2
0–8	6–8	37.7 ± 3.6	
8–10	None	17.5 ± 1.6	5.1
0–10	8–10	22.6 ± 0.7	

Confluent BAC cells were exposed to 10^{-7} M ACTH for the periods indicated after addition of new medium. For the indicated plates (2 per point), AMG (0.5 mM) was added for the final 2 h. Mitochondria was isolated at the end of the incubation, and cholesterol levels were quantitated by gas chromatography.

results in a progressive increase in 2-h mitochondrial-cholesterol accumulation, reaching a maximum at 6–8-h exposure to ACTH. It seems, therefore, that the peak and decline observed during the initial prolonged exposure to ACTH and AMG is not a result of prolonged exposure to ACTH. The most likely possibility is that the cells respond to prolonged accumulation of mitochondrial-cholesterol by generating a removal mechanism. The second interesting feature of this experiment is that 6–8-h exposure to ACTH apparently enhances the capacity of the mitochondria to accumulate cholesterol. Since there is no increase in cellular steroidogenesis during this 6-h period, ACTH is probably not enhancing cholesterol transfer to the mitochondria but rather the storage capacity.

The effect of the protein synthesis inhibitor, cycloheximide, on the ACTH stimulation of steroidogenesis in BAC cells has been examined. The results were very similar to those observed in rat adrenal tissues [9, 12]. Steroidogenesis was inhibited by cycloheximide, but in the presence of AMG there was no effect on transfer of cholesterol to the mitochondria (Table 1). When AMG was removed, cycloheximide proved equally effective in causing mitochondrial-cholesterol accumulation. Like rat adrenals, these mitochondria also failed to show ACTH stimulation of cholesterol metabolism (Fig. 4). In addition, cycloheximide prevented the enhanced P-450$_{scc}$–cholesterol complex formation caused by the combined treatment of ACTH and AMG (Table 3). Again, the consequences of cycloheximide inhibition are realized in the mitochondria as a block to cholesterol combination with P-450$_{scc}$. By analogy with the recent work of Pederson *et al.* on rat adrenals [15], it seems likely that ACTH can activate steroidogenesis in BAC cells at least in part through stimulation of a labile regulatory protein.

The potent environmental toxicant, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exerts a very

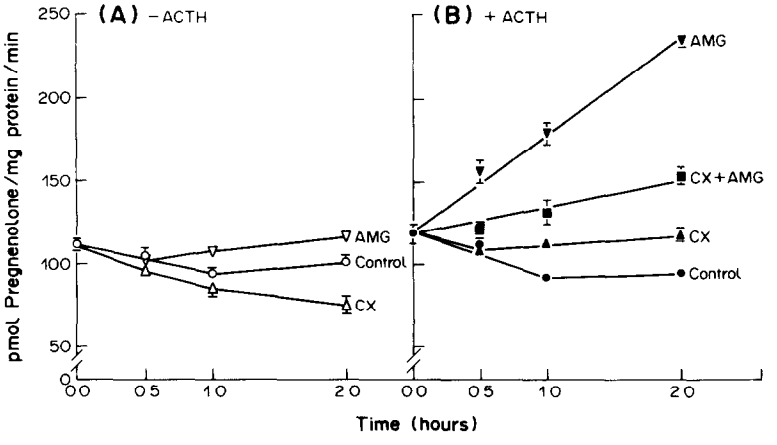

Fig. 4. SCC activity in mitochondria isolated from BAC cell cultures treated with ACTH, cycloheximide, and AMG. Conditions were the same as for Fig. 2 except that indicated incubations contained cycloheximide (0.2 mM, CX). (From Ref. [19] with permission.)

Table 3. Determination of the formation of cholesterol and P-450$_{scc}$ complexes in mitochondria isolated from BAC cell cultures

Treatment	ΔA(390–420)/mg × 10^{-3}	
	−ACTH	+ACTH
Control	14.0	16.0
AMG	14.5	22.5
CX	10.5	13.0
CX + AMG	ND*	13.0

Conditions were as described in Table 5. Complex formation of cholesterol with P-450$_{scc}$ was determined with pregnenolone-induced, reverse Type-I spectral differences as described under Materials and Methods. Each number represents the average of 2 determinations.
*ND: not determined.

different inhibitory effect on steroidogenesis in BAC cells. A 24-h exposure to 10^{-8} M TCDD shifts the ED_{50} for ACTH about 30-fold to higher concentrations [26]. The initial rate of stimulated steroidogenesis was not affected but rather delined from linearity as early as 4 h after stimulation (Fig. 5). Similar results were obtained with cAMP stimulation of BAC cells. Quantitation of mitochondrial-cholesterol levels in these cells showed this to be the source of the inhibitory effect. In cells inhibited with AMG that have also been treated with TCDD, ACTH not only failed to elicit accumulation of mitochondrial-cholesterol but in fact caused a decrease (Table 4). A similar decrease was observed when AMG was omitted. There was no effect of TCDD treatment on unstimulated mitochondrial-cholesterol levels either with or without AMG or on the uptake of LDL cholesterol esters into BAC cells [26]. These changes are consistent with TCDD-induced synthesis of protein(s) that, following activation by ACTH–cAMP, cause cholesterol to redistribute out of the mitochondria. This mechanism may also be related to the depletion of mitochondrial-cholesterol following AMG-enhanced cholesterol accumulation or, alternatively, to a reversal of the ACTH-induced stimulation of the mitochondrial-cholesterol capacity. Since the mitochondria in most tissues are characterized by very low cholesterol levels, it seems reasonable to suppose that adrenal mitochondria have specific ACTH-controlled structures that bind cholesterol in sites that are readily available to cytochrome P-450$_{scc}$. The observation that AMG enhances rat adrenal mitochondrial levels of fatty acid binding protein [17] suggests that this protein may contribute to such mitochondrial-cholesterol storage rather than to transport [16]. Any change in mitochondrial-cholesterol sequestration could lead to the ACTH-induced loss of BAC mitochondrial-cholesterol following TCDD treatment. The time course of the TCDD effect ($t_{1/2}$ = 4 h) is about 2-fold more rapid than typical inductions stimulated by TCDD that are mediated by the Ah-receptor [27].

In order to probe the nature of intramitochondrial-cholestrol movement, we have examined the distribution of cholesterol and phospholipids between the inner and outer membranes of rat adrenal mitochondria. Combined AMG–ACTH treatment of rats for 20 min results in a peak accumulation of mitochondrial cholesterol. About 70% of newly accumulated cholesterol is found in the inner membrane [20] (Table 5). Simultaneous treatment with cycloheximide blocked entry of cholesterol into

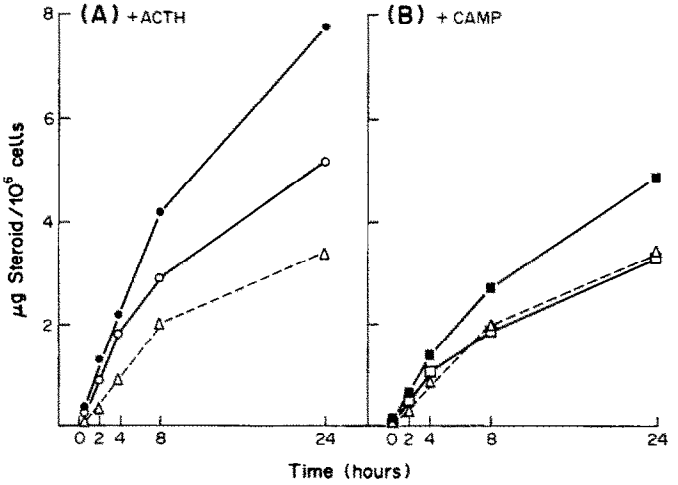

Fig. 5. Time course of steroid synthesis in ACTH-stimulated or cAMP-stimulated BAC cells; effect of TCDD on steroidogenesis. Confluent cultures of BAC cells were exposed to TCDD (10^{-8} M) for 24 h, washed, and incubated in the presence of half-maximum concentrations of ACTH (5×10^{-11} M) (A) or dibutyryl cAMP (0.3 mM) B. Each time point is the average of two incubations. (---△---) Untreated cells, ●, ■; stimulated control cells; ○ and □, stimulated TCDD-treated cells. (From Ref. [26] with permission.)

Table 4. Free cholesterol content in mitochondria and homogenates prepared from primary BAC cell cultures exposed to TCDD, ACTH and aminoglutethimide

Treatment	Free cholesterol (μg cholesterol/mg protein)		
	Mitochondria	Homogenate	Δ*
AMG			
− ACTH	13.2 ± 0.3	13.5 ± 0.5	10.9
+ ACTH (5×10^{-11} M)	18.2 ± 0.5	14.1 ± 0.7	10.5
+ ACTH (5×10^{-9} M)	21.0 ± 0.5	14.0 ± 0.2	9.8
TCDD + AMG			
− ACTH	11.9 ± 0.1	13.0 ± 0.2	10.6
+ ACTH (5×10^{-11} M)	8.1 ± 0.3	14.0 ± 0.03	12.4
+ ACTH (5×10^{-9} M)	8.5 ± 0.05	15.8 ± 0.05	14.1

Conditions were as described for Table 3 except that cells were incubated in the presence of aminoglutethimide (0.5 mM) (AMG) in addition to TCDD (10^{-8} M) and ACTH. Each value is the average ± SEM for 2 samples. The contribution of mitochondrial free cholesterol to total cellular free cholesterol was calculated by multiplying each value for mitochondrial free cholesterol by the fraction of mitochondrial protein in cellular homogenates (20%).

*Cellular free cholesterol − mitochondrial free cholesterol contribution.

Table 5. Distribution of ACTH-induced cholesterol accumulation in rat adrenal mitochondria

Treatment	Treatment-induced cholesterol increase (μg/1 mg mitochondrial protein); membrane fractions			
	Total	Inner	Inter	Outer
10 min				
ACTH†	6.5	2.3 (36)§	1.8 (28)	0.7 (11)
ACTH/CX†	4.6	0	0.9 (20)	2.7 (59)
20 min				
ACTH†	11.8	7.9 (67)	1.5 (13)	1.3 (11)
ACTH/CX†	8.6	2.2 (25)	1.5 (17)	2.4 (28)
ACTH‡	16.5	7.1 (43)	ND	1.6 (10)
ACTH/CX‡	12.6	1.2 (9)	ND	2.5 (20)

Cholesterol increases were calculated by subtraction of zero time values or inhibitor-free determinations, as indicated. Values represent the amount of cholesterol in each membrane fraction from mitochondria containing 1 mg of protein. The per cent distribution of inhibitor-induced accumulation of mitochondrial cholesterol in each membrane fraction is then expressed as a percentage of the total increase in the whole mitochondria.

*All rats receive AMG.

†Increases relative to zero time-pretreatment values (Table 1).

‡Increases in dexamethazone-treated rats relative to 20-min treatment with saline/AMG (Table 2).

§Numbers in brackets represent percentages of the total increase in each fraction.

ND: not determined.

Inter, material separating between inner and outer membrane fractions (fraction 3).

the inner membrane and caused additional accumulation in the outer membrane. It seems, however, that the outer membrane capacity to integrate this cholesterol is exceeded more rapidly than the capacity of the inner membrane. Consequently, within 10 min after ACTH–cycloheximide treatment cholesterol starts to enter a "weakly bound" pool that is lost during membrane isolation. The cycloheximide-sensitive steroid regulatory protein [15] may, therefore, be involved in transferring cholesterol between mitochondrial membranes. SCP_2 may also participate in this transfer [28] since removal of this protein from adrenal cytosol prevents stimulation of cycloheximide-blocked adrenal mitochondria by this fraction.

Phosphatidyl inositol synthesis is rapidly increased in adrenal cells in response to ACTH [29]. This increase is predominantly located in the mitochondria, specifically the outer membrane (Table 6). This process is also rapidly blocked by cycloheximide. Phospholipids such as PI may, therefore, play a role in the facilitated intermembrane transfer of cholesterol in these mitochondria. Some support for this idea is provided by the direct stimulatory effect on steroid synthesis in cycloheximide-blocked mitochondria of di- and tri-phosphatidyl inositol [29]. However, very high unphysiological concentrations are required for such effects.

Studies in bovine adrenal cells indicate very similar regulatory processes to those observed in the rat adrenal, even though steroid synthesis is about 10-fold lower. Interestingly, both the maximum rates of cholesterol transfer to mitochondria and mitochondrial SCC are similarly decreased suggesting a coordinate control of these activities. Four principle activities seem to contribute to ACTH-induced changes in cholesterol distribution in adrenal cells (Fig. 6):

1. Cholesterol transfer to mitochondria. Studies in rat cells suggest that SCP_2 may be involved [28]. Microfilaments that participate in this process in rat cells are not required in bovine cells possibly because serum LDL rather than lipid droplets are the immediate source of cholesterol.

2. Intramitochondrial cholesterol transfer between outer and inner membranes. Cycloheximide-sensitivity implicates the 3.5-kDa sterol regulatory protein isolated by Pederson *et al.* [15]. ACTH and cycloheximide-sensitive changes in outer membrane PI content may play a role in this transfer together with SCP_2.

3. Binding of cholesterol in mitochondrial sites that are available to $P\text{-}450_{scc}$. ACTH appears to increase the level of such sites possibly through protein synthesis. In rat adrenals the ACTH–aminoglutethimide provoked increase in mitochondrial FABP [17] may be involved in such binding rather than cholesterol transport [16].

4. Cholesterol removal from mitochondria. Prolonged elevated mitochondrial-cholesterol levels may be deleterous to mitochondrial function thus requiring a mechanism to effect appropriate removal. TCDD appears to provoke such a response in conjunction with ACTH–cAMP, irrespective of mitochondrial-cholesterol elevation. This may operate through decreased cholesterol binding sites or enhanced removal. One may speculate that the elevated levels of Apo E_1 in adrenal cells have a role in such removal by analogy with their capacity to confer cholesterol-acceptor characteristics on HDL [18].

Table 6. Phospholipid content of inner and outer mitochondrial membranes

	Phospholipid content (μg phosphorus/mg protein)			Upper limit of P values		
	Control	ACTH	ACTH/CX	ACTH vs CONT	CONT vs CX	ACTH vs CX
Outer membrane						
PI	0.7 ± 0.04	1.4 ± 0.1	0.4 ± 0.03	0.01	0.01	0.01
PS	0.45 ± 0.04	0.4 ± 0.01	0.45 ± 0.03			
CL	1.35 ± 0.1	1.9 ± 0.1	1.6 ± 0.1			
PC	9.4 ± 1.5	9.1 ± 1.6	9.3 ± 1.6			
PE	3.8 ± 0.5	5.9 ± 0.7	3.9 ± 0.2		0.05	0.05
Total	15.7 ± 2.2	18.6 ± 2.5	15.7 ± 2.0			
Inner membrane						
PI	0.7 ± 0.1	0.6 ± 0.1	0.2 ± 0.01		0.05	0.05
PS	0.4 ± 0.04	0.3 ± 0.1	0.35 ± 0.05			
CL	2.8 ± 0.5	2.6 ± 0.3	1.6 ± 0.04		0.05	0.05
PC	6.8 ± 1.4	5.4 ± 0.1	5.7 ± 0.3			
PE	4.85 ± 0.4	5.3 ± 0.4	3.4 ± 0.1		0.05	0.02
Total	14.5 ± 2.4	14.2 ± 1.0	11.3 ± 0.5			

Data were determined from the same experiment as described in Table 3 in which rats received injections of both dexamethazone and AMG.

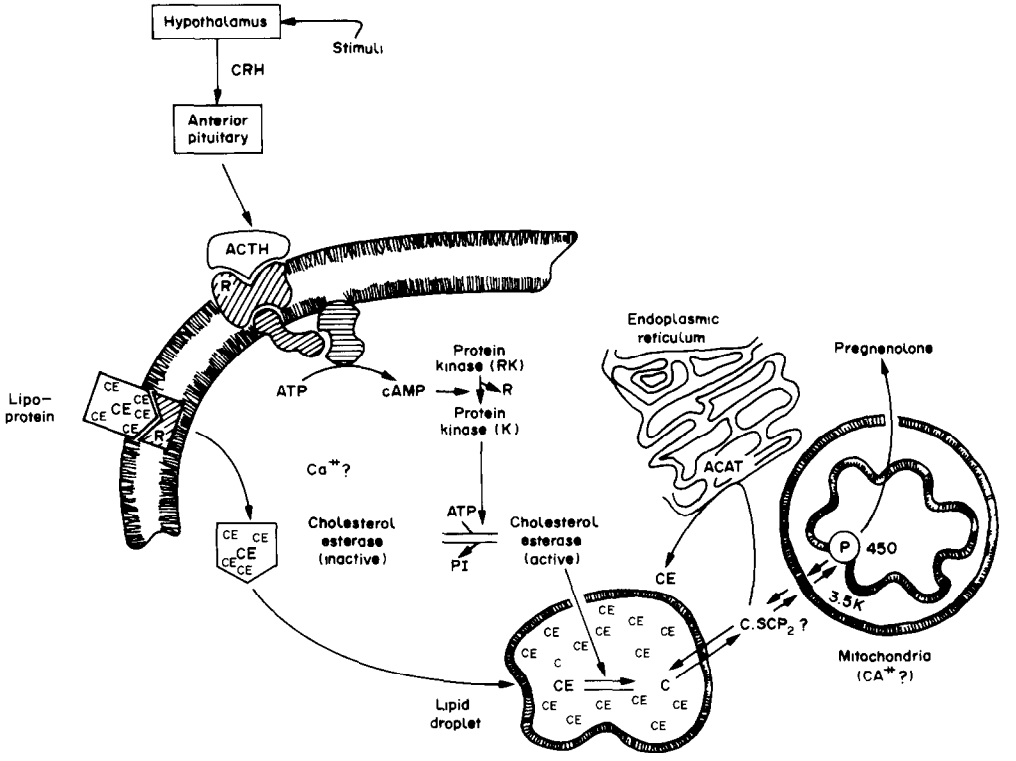

Fig. 6. Schematic representation of the activation of cholesterol metabolism in adrenal cells.

Much work remains to be done to examine the relationship between these activities. Bovine adrenal cell primary cultures seem to provide an excellent model for such studies.

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