J. Steroid Biochem. Molec. Biol. Vol. 43, No. 8, pp. 751–767, 1992
Printed in Great Britain

0960-0760/92 $5.00 + 0.00
Pergamon Press Ltd

REGULATION OF CHOLESTEROL MOVEMENT TO MITOCHONDRIAL CYTOCHROME *P*450scc IN STEROID HORMONE SYNTHESIS

COLIN R. JEFCOATE,* BRIAN C. MCNAMARA, IRINA ARTEMENKO and TAKESHI YAMAZAKI
Department of Pharmacology, University of Wisconsin Medical School, 1300 University Avenue, Madison, WI 53706, U.S.A.

Summary—Transfer of cholesterol to cytochrome *P*450scc is generally the rate-limiting step in steroid synthesis. Depending on the steroidogenic cell, cholesterol is supplied from low or high density lipoproteins (LDL or HDL) or *de novo* synthesis. ACTH and gonadotropins stimulate this cholesterol transfer prior to activation of gene transcription, both through increasing the availability of cytosolic free cholesterol and through enhanced cholesterol transfer between the outer and inner mitochondrial membranes. Cytosolic free cholesterol from LDL or HDL is primarily increased through enhanced cholesterol ester hydrolysis and suppressed esterification, but increased *de novo* synthesis can be significant. Elements of the cytoskeleton, probably in conjunction with sterol carrier protein$_2$ (SCP_2), mediate cholesterol transfer to the mitochondrial outer membranes. Several factors contribute to the transfer of cholesterol between mitochondrial membranes; steroidogenesis activator peptide acts synergistically with GTP and is supplemented by SCP_2. 5-Hydroperoxyeicosatrienoic acid, endozepine (at peripheral benzodiazepine receptors), and rapid changes in outer membrane phospholipid content may also contribute stimulatory effects at this step. It is suggested that hormonal activation, through these factors, alters membrane structure around mitochondrial intermembrane contact sites, which also function to transfer ADP, phospholipids, and proteins to the inner mitochondria. Cholesterol transfer may occur following a labile fusion of inner and outer membranes, stimulated through involvement of cardiolipin and phosphatidylethanolamine in hexagonal phase membrane domains. Ligand binding to benzodiazepine receptors and the mitochondrial uptake of 37 kDa phosphoproteins that uniquely characterize steroidogenic mitochondria could possibly facilitate these changes. ACTH activation of rat adrenals increases the susceptibility of mitochondrial outer membranes to digitonin solubilization, suggesting increased cholesterol availability. Proteins associated with contact sites were not solubilized, indicating that this part of the outer membrane is resistant to this treatment. Two pools of reactive cholesterol within adrenal mitochondria have been distinguished by different isocitrate- and succinate-supported metabolism. These pools appear to be differentially affected *in vitro* by the above stimulatory factors.

OUTLINE

Proceedings of the First International Symposium on A Molecular View of Steroid Biosynthesis and Metabolism, Jerusalem, Israel, 14–17 October 1991.

*To whom correspondence should be addressed.

1. ACUTE HORMONAL REGULATION OF STEROIDOGENESIS

The metabolism of cholesterol to pregnenolone by cytochrome *P*450scc is the rate-limiting step in the production of *total* steroids for all steroid-producing tissues, even though formation of individual downstream steroids may be selectively controlled [1–3]. For each cell type, cholesterol metabolism consists of three distinct processes, each of which involves multiple steps and each of which can potentially be rate limiting: cholesterol supply, intracellular transfer, and metabolism. Cholesterol

is an essential component of cell membranes, and consequently steroidogenesis depends on a sufficient supply of cell cholesterol that exceeds constitutive cellular requirements. This cholesterol must be transferred to cytochrome *P*450scc on the inner membranes of the mitochondria [4] prior to metabolic cleavage to pregnenolone by the cytochrome.

Hormonal stimulation of cholesterol metabolism may occur within minutes but may also change over the course of days. Acute stimulation inevitably involves activation of metabolism without increased gene expression and depends exclusively on enhanced cholesterol availability. Slower stimulation (>3 h) typically involves increased gene transcription, resulting in increased levels of proteins involved in cholesterol metabolism, including cytochrome *P*450scc and those involved in mobilization of cholesterol. Slow changes (several days) may additionally involve changes in the numbers of specific steroidogenic cells. The overall process of cholesterol side-chain cleavage is always hormonally controlled in steroidogenic cells, although the relative rates, points of activation, and detailed mechanism may be cell specific. Thus cholesterol can be supplied from serum as various cholesterol esters by transfer from either low or high density lipoproteins (LDL or HDL) [5, 6], or cholesterol may be provided by enhanced *de novo* synthesis of cholesterol. The various processes for internal transfer of cholesterol that will be described in this review may additionally depend on the source of the cholesterol.

Enzymatic cleavage of cholesterol in the mitochondria requires a high NADPH/NADP ratio, two electron transfer proteins, adrenodoxin (ADX) and NADPH–ADX-reductase, together with cytochrome *P*450scc [7, 8]. *P*450scc and ADX are present in approximately stoichiometric amounts, substantially in excess of ADX-reductase [9]. ADX functions by shuttling between the reductase and *P*450scc, forward in the reduced state and back in the oxidized state [7, 10]. The rate of cholesterol side-chain cleavage when cholesterol is optimally available is relatively high and is only likely to limit pregnenolone formation if expression of *P*450scc (or reductases) is extremely low. In ACTH-stimulated rat adrenal glands, ADX is fully reduced in the mitochondria, even though these conditions provide the highest rate of pregnenolone formation in any tissue [11]. Thus, the supply of reducing equivalents is unlikely to be a limiting factor *in vivo*, at least in adrenal mitochondria. During *in vivo* turnover in the rat adrenal, the *P*450scc is nearly fully depleted of cholesterol, consistent with rate-limiting cholesterol availability [12].

Steroidogenic cells clearly share many mechanisms of cholesterol regulation that can be controlled externally by a variety of cell-specific hormonal processes (e.g. ACTH for adrenal fasciculata cells, LH for testis Leydig cells). The intracellular processes that stimulate steroid synthesis can be activated either by hormonal activation of cAMP (ACTH/adrenal fasciculata) [13] or through elevation of cytosolic Ca^{2+} (angiotensin/adrenal glomerulosa) [14]. Both mechanisms may also operate in the same cells. Thus, cholesterol metabolism in cultured bovine adrenal fasciculata cells can be stimulated effectively by both ACTH and angiotensin [15], although significant differences in the regulation of subsequent metabolic steps typically occur. In these bovine adrenal cells, cholesterol is mobilized within minutes by ACTH and cAMP through an overall process that shows very similar characteristics to those of rat adrenals *in vivo* [16]. However, stimulation of the transcription of the various steroidogenic genes peaks only after 24–36 h [17], and recent work suggests that cAMP may be insufficient to account for the stimulatory activity of ACTH [18].

The capacity of a hormone to stimulate a particular cell type will be determined by the extent of expression of the corresponding cell surface receptors and on the coupling of these receptors to signalling enzymes through G proteins [19]. The expression of receptors and G protein, together with their mutual interactions, may change substantially when cells are cultured, depending on many factors, including the culture medium and time in culture [20, 21]. While a hormone such as ACTH may be absolutely dependent on cAMP and protein kinase A in cultured adrenal cells [22], this does not exclude additional regulatory factors (e.g. Ca-dependent steps). Irrespective of whether signalling is mediated by cytosolic cAMP or Ca^{2+}, a sequence of protein phosphorylation steps presumably follows the stimulation of adrenal steroidogenesis by ACTH [23] and mediates hormonal control of the steroidogenic pathways. One of the major challenges in understanding the activation of steroidogenesis is the identification of these key protein phosphorylation steps.

2. REGULATION OF CYTOSOLIC CHOLESTEROL

Many questions remain about whether cholesterol regulatory mechanisms in steroidogenic cells differ fundamentally from those in other cells, such as, for example, macrophage, where specialized cholesterol scavenging functions have been identified [24]. We will examine whether the exceptional transfer of cholesterol to the mitochondria of steroidogenic cells requires processes that are only found in these cells or whether hormones activate exceptionally high levels of activities found in most cells.

2.1. Lipoprotein uptake and processing

For the most part, current evidence suggests that the availability of cholesterol in steroidogenic cells is determined by processes that are equivalent to those analyzed in non-steroidogenic cells [25]. These various steps are summarized in Fig. 1. In most steroidogenic cells, cholesterol esters are provided through endocytotic uptake of LDL via a receptor that binds either apo B or apo E protein components of LDL [7, 23, 24]. After uptake, LDL is processed in lysosomes where cholesterol ester is hydrolyzed to free cholesterol by an acidic cholesterol esterase [26]. This process is inhibited by chloroquine and by other agents that raise intralysosomal pH [27]. HDL can also supply cholesterol esters to cells, and this is the favored pathway in rat adrenal cells [28]. Here uptake involves saturable binding of the lipoprotein particle with the plasma membrane to an, as yet, uncharacterized binding site [6, 28, 29]. This is followed by direct transfer of cholesterol ester to the cytosol, where lipid droplets form after sufficient accumulation. Hydrolysis of lipid droplet esters involves a distinct neutral cholesterol ester hydrolase [30] that is selectively inhibited by 10 μM diethyl phosphate [31].

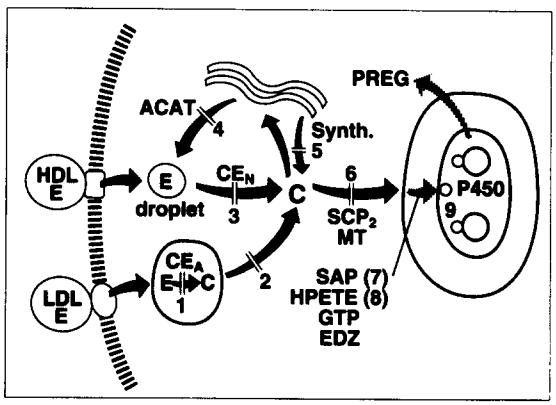

Fig. 1. Steps involved in the transfer of cholesterol to mitochondrial cytochrome *P*450scc in steroidogenic tissues. The figure shows transfer between lysosomes, endoplasmic reticulum, and mitochondria. Numbers 1–9 refer to specific inhibitors listed in Table 1. E represents cholesterol esters, and MT refers to microfilaments and microtubules. Other abbreviations are referred to in the text.

The transfer of LDL–cholesterol from lysosomes to other cell membranes is relatively slow ($t_{1/2}$ = 10–30 min) [32, 33] and can be selectively inhibited by a variety of drugs that appear to contain both a lipophilic region and an amine functional group, such as steroid amines [34] and imipramine [35]. A major part of cholesterol transfer between cell membranes involves transfer of membrane segments that are targeted, in part, by lipid constituents [36]. This plasma membrane, golgi, and lysosomes contain a far higher proportion of sphingomyelin than is found in endoplasmic reticulum and mitochondria. The high concentration of cholesterol in plasma membranes is dependent on sphingomyelin, such that enzymatic hydrolysis releases cholesterol to other membranes [37]. A human deficiency in cholesterol processing known as the Niemann-Pick Type C syndrome is located at the lysosomal cholesterol redistribution step since, like imipramine inhibition, it is associated with accumulation of free cholesterol in lysosomes [38]. Growth of cells in the presence of imipramine or these aminosteroids is highly inhibited as cells are effectively starved of cholesterol, as evidenced from a failure of this lysosomal cholesterol to regulate cholesterol synthesis or esterification. Variant cells that escape this block have recently been isolated [39] and may provide insight into the mechanism of cholesterol transfer from lysosome to other cellular locations.

The regulation of cholesterol distribution and of many cholesterol-related proteins in cells is

Table 1. Selective inhibitors of cholesterol metabolism

Process	
1. Lysosomal cholesterol esterase	Chloroquine
2. Transfer of cholesterol from lysosome to cytosol	Imipramine, 3β-(diethylamino)-Ethoxyandrostenedione
3. Neutral cholesterol esterase	Diethyl phosphate
4. ACAT	Sandoz 58035
5. Cholesterol synthesis	Lovastatin
6. Cytoskeletal transfer of cholesterol to mitochondria	Cytochalasins, vinblastine
7. Steroidogenesis activator peptide	Cycloheximide
8. 5-HPETE	Nordihydroguaretic acid
9. Cytochrome *P*450scc	Aminoglutethimide

Steps 1–9 are shown in Fig. 1.

determined by the concentration of free cholesterol in the cytosol or endoplasmic reticulum. This concentration depends on the relative rates of cholesterol supply from the net effects of ester hydrolysis, cholesterol esterification by the microsomal acyl CoA cholesterol acyl transferase (ACAT) enzyme and *de novo* synthesis [40], which are enhanced (hydrolysis, synthesis) or suppressed (esterification) by increased cholesterol availability. Many of the genes that are responsive to changes in cytosolic cholesterol have sterol-response elements that mediate increased or decreased transcription as cholesterol levels change [41]. These changes may be directly affected by 27-hydroxycholesterol that can be formed by a mitochondrial *P*450-mediated reaction [42]. A selective binding protein for this sterol has been isolated, but its role in regulation is unknown [43].

Separate measurement of acidic (lysosomal) and neutral cholesterol esterases following stimulation of rat adrenals indicates elevation of both activities [44], although the neutral esterase is much more active. This neutral cholesterol esterase is identical or closely homologous to hormone-sensitive lipase [45]. This 84 kDa protein is regulated by phosphorylation of serine groups at positions 563 and 565 in a mutually exclusive manner. cAMP-dependent protein kinase activates the enzyme by phosphorylation of Ser-563 AMP-dependent, and Ca^{2+}/calmodulin-dependent protein kinases target Ser-565 and, consequently, prevent activation. In rat adrenal fasciculata cells, the most effective stimulant of cholesterol esterase activity appears to be γ-MSH (melanocyte stimulatory hormone), rather than ACTH. However, like ACTH, γ-MSH is formed by cleavage of pro-opiomelanocortin [46, 47]. The intracellular mediator for γ-MSH is unknown, but it seems likely that the regulation by ACTH and γ-MSH involves a combination of the two phosphorylation steps on the esterase/lipase. ACTH also elevates free cholesterol by suppressing ACAT activity, as indicated by use of the specific ACAT inhibitor, Sandoz 58035 [48]. Inhibitors that selectively inhibit each of the steps in cholesterol distribution are listed in Table 1.

When cholesterol influx into adrenal cells is depleted by removal of serum lipoproteins, the availability of free cholesterol can be partially retained through stimulation of *de novo* cholesterol biosynthesis [49]. During adrenal cell culture with LDL-deficient media, ACTH elevates HMG CoA reductase [49, 50]. Transcription of the HMG CoA reductase gene is probably increased in response to a lowering of cytosolic cholesterol. Following ACTH stimulation of adrenal cells [40] or gonadotropin stimulation of luteal cells [51]. Steroid synthesis depends on HDL cholesterol esters. In the adrenals of lipoprotein-suppressed rats cholesterol synthesis is insufficient to maintain corticosterone synthesis at levels seen with normal rats. However, in the testis, where steroid synthesis is much slower, cholesterol biosynthesis probably plays the major role [52].

2.2. Transfer to mitochondria

In MA-10 Leydig tumor cells, the slow release of cholesterol from lysosomes probably limits redistribution of LDL cholesterol to the plasma membrane which, when LDL is removed, becomes the primary source for cholesterol side-chain cleavage [33, 53]. Under LDL-deficient conditions, cAMP stimulates depletion of plasma membrane cholesterol equivalent to mitochondrial metabolism. Inhibition of metabolism prevents this depletion, indicating that cholesterol is not actively transferred to the mitochondria but rather that cholesterol moves to the mitochondria to meet the deficiency produced by metabolism. However, in the presence of lipoproteins, studies in several other cell types and *in vivo* indicate that cAMP can increase mitochondrial cholesterol levels when metabolism is inhibited, suggesting an alternative active transfer to the mitochondria in these tissues [54, 55].

Several studies have shown that cholesterol transfer to the mitochondria is dependent on an intact cytoskeleton. Transfer to mitochondria in Y-1 adrenal tumor cells is blocked by cytochalasins [56] and by intracellular administration of anti-actin antibodies [57]. In rat adrenal cells, drugs that disrupt microfilaments and microtubules are equally effective in preventing cholesterol transfer to the mitochondria [58]. It has been reported that cytochalasins are ineffective in bovine adrenal cells [59] but under conditions where the source of cholesterol is intracellular. In these experiments, there was no extracellular LDL, suggesting that steroidogenesis may be dependent on cholesterol synthesized within the cells. Recent work described in this issue [60] shows that cytosolic lipid droplets bind directly to vimentin in intermediate filaments, thus providing a mechanism for involvement of the cytoskeleton. ACTH

causes a rounding up of bovine adrenal cells through dissolution of microfilaments, but this process can be prevented by inhibition of the proteolytic enzyme, urokinase, without effect on steroidogenesis [61]. This suggests that this major effect of ACTH on the cytoskeleton is not a necessary part of the cholesterol transfer process.

2.3. Apoprotein E

ACTH stimulation of adrenal glands is associated with rapid depletion of cytosolic cholesterol esters from the gland [3]. Apoprotein E (Apo E) mediates binding to LDL receptors and has been associated with reverse cholesterol transfer to plasma HDL [24, 62]. It has been suggested that association of Apo E with intracellular cholesterol/phospholipid particles may target them for exocytosis [24]. Apo E is expressed at high levels in steroidogenic cells [63, 64] and, as in macrophage, the synthesis of this protein is probably triggered by elevated free cytosolic cholesterol, thus providing an additional homeostatic mechanism for cellular cholesterol [62]. Elevation of Apo E in Y-1 adrenal cells by transfection results in suppression of cholesterol transport to mitochondria [64], but surprisingly this was accompanied by suppression of cAMP-dependent activation of the transcription of several steroidogenic genes. Although Apo E may participate in directing cholesterol to the plasma membrane, there is no evidence of Apo E-mediated transfer to the mitochondria.

3. INTRAMITOCHONDRIAL CHOLESTEROL TRANSPORT

Transfer of cholesterol within the mitochondria is a critical part of the steroidogenic process and is also under hormonal control. Several contributors to the intramitochondrial cholesterol transfer have been suggested and will be discussed in the following sections.

3.1. Protein synthesis and steroidogenesis activator peptide (SAP)

In adrenal cells, ACTH activation greatly enhances transfer of cholesterol from the outer to the inner membrane by a process that is contingent upon ongoing protein synthesis [65–67]. These effects of ACTH are retained, at least in part, by isolated mitochondria. Following ACTH stimulation *in vivo*, rat adrenal mitochondria retain a pool of reactive cholesterol and exhibit enhanced cholesterol *P*450scc–cholesterol complex formation, as indicated by optical and EPR spectroscopy [68–70]. The presence in intact cells of inhibitors of protein synthesis, such as cycloheximide, very rapidly (< 10 min) blocks this intermembrane transfer with a consequent ACTH-stimulated accumulation of cholesterol in the outer membrane that is unavailable to *P*450scc, as evidenced by low metabolism and complex formation [68]. This inhibition has no effect on the metabolism of more soluble hydroxy-cholesterol analogs, which readily transfer across the intermembrane space [70]. Similar effects of cycloheximide have been seen in mitochondria from the ovaries of luteinized rats [71]. Complex formation between cholesterol and *P*450scc in isolated rat adrenal mitochondria parallels the size of the pool of reactive cholesterol. A comparison of these parameters following different adrenal excision procedures indicates that most of this cholesterol accumulates when the gland is deprived of oxygen [12]. This change corresponds in part to the accumulation seen after inhibition of *P*450scc with aminoglutethimide, suggesting that cholesterol transfer continues in the gland after cessation of metabolism at *P*450scc through lack of oxygen [54, 55, 65]. When these mitochondria are disrupted by sonication, these effects of pretreatment are lost, presumably because of a loss of the intermembrane barrier [70].

A hormonally stimulated cycloheximide-sensitive SAP has been isolated from adrenal and testis cells [72–74]. This 30 amino acid peptide corresponds to the C-terminus of the glycosylation regulatory protein (GRP-78), a regulator of cellular protein glycosylation and turnover [75]. SAP is probably produced by an ACTH-stimulated co-translational proteolysis as GRP-78 is transferred from the ribosome into the lumen of the endoplasmic reticulum [76]. The stimulation of intramitochondrial cholesterol transfer by direct addition of SAP is, however, far smaller than would account for activation of mitochondrial steroidogenesis seen in intact cells and may, as discussed later, require other participants. Nevertheless, the level of this peptide increases within minutes in all steroidogenic tissues and shows very similar hormonal dependence to the steroidogenesis response.

3.2. Lipoxygenase metabolites

Several experiments implicate 5-lipoxygenase metabolites in the mitochondrial transfer of cholesterol [76, 77]. In rat adrenal cells, ACTH stimulates by 5- to 10-fold the synthesis of the lipoxygenase-dependent products, 5-hydroxyeicosatrienoic acid (5-HETE) and leukotriene B_4 (LTB_4). ACTH also stimulates the formation of each of the major prostanoids formed from an initial endoperoxide synthase reaction ($PGF_{2\alpha} > PGI_2 > PGE_2 > TXA_2$). Indomethacin, which substantially decreases prostanoid levels through inhibition of the synthase, however, has no effect on steroidogenesis. A specific lipoxygenase inhibitor (AA861—a derivative of 2,3,5-trimethyl-*p*-benzoquinone) inhibited formation of 5-HETE and LTB_4 while also decreasing steroidogenesis [76]. However, dibutyryl cAMP (dbcAMP) stimulated steroidogenesis in rat adrenal cells but had no effect on 5-lipoxygenase activities. Unfortunately the effect of AA861 on steroidogenesis stimulated by db cAMP was not examined. Nordihydroguaretic acid (NDGA), another lipoxygenase inhibitor, also inhibited ACTH-induced steroidogenesis in rat adrenal cells [77]. NDGA inhibited dbcAMP-stimulated steroidogenesis in these cells but was less effective at high levels of dbcAMP than at lower levels or following ACTH stimulation (50 vs 80%). 5-Hydroperoxyeicosatrienoic acid (5-HPETE), the initial product of the 5-lipoxygenase, reversed the action of AA861, suggesting that this may be the active product. In support of this conclusion, other products were inactive. Leukotriene A_4 (LTA_4), the intermediate metabolite from 5-HPETE, was inhibitory, while LTB_4 had no effect. A similar involvement of lipoxygenase products has been deduced from inhibitor effects on LH-stimulated steroidogenesis in rat Leydig cells [78, 79].

Lipoxygenase products probably affect the transfer of cholesterol to *P*450scc. As with protein synthesis inhibitors, there was no effect of these inhibitors on 25-hydroxycholesterol metabolism to pregnenolone or on steroid synthesis from pregnenolone. AA861, like protein synthesis inhibitors, increased mitochondrial cholesterol levels, indicating a block to mitochondrial cholesterol transfer [76]. This study did not, however, exclude AA861 inhibition of protein synthesis, which would be sufficient to block cholesterol transport to *P*450scc. Nevertheless, these studies suggest that SAP and 5-HPETE (or a metabolite) act together and perhaps synergistically on transfer of cholesterol between mitochondrial membranes. In other cells, 5-HPETE has been associated with calcium-dependent processes [80], and calcium sensitivity of the cholesterol metabolism in adrenal mitochondria has been reported previously [81]. Several other arachidonic acid metabolites (14,15-epoxide, 12- and 15-lipoxygenase products) have been reported in glomerulosa cells following angiotensin-II stimulation, and 12-lipoxygenase products have been implicated in aldosterone synthesis [82]. These additional products could also contribute to metabolism in adrenal fasciculata cells.

3.3. Sterol carrier protein$_2$ (SCP_2)

SCP_2, a 13 kDa protein that has been purified from liver, stimulates the activity of several microsomal enzymes involved in the synthesis and utilization of cholesterol. For example, cholesterol esterification in microsomal membranes catalyzed by ACAT is stimulated by SCP_2 [83, 84], probably through enhanced delivery of the substrate. SCP_2 enhances transfer of cholesterol from lipid droplets to mitochondria and also weakly stimulates mitochondrial steroidogenesis [85, 86]. SCP_2 is synthesized from a 60 kDa precursor protein, and both precursor and mature forms are primarily located in peroxisomes [87, 88]. Significant amounts can be detected in rat adrenal mitochondria, but this is not elevated by ACTH during acute stimulation [87]. However, treatment of rat adrenal cells for 24 h with ACTH elevated SCP_2 several fold [89]. Likewise, in rat corpus luteum, estradiol elevated SCP_2 while increasing the availability of cholesterol to *P*450scc [90]. In Leydig cells, hormonal stimulation is associated with redistribution of SCP_2 [91]. In rat adrenal cells, steroidogenesis is partially suppressed by intracellular administration of anti-SCP_2 IgG [92]. Overexpression of SCP_2 in COS cells also enhances steroidogenesis, but the site of this effect has not been defined and overall rates of steroid production remain very low [93].

SCP_2 has also been characterized as a non-specific phospholipid transfer protein that facilitates the transfer of several types of phospholipid between cell membranes [94]. At most, this protein binds cholesterol very weakly, and this interaction is certainly weaker than the

Table 2. Stimulation of rat adrenal mitochondria by rat adrenal cytosols (from Ref. [100])

Stimulant	Reductant	Increased activity (nmol pregnenolone/mg protein/10 min) Total[a]	SCP_2-derived[b]	ACTH sensitive[c]
Ex. 1				
Control cytosol	Isocitrate	0.32 (35)[d]	0.25	—
	Succinate	0.45 (96)	0.29	—
ACTH cytosol	Isocitrate	0.70 (76)	0.29	0.38
	Succinate	1.06 (225)	0.33	0.61
Ex. 2				
SCP_2 (1 μM)	Isocitrate	0.60 (38)		
	Succinate	0.50 (145)		

Rat adrenal mitochondria were isolated from rats treated for 15 min with ACTH and cycloheximide. Mitochondria (0.5 mg/ml) were incubated for 10 min with and without the designated cytosol (30/50 μl incubation) plus the indicated reductant. Cytosols were obtained from adrenals isolated (6/2 ml homogenate) from rats treated for 20 h with dexamethasone to suppress ACTH and then ACTH or saline for the final 15 min. Cytosols contributed ~0.1 μM SCP_2 to the incubations.

[a](Activity in presence of cytosol) – (activity in absence of cytosol).

[b](Activity after control IgG treatment) – (activity after anti-SCP_2 treatment).

[c]Total stimulation = (stimulation by ACTH cytosol) – (stimulation by control cytosol). ACTH-sensitive increases were halved by co-treatment with cycloheximide.

[d]Numbers in parentheses represent percent stimulation.

interaction with phospholipids. This affinity may only be sufficient to enhance cholesterol transfer from lipid droplets or aqueous suspensions but not from the stronger interactions involved in phospholipid-cholesterol vesicles. The stimulatory effect on cholesterol metabolism in adrenal mitochondria is low affinity and increases linearly up to the highest SCP_2 concentration (10 μM), where several-fold stimulation can be observed [86]. Facilitated transfer of cholesterol from outer to inner mitochondrial membranes has been reported [95], but this effect may well depend on perturbation of mitochondrial membrane structure, similar to that seen after addition of hexane and cardiolipin to adrenal mitochondria [96].

3.4. *Cytosolic factors*

Several laboratories have noted that cytosol from ACTH-stimulated adrenal cells can stimulate cholesterol metabolism in adrenal mitochondria isolated from cycloheximide-inhibited or from unstimulated rats [97–99]. After Sephadex fractionation of cytosol, the only stimulatory fraction was more strongly retained by the column than even small peptides (5 kDa). The active fraction was rich in phospholipids, and cardiolipin was identified as the major mitochondrial stimulant [98]. Since cytosol also contains both SCP_2 and SAP, we attempted to resolve their contributions [100] (Table 2). Anti-SCP_2 IgG decreased the magnitude of cytosolic stimulation and indicated that the contribution of SCP_2 was independent of ACTH stimulation. Thus, SCP_2 contributed nearly half of the activation from unstimulated cytosol. Quantitation of SCP_2 in these cytosol preparations confirmed that levels were not elevated by ACTH but also showed that the activity of SCP_2 in the cytosol fraction was 10 times higher active than purified liver SCP_2 with respect to mitochondrial activation. *In vivo* cycloheximide treatment approximately halved the increased stimulatory effect of cytosol following ACTH treatment, consistent with a cycloheximide-induced decline in SAP levels [72].

3.5. *GTP*

When isolated rat adrenal mitochondria are pre-incubated with GTP, metabolism of exogenously added cholesterol is stimulated [101]. This stimulation seems to reflect enhanced transfer of cholesterol from outer to inner mitochondrial membranes. Recent studies indicate that while SAP is essentially inactive in stimulating mitochondrial cholesterol metabolism, stimulation by GTP is doubled when added with SAP [101]. The effect of SCP_2 is independent of both GTP and SAP but is approximately additive with the combined SAP/GTP stimulation. The direct proportionality between the rate of cholesterol metabolism and SCP_2 levels is consistent with cholesterol transfer to the mitochondria through a complex with SCP_2. The effect of GTP is dependent on preincubation of the mitochondria with the nucleotide and is enhanced by use of a GTP generator, which maintains the levels in the face of rapid hydrolysis. GTPγS is inactive, suggesting that hydrolysis of the nucleotide at a mitochondrial G protein may mediate intermembrane cholesterol transfer. GTP activates the metabolism of exogenous cholesterol but not endogenous

cholesterol. In this laboratory, we find that GTP stimulation depends critically on the medium used for mitochondrial suspension.

3.6. Heterogeneity of mitochondrial cholesterol pool

Studies in this laboratory point towards additional requirements for metabolism of cholesterol within the inner mitochondria [102, 103]. Optimum cholesterol metabolism in rat adrenal mitochondria is obtained with 1 mM isocitrate, while only about 50% of this reactive mitochondrial cholesterol is available to metabolism supported by maximal levels of succinate. The reactive cholesterol pool is turned over in 2–10 min, depending on the amount of cholesterol in the inner membranes. Addition of 20α-hydroxycholesterol after cessation of succinate-supported cholesterol metabolism restarts pregnenolone formation, indicating that reductant transfer and *P*450scc remain fully functional. Succinate also sustains NADPH for 11β-hydroxylation for 30 min, although again less effectively than isocitrate. Apparently, succinate can serve as a source of reducing equivalents for only about half of the inner membrane *P*450scc and a corresponding proportion of the total reactive cholesterol pool.

Figure 2 shows a model for metabolism of cholesterol located both on the inner membrane and on matrix vesicles that are uniquely characteristic of the most active steroidogenic mitochondria [104, 105]. Succinate generates NADPH through a potential-driven NADH/NADP transhydrogenase that is linked to succinate dehydrogenase [106]. Our recent data indicate two separable deficiencies in cholesterol metabolism supported by succinate. First, NADPH generation is less effective, as evidenced by lower activities for metabolism of 20α-hydroxycholesterol at *P*450scc and deoxycorticosterone at *P*450c11. An additional factor is, however, indicated by the observation that cholesterol metabolism supported by succinate is almost completely lost after a 2 min/37° preincubation prior to addition of reductant. NADPH generation, as evidenced by succinate-dependent metabolism of 20α-hydroxycholesterol or deoxycorticosterone, is only slightly decreased. We find that this preincubation-induced transition corresponds closely to a loss of capacity for succinate-dependent ATP generation. Succinate-dependent reactions at *P*450scc and *P*450c11, together with ATP generation, are enhanced and protected against

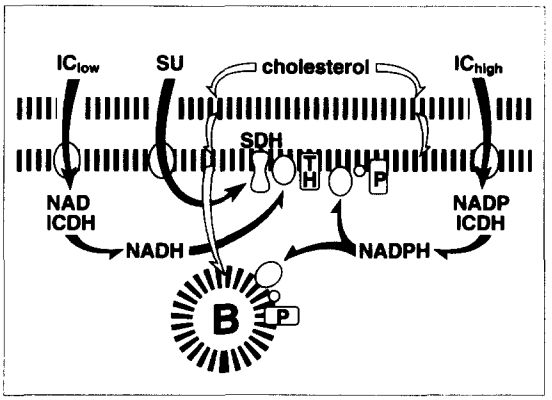

Fig. 2. A model for cholesterol transfer and metabolism in adrenal mitochondria. Cholesterol is transferred from outer to inner membrane mediated by various factors at contact sites and then moves to intramatrix vesicles (B). In this model, only metabolism in the inner membrane (Pool A) is mediated by succinate dehydrogenase (SDH) and NADH/NADP transhydrogenase (TH). NADPH generated by NADP/isocitrate dehydrogenase mediates metabolism at all *P*450scc (P). A high affinity isocitrate reaction is also mediated by the transhydrogenase.

preincubation losses by sequestering Ca^{2+} and fatty acids with, respectively, EDTA and defatted bovine serum albumin [102, 103]. This suggests that Ca^{2+} and fatty acids may cause inactivation of succinate-mediated cholesterol metabolism, possibly through effects on membrane permeability. Succinate-dependent NADPH generation requires membrane integrity because of dependence on the transhydrogenase. Cholesterol availability to *P*450scc, at least when succinate is the reductant, is evidently still more sensitive, possibly through changes in membrane fluidity with mitochondrial energy state.

Cholesterol, unlike 20α-hydroxycholesterol or deoxycorticosterone, is a membrane component and succinate-dehydrogenase, unlike NADP-dependent isocitrate dehydrogenase, is an integral membrane protein. NADPH generated from succinate via the transhydrogenase may therefore preferentially mediate metabolism of cholesterol at *P*450scc located on surrounding inner membrane. Thus, NADPH transfer from transhydrogenase to ADX-reductase may parallel the high localized enzyme to enzyme transfer of NAD/NADH reported for complexed mitochondrial enzymes [107]. Isocitrate may, by contrast, provide NADPH to all matrix locations through the soluble NADP-isocitrate dehydrogenase [108]. Interestingly, a similar decrease in the malate-mediated reactive cholesterol pool in rat adrenal mitochondria is seen when the O_2 concentration is

decreased [109]. In bovine adrenal mitochondria, the diminished activity of succinate is not seen. Here, the malate enzyme is very active in NADPH generation [110] and probably provides the preferred route for succinate-dependent cholesterol metabolism, which is typically faster than isocitrate-driven cholesterol metabolism.

3.7. Contact sites

Rapid transfer of cholesterol between the outer and inner mitochondrial membranes that is seen in activated adrenal mitochondria probably involves contact between the two membranes since cholesterol exchange between separate membranes is typically very slow ($t_{1/2}$ 1–2 h) [111]. Figure 3 shows a diagrammatic presentation of a contact site involving features that will be described in the following sections. Such contact sites provide the location for the import of mitochondrial proteins that are synthesized on cytoplasmic ribosomes [112]. This process requires an organized complex of proteins juxtaposed on the outer and inner membranes [113] involving about 10% of the mitochondrial surface and 5000 sites per mitochondria [112]. Contact sites have also been implicated in the transfer of phospholipids [114, 115] and in the transfer of mitochondrial energy to the cytoplasm [116]. Energy transfer involves a complex formed between the ADP/ATP translocator protein that spans the inner membrane and creatine kinase (CK) [116]. When this complex is formed, intramitochondrial ATP is used by CK in preference to added ATP [117], possibly because an intermembrane compartment is formed by these contact interactions. A special mitochondrial form of CK that forms both dimers and octamers [118] has been immunohistochemically located at contact sites [119]. CK binds to both outer and inner membranes with higher affinity when in the octomeric form and probably also interacts with the ATP/ADP translocator in the contact site [119, 120]. Octamer formation is selectively opposed by phosphate ions and by increased pH in the range 6–8, suggesting a mechanism for physiological coupling to oxidative phosphorylation.

The interaction of CK with mitochondrial membranes is facilitated by cardiolipin [121], which also enhances the affinity of *P*450scc for cholesterol [122], thus suggesting the possibility of enhanced cholesterol transfer to *P*450scc at contact sites. Since cardiolipin induces non-

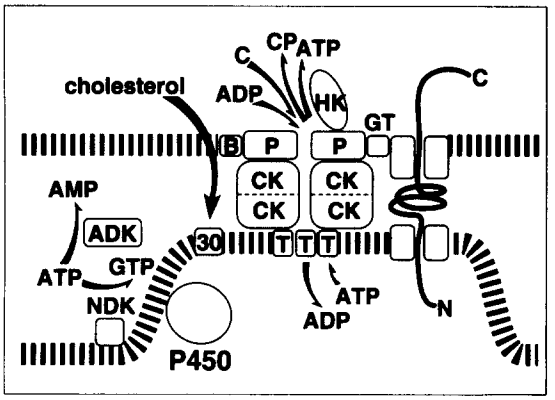

Fig. 3. A model for cholesterol transfer at contact sites. Contact sites comprise ADP/ATP translocator (T), octameric creatine kinase (CK), porin (P), hexokinase (HK), benzodiazepine receptor (B), glutathione-transferase (GT), and protein translocation complex. The inner membrane contains a 30 kDa phosphoprotein [30] which is associated with active steroidogenesis and which may facilitate cholesterol transfer. Adenylate kinase (ADK) and nucleoside diphosphate kinase (NDK) are kinases located in the intermembrane space.

bilayer membrane structures that also enhance membrane fusion [123], a model can be conjectured in which *P*450scc preferentially accepts cholesterol in a cardiolipin-rich region of the inner membrane that has been observed in contact sites [124]. A linkage between CK and the membrane environment of *P*450scc is supported by a recent observation that salt-mediated dissociation of CK from liver mitoplasts produces a change in inner membrane fluidity [116].

The contact site is also associated with porin, a 30 kDa outer membrane protein that permits entry of small molecules into the intermembrane space, by hexokinase bound to the extra-mitochondrial surface of porin by nucleoside diphosphate kinase (NDK), and by a membrane-bound glutathione, S-transferase [119, 125]. The channel formed by oligomeric porin is voltage dependent, while at contact sites the intermembrane distance is so close that inner membrane charges probably affect outer membrane permeability through porin channels [126]. At voltages more positive than 30 mV, large anions (ADP, ATP, and creatine phosphate) are partially excluded while cation transport is retained [126]. Depletion of Ca^{2+} (EGTA wash) or addition of fatty acyl CoA results in a decrease in these contact compartments, as observed directly by freeze fracture electron microscopy [127]. This observation suggests a connection between the number of contact sites and succinate-supported

cholesterol metabolism, which is similarly sensitive.

The interactions at contact sites may directly affect the characteristics of the surrounding membrane, which may in turn have a major effect on cholesterol transfer. Suspension of mitochondria in 0.25 M sucrose facilitates coupling between oxidative phosphorylation and creatine kinase that is lost after suspension in isotonic 0.125 M KCl. When rat adrenal mitochondria are preincubated in 0.125 M KCl, succinate-supported metabolism of endogenous cholesterol increases while the opposite effect is seen in sucrose media (McNamara and Jefcoate, unpublished data). Since this relative high ionic strength induces dissociation of CK from membranes, a link between contact sites and cholesterol distribution is again suggested. In hepatocytes, the number of contact sites seems to be sensitive to hormonal regulation. Thus, α receptor-stimulation (elevated Ca^{2+}) increases mitochondrial contacts, while glucagon (elevated cAMP) decreases contacts [54]. It is, however, unknown whether this is a direct effect of phosphorylation at the contact site or due to secondary metabolic changes.

3.8. Endozepine/benzodiazepines

The high concentration and activity of peripheral benzodiazepine receptors in adrenal mitochondrial outer membranes [128] may be associated with regulation of contact sites. These receptors differ from the benzodiazepine receptors of the central nervous system with respect to ligand specificity. Thus, R05-4864 is specific to peripheral sites but is without effect on the receptors in the central nervous system. Peripheral benzodiazepine receptors are 18 kDa proteins [129] that are increased in steroidogenic tissues by hormonal stimulation, presumably through enhanced transcription [130]. This receptor is present in many non-steroidogenic cell types where benzodiazepines are active (pituitary, salivary gland, nasal epithelium, lung, kidney, uterus). In many cases, secretory functions are implicated, suggesting some more general function on ion fluxes and membranes.

Benzodiazepine agonists stimulate steroidogenesis in adrenal, Leydig, and granulosa cells and are also effective when added to isolated mitochondria from adrenal or testis tissue [130–132]. This stimulation of isolated mitochondria partially overcomes the block caused by treatment *in vivo* with cycloheximide (SAP depletion) and results in enhanced cholesterol transfer from outer to inner membrane [133]. Active benzodiazepines, however, stimulate cholesterol metabolism in steroidogenic cells to a much smaller degree (3- to 4-fold) than the appropriate hormone. Co-addition of benzodiazepines with ACTH in Y-1 adrenal cells did not increase the sensitivity to the hormone, suggesting that activity at the benzodiazepine receptor is not a limiting factor in the cellular process. Hormone-stimulated steroidogenesis in these cells and in MA-10 Leydig cells, but not basal activity, was, however, inhibited 50% by the partial agonist, flunitrazepam, implying a role for the receptor in the activation of these mitochondria by cAMP [133].

An 8.5 kDa peptide has been isolated from bovine adrenal tissue that stimulates mitochondrial steroidogenesis [134, 135]. This protein is identical to [des-Gly-Ile]endozepine, a natural agonist for both central and peripheral receptors which interact with different domains of the polypeptide [136]. Endozepine is also identical to the acyl CoA binding protein which has been purified from liver [137]. Certainly, regulation of the multiple activities at contact sites would seem a necessary cell function that interrelates cytosolic and mitochondrial compartments. Recent work indicates that, in MA-10 Leydig cells and Y-1 adrenal cells, hormonal stimulation of steroid synthesis is not associated with elevated endozepine peptide [138]. However, acyl CoA, through binding to endozepine, may also regulate binding to the receptor and thus affect contact sites.

In view of the possible effects of intracellular mediators on contact sites, we have recently carried out experiments to determine whether enzymes that characterize contact sites are affected by ACTH stimulation of adrenal tissue. Although adrenal mitochondria exhibited clearly measurable activities for the enzymes that associate with contact sites (CK, NDK, hexokinase, and glutathione-transferase), these activities were completely insensitive to prolonged ACTH activation (Table 3) and to loading the inner or outer mitochondrial membranes with cholesterol. An intermembrane enzyme, adenylate kinase, was also not significantly affected by these changes. Hormonal sensitivity was however seen with digitonin treatment, which selectively fragments the outer membrane through complexes with cholesterol.

This treatment produced a differential release of these enzymes from mitochondria. Adenylate kinase and rotenone-insensitive NADH–cytochrome *c* reductase (outer membrane marker) were predominantly released, while a major proportion of the octomeric CK, hexokinase, NDK, and glutathione-transferase were unaffected by digitonin, suggesting location of these enzymes at a resistant contact site [119]. Interestingly, suppression of ACTH *in vivo* with hypophysectomy (Table 3) or with dexamethasone substantially decreased the sensitivity of rat adrenal mitochondria to digitonin independently of cholesterol content. We observed decreased release of intermembrane adenylate kinase and higher residual levels of CK and NDK. This suggests a considerable change in the outer mitochondrial membrane. We currently hypothesize that ACTH stimulates the availability of outer membrane cholesterol to digitonin complexation, possibly through changes in outer membrane lipids which we describe in a later section. There may also be a cAMP-mediated *decrease* in contact sites, consistent with observations in the liver [139].

These observations are counter to our expectation that cholesterol transfer would be favored by a stabilization of contact sites and instead suggest that transfer occurs at less stable, more mobile contacts. The resistance to digitonin following hypophysectomy is exceptional compared to what we see in liver and kidney mitochondria, suggesting unusually stable contacts and membranes in these mitochondria that are modified to a more normal status by hormonal stimulation. We have recently shown that a far shorter 30 min ACTH stimulation substantially elevates metabolism of endogenous mitochondrial cholesterol without increasing digitonin susceptibility. The membrane state that is observed through this decreased digitonin sensitivity clearly is not the cause of low intramitochondrial cholesterol transfer. However small membrane changes produced by brief exposure to ACTH may be sufficient to elevate mitochondrial cholesterol metabolism without being detected by digitonin.

3.9. Mitochondrial p30 proteins

One further participant in mitochondrial steroidogenesis has been identified from two-dimensional electrophoresis of steroidogenic cells. This technique shows that hormonal activation changes the levels of a set of four 30 kDa mitochondrial proteins [140–143]. A p37 precursor is processed in the mitochondria to a p32 intermediate and then to a pair of p30 mature inner membrane proteins. Both precursors and mature proteins can be phosphorylated, and indeed in the adrenal the precursors have only been detected as phosphoproteins. The near identity of proteolysis products establishes that the four p30 proteins arise from two distinct modifications of a single precursor protein. Coadministration of cycloheximide with cAMP prevented formation of the phosphoproteins,

Table 3. Digitonin solubilization of outer membrane and intermembrane enzymes in rat adrenal mitochondria

	Enzyme activity[a]			
	AK	CK	NDK	HK
HYPOX (24 h)				
Intact	130	8	3	41
0.25[b]	130 (96 ± 3)[c]	12.5 (156)[d]	4	28 (64 ± 7)[c]
0.45	40 (31)	7 (88)	2	23 (56)
HYPOX + ACTH (24 h)				
Intact	192	8	3	43
0.25	152 (69 ± 6)[c]	8 (100)	3.5	23 (48 ± 5)[c]
0.45	29 (15)	4 (50)	3.5	—
Untreated				
Intact	176	12	11	38
0.25	82 (46)	11 (92)	4	19 (50)
0.45	26 (15)	11 (92)	5	15 (39)

Female rats were hypophysectomized 24 h prior to sacrifice. Half of the animals were injected after hypophysectomy and after a further 12 h with long-acting ACTH. Digitonin treatment of isolated mitochondria was conducted at 10 mg protein/ml, as described previously [119].

[a]Activities are expressed in mU/mg protein using coupled assays in which NADPH is followed spectrophotometrically [119].

[b]Ratio of digitonin/protein.

[c]Mean + SD for 4 (AK) or 3 (HK) separate experiments.

[d]( )Percent of activity in intact mitochondria—activities expressed per mg protein were more reproducible than corresponding numbers for retention of total mitochondrial activity. Approximately 20–30% of outer membrane and intermembrane proteins were released by digitonin.

suggesting phosphorylation concomitant with translation [141]. Since, however, the 37 kDa precursor is apparently rapidly taken into the mitochondria, a cAMP-dependent phosphorylation of p37 *after* release from the ribosome could produce similar kinetics through competition with uptake. The association of ribosomes with contact sites [144] suggests a concerted process of synthesis, phosphorylation, and uptake. The appearance of p30 exhibits the same time course and hormonal concentration dependence as steroidogenesis, while cycloheximide sensitivity in part parallels the effect on cholesterol metabolism. However, the rapid decay of activity following this inhibition of steroidogenesis more closely follows the decay of intermediates (p37, p32) rather than any of the p30 variants. These proteins appear to localize in the inner mitochondrial membrane [141, 142], and, therefore, the precursor protein should span the contact site prior to processing and inner membrane insertion. This could provide a mechanism for additional manipulation of the contact site that might facilitate cholesterol transfer.

4. CHANGES IN ADRENAL LIPIDS

ACTH stimulates a very rapid net synthesis of phospholipids, apparently secondary to *de novo* synthesis of phosphatidic acid [145, 146]. This increase is distinct from the rapid turnover of PI stimulated by angiotensin in adrenal glomerulosa cells that releases intracellular Ca^{2+} and is associated with translocation of protein kinase C activity to cell membranes. This increase in lipid synthesis results in an elevation of PI and PE in the mitochondrial outer membrane (Table 4) [147]. Other studies indicate an ACTH-induced enrichment of the PE fraction with polyunsaturated (22:6) adrenic acid [148]. Adrenic acid-substituted PE, like cardiolipin, directly stimulates mitochondrial steroidogenesis [148]. This, taken together with the enrichment of cardiolipin in contact sites [124], suggests that contact sites may mediate cholesterol transfer through hexagonal phase structures in which the normal bilayer inverts. This membrane inversion is favored by both polyunsaturated PE and cardiolipin [123] and has been observed in mitochondria at suspected contact sites [115]. Possibly, benzodiazepine receptors, when bound by appropriate ligands, could favor such hexagonal phase structures.

Table 4. ACTH stimulation of specific phospholipids in the outer membrane of rat adrenal mitochondria (from Ref. [147])

	Phospholipid content (μg phosphorus/mg protein)	
	Control	ACTH
Outer		
PI	0.7 ± 0.04	1.4 ± 0.1 ($P < 0.01$)
PS	0.45 ± 0.04	0.4 ± 0.05
CL	1.35 ± 0.1	1.9 ± 0.1
PC	9.4 ± 1.5	9.1 ± 1.6
PE	3.8 ± 0.5	5.9 ± 0.7 ($P < 0.05$)
Total	15.7 ± 2.2	18.6 ± 2.9
Inner		
PI	0.7 ± 0.1	0.6 ± 0.1
PS	0.4 ± 0.04	0.3 ± 0.1
CL	2.8 ± 0.5	2.6 ± 0.03
PC	6.8 ± 1.4	5.4 ± 0.1
PE	4.9 ± 0.4	5.3 ± 0.4
Total	14.5 ± 2.4	14.2 ± 1.0

Adrenal mitochondria were isolated from rats pretreated for 20 min with ACTH or left untreated. Inner and outer membranes were separated by hyposmotic shock and ultracentrifugation through Ficoll, as described previously [65].

5. OTHER EXTRACELLULAR MODULATORS OF CHOLESTEROL DISTRIBUTION

In addition to direct hormonal stimulation of cholesterol mobilization, recent work indicates the involvement of other modulators of these processes. The participation of γ-MSH has already been described as a stimulant at least of cholesterol esterase [45]. The two best characterized modulators are $TGF_{\beta 1}$, which partially inhibits steroidogenesis in most steroidogenic cells [149, 150], and atrial natrietic peptide (ANP), which decreases steroid synthesis in adrenal glomerulosa cells [151]. $TGF_{\beta 1}$ down-regulates several steroidogenic enzymes at the transcriptional level, most notably *P*45017α [150], but also decreases cholesterol mobilization [149]. In bovine adrenal cells, this effect in part involves decreased cholesterol uptake through the LDL pathway [149]. Interleukins 1α and 1β similarly suppress steroidogenesis, most obviously by modulating expression of *P*450 cytochromes, but changes in cholesterol transfer mechanisms have typically not been excluded [152, 153]. ANP decreases aldosterone synthesis, at least in part through decreased cholesterol availability to *P*450scc, probably mediated by cGMP and antagonism of the Ca^{2+}-mediated phosphorylation step [151]. The complexity of the cholesterol transfer processes thus provides many opportunities for stimulation or inhibition by a variety of autocrine and paracrine processes.

REFERENCES

1. Stone D. and Hechter O.: Studies on ACTH action in perfused bovine adrenals: Site of action of ACTH in corticosteroidogenesis. *Archs Biochem. Biophys.* **51** (1954) 457–469.
2. Karaboyas G. C. and Koritz S. B.: Identity of the site of action of cAMP and ACTH in corticosteroidogenesis in rat adrenal and beef adrenal cortex slices. *Biochemistry* **4** (1954) 462–468.
3. Garren L. D., Gill G. N., Masui H. and Walton G. M.: On the mechanism of action of ACTH. *Recent Prog. Horm. Res.* **27** (1971) 433–478.
4. Churchill P. F. and Kimura T.: Topological studies of cytochromes $P450_{scc}$ and $P450_{11\beta}$ in bovine adrenocortical inner mitochondrial membranes. *J. Biol. Chem.* **254** (1979) 10443–10448.
5. Kovanen P. T., Goldstein J. L., Chappell D. A. and Brown M. S.: Regulation of low density lipoprotein receptors by adrenocorticotropin in the adrenal gland of mice and rats *in vivo*. *J. Biol. Chem.* **255** (1980) 5591–5598.
6. Gwynne J. T. and Mahaffee D. D.: Rat adrenal uptake and metabolism of high density lipoprotein cholesteryl ester. *J. Biol. Chem.* **264** (1989) 8141–8150.
7. Lambeth J. D., Seybert D. W. and Kamin H.: Ionic effects on adrenal steroidogenic electron transport. The role of adrenodoxin as an electron shuttle. *J. Biol. Chem.* **254** (1979) 7255–7264.
8. Tuls J., Geren L., Lambeth J. D. and Millett F.: The use of a specific fluorescence probe to study the interaction of adrenodoxin with adrenodoxin reductase and cytochrome $P450_{scc}$. *J. Biol. Chem.* **262** (1987) 10020–10025.
9. Hanukoglu I. and Hanukoglu Z.: Stoichiometry of mitochondrials, cytochromes *P*450, adrenodoxin and adrenodoxin reductase in adrenal cortex and corpus luteum. *Eur. J. Biochem.* **157** (1986) 27–31.
10. Hanukoglu I. and Jefcoate C. R.: Adrenal mitochondrial cytochrome $P450_{scc}$: Mechanism of electron transport by adrenodoxin. *J. Biol. Chem.* **255** (1980) 3057–3061.
11. Williams-Smith D. L. and Cammack R.: Oxidation-reduction potentials of cytochromes *P*450 and ferredoxin in the bovine adrenal. Their modification by substrates and inhibitors. *Biochim. Biophys. Acta* **499** (1977) 432–442.
12. Jefcoate C. R. and Orme-Johnson W. H.: Cytochrome *P*450 of adrenal mitochondria: *in vitro* and *in vivo* changes in spin state. *J. Biol. Chem.* **250** (1975) 4671–4677.
13. Richardson M. C. and Schulster D.: The role of protein kinase activation in the control of steroidogenesis in the adrenal cortex. *Biochem. J.* **136** (1973) 993–998.
14. Kojima I., Kojima K. and Rasmussen H.: Role of calcium and cAMP in the action of ACTH on aldosterone secretion. *J. Biol. Chem.* **260** (1985) 4248–4256.
15. Walker S. W., Lightly E. R. T., Clyne C., Williams B. C. and Bird I. M.: Adrenergic and cholinergic regulation of cortisol secretion from the zona fasciculata/reticularis of bovine adrenal cortex. *Endocrine Res.* **17** (1991) 237–265.
16. DiBartolomeis M. J. and Jefcoate C. R.: Characterization of the acute stimulation of steroidogenesis in primary bovine adrenal cortical cell cultures. *J. Biol. Chem.* **259** (1984) 10159–10167.
17. Kramer R. E., Ramey W. E., Funkenstein B., Dee A., Simpson E. R. and Waterman M. R.: Induction of synthesis of mitochondrial steroidogenic enzymes of bovine adrenal cortical cells by analogs of cAMP. *J. Biol. Chem.* **259** (1984) 707–715.
18. Hanukoglu I., Feuchtwanger R. and Hanukoglu A.: Mechanism of corticotropin and cAMP induction of mitochondrial cytochrome *P*450 system enzymes in adrenal cortex. *J. Biol. Chem.* **265** (1990) 20602–20608.
19. Langlois D., Saez J. M. and Begeot P.: The potenting effects of phorbol ester on ACTH cholera toxin- and forskolin-induced cAMP production in cultured bovine adrenal cells is not mediated by inactivation of α-subunit of G_i protein. *Biochem. Biophys. Res. Commun.* **146** (1987) 517–523.
20. Hornsby P. J. and Gill G. N.: Regulation of responsiveness of cultured adrenal cells to adrenocorticotropin and prostaglandin E_1: Cell density, cell division, and inhibitors of protein synthesis. *Endocrinology* **108** (1981) 183–188.
21. Hornsby P. J.: Regulation of steroid hydroxylases in normal and SV40 T antigen-transfected bovine adrenocortical cells in long-term culture. *Endocrine Res.* **17** (1991) 109–134.
22. Wong M. and Schimmer B. P.: Recovery of responsiveness to ACTH and cAMP in a protein kinase-defective adrenal cell mutant following transfection with a protein kinase A gene. *Endocrine Res.* **15** (1989) 49–65.
23. Koroscil T. M. and Gallant S.: The phosphorylation of adrenal proteins in response to adrenocorticotropic hormone. *J. Biol. Chem.* **256** (1981) 6700–6707.
24. Johnson W. J., Mahlberg F. H., Rothblat G. H. and Phillips M. C.: Cholesterol transport between cells and high-density lipoproteins. *Biochim. Biophys. Acta* **1085** (1991) 273–298.
25. Brown M. S. and Goldstein J. L.: Receptor-mediated endocytosis: Insights from the lipoprotein receptor system. *Proc. Natn. Acad. Sci. U.S.A.* **76** (1979) 3330–3337.
26. Fowler S. D. and Brown W. J.: Lysosomal acid lipase in lipases. In *Lipases* (Edited by B. Bergstrom and H. L. Brockman). Elsevier Science, New York (1984) pp. 329–364.
27. Poole B. and Ohkuma S.: Effect of weak bases on the intralysosomal pH in mouse peritoneal macrophages. *J. Cell. Biol.* **90** (1981) 665–669.
28. Gwynne J. T. and Strauss J. F. III: The role of lipoproteins in steroidogenesis and cholesterol metabolism in steroidogenic glands. *Endocrine Rev.* **3** (1982) 299–329.
29. Pittman R. C., Knecht T. P., Rosenbaum M. S. and Taylor C. A. Jr: A nonendocytotic mechanism for the selective uptake of high density lipoprotein-associated cholesterol esters. *J. Biol. Chem.* **262** (1987) 2443–2450.
30. Brown M. S., Goldstein J. L., Krieger M., Ho Y. K. and Anderson R. G. W.: Reversible accumulation of cholesterol esters in macrophages incubated with acetylated lipoproteins. *J. Cell. Biol.* **82** (1979) 597–613.
31. Harrison E. H., Bernard D. W., Scholm P., Quinn D. M., Rothblat G. H. and Glick J. M.: Inhibitors of neutral cholesteryl ester hydrolase. *J. Lipid Res.* **31** (1990) 2187–2193.
32. Johnson W. J., Chacko G. K., Phillips M. C. and Rothblat G. H.: Efflux of lysosomal cholesterol from cells. *J. Biol. Chem.* **265** (1990) 5546–5553.
33. Freeman D. A.: cAMP mediated modification of cholesterol traffic in Leydig tumor cells. *J. Biol. Chem.* **262** (1987) 13061–13068.
34. Liscum L. and Faust J. R.: The intracellular transport of low density lipoprotein-derived cholesterol is inhibited in Chinese hamster ovary cells cultured with 3-β-[2-(diethylamino)ethoxy]androst-5-en-17-one. *J. Biol. Chem.* **264** (1989) 11796–11806.
35. Rodriguez-Lafrasse C., Rousson R., Bonnet J., Pentchev P. G., Louisot P. and Vanier M. T.: Abnormal cholesterol metabolism in imipramine-

treated fibroblast cultures. Similarities with Niemann-Pick type C disease. *Biochim. Biophys. Acta* **1043** (1990) 123–128.
36. van Meer G.: Lipid traffic in animal cells. *A. Rev. Cell Biol.* **5** (1989) 247–275.
37. Slotte J. P. and Bierman E. L.: Depletion of plasma membrane sphingomyelin rapidly alters the distribution of cholesterol between plasma membranes and intracellular cholesterol pools in cultured fibroblasts. *Biochem. J.* **250** (1988) 653–658.
38. Blanchette Mackie E. J., Dwyer N. K., Vanier M. T., Sokol J., Merrick H. F., Comly M. E., Argoff C. E. and Pentchev P. G.: Type C Niemann-Pick disease: dimethyl sulfoxide moderates abnormal LDL-cholesterol processing in mutant fibroblasts. *Biochim. Biophys. Acta* **1006** (1989) 219–226.
39. Liscum L. and Collins G. J.: Characterization of Chinese hamster ovary cells that are resistant to 3-β-[2-(diethylamino)ethoxy]androst-5-en-17-one inhibition of low density lipoprotein-derived cholesterol metabolism. *J. Biol. Chem.* **266** (1991) 16599–16606.
40. Faust J. R., Goldstein J. L. and Brown M. S.: Receptor-mediated uptake of LDL and utilization of its cholesterol for steroid synthesis in cultured mouse adrenal cells. *J. Biol. Chem.* **252** (1977) 4861–4871.
41. Goldstein J. L. and Brown M. S.: Regulation of the mevalonate pathway. *Nature* **343** (1990) 425–430.
42. Taylor F. R., Kandutsch A. A., Anzalone L., Phirwa S. and Spencer T. A.: Photoaffinity labeling of the oxysterol receptor. *J. Biol. Chem.* **263** (1988) 2264–2269.
43. Cali J. J. and Russell D. W.: Characterization of human sterol 27-hydroxylase. *J. Biol. Chem.* **266** (1991) 7774–7778.
44. Nishikawa T., Mikami K., Saito Y., Tamura Y. and Kumagai A.: Studies on cholesterol esterase in the rat adrenal. *Endocrinology* **108** (1981) 932–936.
45. Yeaman S. J.: Hormone-sensitive lipase—a multipurpose enzyme in lipid metabolism. *Biochim. Biophys. Acta* **1052** (1990) 128–152.
46. Pedersen R. C. and Brownie A. C.: Immunoreactive γ-melanotropin in rat pituitary and plasma. A partial characterization. *Endocrinology* **110** (1982) 825–834.
47. Pedersen R. C., Brownie A. C. and Ling N.: Pro-adrenocorticotropin/endorphin-derived peptides: Coordinate action on adrenal steroidogenesis. *Science* **208** (1980) 1044–1046.
48. Jamal Z., Suffolk R. A., Boyd G. S. and Suckling K. E.: Metabolism of cholesteryl ester in monolayers of bovine adrenal cortical cells. Effect of an inhibitor of acyl-CoA: cholesterol acyltransferase. *Biochim. Biophys. Acta* **834** (1985) 230–237.
49. Rainey W. E., Shay J. W. and Mason J. I.: ACTH induction of 3-hydroxy-3-methylglutaryl coenzyme A reductase, cholesterol biosynthesis, and steroidogenesis in primary cultures of bovine adrenocortical cells. *J. Biol. Chem.* **261** (1986) 7322–7326.
50. Lehoux J.-G., Lefebvre A., Belisle S. and Bellabarba D.: Effect of ACTH suppression on adrenal 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA in 4-aminopyrazolopyrimidine-treated rats. *Molec. Cell. Endocr.* **69** (1990) 41–49.
51. Azhar S., Menon M. and Menon K. M. J.: Receptor-mediated gonadotropin action in the ovary. Demonstration of acute dependence of rat luteal cells on exogenously supplied sterols. *Biochim. Biophys. Acta* **665** (1981) 362–375.
52. Quinn P. G., Dombransky L. J., Chen Y. J. and Payne A. H.: Serum lipoproteins increase testosterone production in HCG-sensitized Leydig cells. *Endocrinology* **109** (1981) 1790–1792.
53. Freeman D. A.: Plasma membrane cholesterol: removal and insertion into the membrane and utilization as a substrate for steroidogenesis. *Endocrinology* **124** (1989) 2527–2535.
54. Mahaffee D., Reitz R. C. and Ney R. L.: Mechanism of action of ACTH-role of mitochondrial cholesterol accumulation in regulation of steroidogenesis. *J. Biol. Chem.* **249** (1974) 227–233.
55. Simpson E. R., McCarthy J. L. and Peterson J. A.: Evidence that the cycloheximide-sensitive site of ACTH action is in the mitochondrion. *J. Biol. Chem.* **253** (1979) 3135–3139.
56. Mrotek J. and Hall P. F.: Response of adrenal tumor cells to ACTH: site of inhibition by cytochalasin B. *Biochemistry* **16** (1977) 3177–3181.
57. Hall P. F., Charponnier C., Nakamura M. and Gabbiani G.: The role of microfilaments in the response of adrenal tumor cells to ACTH. *J. Biol. Chem.* **254** (1979) 9080–9084.
58. Crivello J. F. and Jefcoate C. R.: Mechanism of corticotropin action in rat adrenal cells. 1. Effect of inhibitors of protein synthesis and of microfilament formation on corticosterone synthesis. *Biochim. Biophys. Acta* **542** (1978) 315–329.
59. Ramey W. E., Shay J. W. and Mason J. J.: The effect of cytochalasin D on steroid production and stress fiber organization in cultured bovine adrenal cortical cells. *Molec. Cell. Endocr.* **35** (1984) 189–197.
60. Hall P. F. and Almahbobi G.: The role of the cytoskeleton in the regulation of steroidogenesis. *J. Steroid Biochem. Molec. Biol.* **43** (1992) 769–777.
61. Hornsby P. J., Sepehr S. M., Cheng V. and Cheng C. Y.: cAMP-mediated cytoskeletal effects in adrenal cells are modified by serum, insulin, insulin-like growth factor-J and an antibody against urokinase plasminogen activator. *Molec. Cell. Endocr.* **67** (1989) 185–193.
62. Dory L.: Synthesis and secretion of apoE in thioglycolate-elicited mouse peritoneal macrophages: effect of cholesterol efflux. *J. Lipid Res.* **30** (1989) 809–816.
63. Dyer C. A. and Curtiss L. K.: Apoprotein E-rich high density lipoproteins inhibit ovarian androgen synthesis. *J. Biol. Chem.* **263** (1988) 10965–10973.
64. Reyland M. E. and Williams D. L.: Suppression of cAMP-mediated signal transduction in mouse adrenocortical cells which express apolipoprotein E. *J. Biol. Chem.* **266** (1991) 21099–21200.
65. Privalle C. T., Crivello J. F. and Jefcoate C. R.: Regulation of intramitochondrial cholesterol transfer to side-chain cleavage cytochrome $P450_{scc}$ in rat adrenal gland. *Proc. Natn. Acad. Sci. U.S.A.* **80** (1983) 702–706.
66. Cheng B., Hsu D. K. and Kimura T.: Utilization of mitochondrial membrane cholesterol by cytochrome *P*450-dependent cholesterol side-chain cleavage reaction in bovine adrenocortical mitochondria: steroidogenic and non-steroidogenic pools of cholesterol in the mitochondrial inner membranes. *Molec. Cell. Endocr.* **40** (1985) 233–243.
67. Ohno Y., Yanagibashi K., Yonezawa Y., Ishiwatari S. and Matsuba M.: A possible role of "steroidogenic factor" in the corticoidogenic response to ACTH; Effect of ACTH, cycloheximide and aminoglutethimide on the content of cholesterol in the outer and inner mitochondrial membrane of rat adrenal cortex. *Endocr. Jap.* **30** (1983) 335–338.
68. Simpson E. R., Brownie A. C., Jefcoate C. R. and Boyd G. S.: Effect of ether stress on cholesterol side chain cleavage and cytochrome *P*450 in rat adrenal mitochondria. *Eur. J. Biochem.* **28** (1972) 443–450.
69. Brownie A. C., Alfano J., Jefcoate C. R., Beinert H. and Orme-Johnson W. H.: Effect of ACTH on adrenal mitochondrial cytochrome *P*450 in rat. *Ann. N.Y. Acad. Sci.* **212** (1973) 344–360.

70. Jefcoate C. R., Simpson E. R. and Boyd G. S.: Spectral properties of rat adrenal-mitochondrial cytochrome P450. *Eur. J. Biochem.* **42** (1974) 539–551.
71. Arthur J. R. and Boyd G. S.: The effect of inhibitors of protein synthesis on cholesterol side-chain cleavage in the mitochondria of luteinized rat ovaries. *Eur. J. Biochem.* **49** (1976) 117–127.
72. Frustaci J., Mertz L. M. and Pedersen R. C.: Steroidogenesis activator polypeptide (SAP) in the guinea pig adrenal cortex. *Molec. Cell. Endocr.* **64** (1989) 137–143.
73. Pedersen R. C. and Brownie A. C.: Cholesterol side-chain cleavage in the rat adrenal cortex: Isolation of a cycloheximide-sensitive activator peptide. *Proc. Natn. Acad. Sci. U.S.A.* **80** (1983) 1882–1886.
74. Pedersen R. C. and Brownie A. C.: Steroidogenesis-activator polypeptide isolated from a rat Leydig cell tumor. *Science* **236** (1987) 188–190.
75. Mertz L. M. and Pedersen R. C.: The kinetics of steroidogenesis activator polypeptide in the rat adrenal cortex. *J. Biol. Chem.* **264** (1989) 15274–15279.
76. Mikami K., Omura M., Tamura Y. and Yoshida S.: Possible site of action of 5-hydroperoxyeicosatetraenoic acid derived from arachidonic acid in ACTH-stimulated steroidogenesis in rat adrenal glands. *J. Endocr.* **125** (1990) 89–96.
77. Jones D. B., Marante D., Williams B. C. and Edwards C. R. W.: Adrenal synthesis of corticosterone in response to ACTH in rats is influenced by leukotriene A_4 and by lipoxygenase intermediates. *J. Endocr.* **112** (1987) 253–258.
78. Dix C. J., Habberfield A. D., Sullivan M. H. F. and Cooke B. A.: Inhibition of steroid production in Leydig cells by non-steroidal anti-inflammatory and related compounds: evidence for the involvement of lipoxygenase products in steroidogenesis. *Biochem. J.* **219** (1984) 529–537.
79. Dix C. J., Habberfield A. D., Sullivan M. H. and Cooke B. A.: Evidence for the involvement of lipoxygenase products in steroidogenesis. *Biochem. Soc. Trans. B* (1985) 60–63.
80. Przylipiak A., Kiesel L., Habenicht A. J. R., Przylipiak M. and Runnebaum B.: Exogenous action of 5-lipoxygenase by its metabolites on luteinizing hormone release in rat pituitary cells. *Molec. Cell. Endocr.* **69** (1990) 33–39.
81. Arthur J. R., Mason J. I. and Boyd G. S.: Effect of calcium ions on metabolism of exogenous cholesterol by rat adrenal mitochondria. *FEBS Lett.* **66** (1976) 206–209.
82. Campbell W. B., Brady M. T., Rosolowsky L. J. and Falck J. R.: Metabolism of arachidonic acid by rat adrenal glomerulosa cells: Synthesis of hydroxyeicosatetraenoic acids and epoxyeicosatrienoic acids. *Endocrinology* **128** (1991) 2183–2194.
83. Poorthuis N. M. and Wirtz K. W. A.: Increased cholesterol esterification in rat liver microsomes by purified non-specific phospholipid transfer protein. *Biochim. Biophys. Acta* **710** (1982) 99–105.
84. Gavey K. L., Noland B. J. and Scallen T. J.: The participation of sterol carrier $protein_2$ in the conversion of cholesterol to cholesterol ester rat liver microsomes. *J. Biol. Chem.* **256** (1981) 2993–2999.
85. Chanderbhan R., Noland B. J., Scallen T. J. and Vahouny G. V.: Sterol carrier $protein_2$: Delivery of cholesterol from adrenal lipid droplets to mitochondria for pregnenolone synthesis. *J. Biol. Chem.* **257** (1982) 8928–8934.
86. Vahouny G. V., Chanderbhan R., Noland B. J., Irwin D., Dennis P., Lambeth J. D. and Scallen T. J.: Sterol carrier $protein_2$: Identification of adrenal sterol carrier $protein_2$ and site of action for mitochondrial cholesterol utilization. *J. Biol. Chem.* **258** (1983) 11731–11737.
87. van Amerongen A., van Noort M., van Beckhoven J. R. C. M., Rommerts F. F. G., Orly J. and Wirtz K. W. A.: The subcellular distribution of the non-specific lipid transfer protein (sterol carrier protein 2) in rat liver and adrenal gland. *Biochim. Biophys. Acta* **1001** (1989) 243–248.
88. Fujiki Y., Tsuneoka M. and Tashiro Y.: Biosynthesis of nonspecific lipid transfer protein (sterol carrier protein 2) on free polyribosomes as a larger precursor in rat liver. *J. Biochem.* **106** (1989) 1126–1131.
89. Trzeciak W. H., Simpson E. R., Scallen T. J., Vahouny G. V. and Waterman M. R.: Studies on the synthesis of SCP-2 in rat adrenal cortical cells in monolayer culture. *J. Biol. Chem.* **262** (1987) 3713–3720.
90. McLean M. P., Puryear T. K., Khan I., Azhar S., Billheimer J. T., Orly J. and Gibori G.: Estradiol regulation of sterol carrier protein-2 independent of cytochrome P450 side-chain cleavage expression in the rat corpus luteum. *Endocrinology* **125** (1989) 1337–1344.
91. Van Noort M., Rommerts F. F. G., Van Amerongen A. and Wirtz K. W. A.: Regulation of SCP_2 in the soluble fraction of rat Leydig cells. Kinetics and possible role of calcium influx. *Molec. Cell. Endocr.* **56** (1988) 133–138.
92. Chanderbhan R. F., Kharroubi A. T., Noland B. J., Scallen T. J. and Vahouny G. V.: SCP_2: Further evidence for its role in adrenal steroidogenesis. *Endocrine Res.* **12** (1986) 351–360.
93. Yamamoto R., Kallen C. B., Babalola G. O., Rennert H., Billheimer J. T. and Strauss J. F. III: Cloning and expression of a cDNA encoding human sterol carrier protein 2. *Proc. Natn. Acad. Sci. U.S.A.* **88** (1991) 463–467.
94. Westerman J. and Wirtz K. W. A.: The primary structure of the nonspecific lipid transfer protein (sterol carrier protein 2) from bovine liver. *Biochem. Biophys. Res. Commun.* **127** (1985) 333–338.
95. Vahouny G. V., Dennis P., Chanderbhan R., Fiskum G., Noland B. J. and Scallen T. J.: Sterol carrier $protein_2$ (SCP_2)-mediated transfer of cholesterol to mitochondrial inner membranes. *Biochem. Biophys. Res. Commun.* **122** (1984) 509–515.
96. McNamara B. C. and Jefcoate C. R.: Synergistic stimulation of pregnenolone synthesis in rat adrenal mitochondria by *n*-hexane and cardiolipin. *Archs Biochem. Biophys.* **260** (1988) 780–788.
97. Ray P. and Strott C. A.: Cytosol stimulation of pregnenolone synthesis by isolated adrenal mitochondria. *Life Sci.* **28** (1981) 1529–1534.
98. Farese R. V. and Sabir A. M.: Polyphosphoinositides: stimulator of mitochondrial cholesterol side chain cleavage and possible identification as an adrenocorticotropin-induced, cycloheximide-sensitive, cytosolic, steroidogenic factor. *Endocrinology* **106** (1980) 1869–1979.
99. Xu X., Xu T., Robertson D. G. and Lambeth D. J.: GTP stimulates pregnenolone generation in isolated rat adrenal mitochondria. *J. Biol. Chem.* **264** (1989) 17674–17680.
100. McNamara B. C. and Jefcoate C. R.: The role of SCP_2 in stimulation of steroidogenesis in rat adrenal mitochondria by adrenal cytosol. *Archs Biochem. Biophys.* **275** (1989) 53–62.
101. Xu T., Bowman E. P., Glass D. V. and Lambeth J. D.: Stimulation of adrenal mitochondrial cholesterol side-chain cleavage by GTP, steroidogenesis activator polypeptide (SAP), and sterol carrier protein. *J. Biol. Chem.* **266** (1991) 6801–6807.
102. McNamara B. C. and Jefcoate C. R.: Heterogeneous pools of cholesterol side-chain cleavage activity in adrenal mitochondria from ACTH-treated rats:

differential responses to different reducing precursors. *Molec. Cell. Endocr.* **73** (1990) 123–134.
103. McNamara B. C. and Jefcoate C. R.: Heterogenous pools of cholesterol side-chain cleavage activity in adrenal mitochondria from adrenocorticotropic hormone-treated rats: Reconstitution of the isocitrate response with succinate and low concentrations of isocitrate. *Archs Biochem. Biophys.* **283** (1990) 464–471.
104. Farkash Y., Timberg R. and Orly J.: Preparation of antiserum to rat cytochrome *P*-450 cholesterol side chain cleavage, and its use for ultrastructural localization of the immunoreactive enzyme by protein A-gold technique. *Endocrinology* **118** (1986) 1353–1365.
105. Allmann D. W., Wakabayashi T., Korman E. F. and Green D. E.: Studies on the transition of the cristae membrane from the orthodox to the aggregated configuration I: Topology of bovine adrenal cortex mitochondria in the orthodox configuration. *Bioenergetics* **1** (1970) 73–86.
106. Robinson J. and Stevenson P. M.: The energy-linked reduction of $NADP^+$ by succinate, and its relationship to cholesterol side-chain cleavage. *FEBS Lett.* **23** (1972) 327–331.
107. Fahien L. A., MacDonald M. J., Teller J. K., Fibich B. and Fahien C. M.: Kinetic advantages of hetero-enzyme complexes with glutamate dehydrogenase and the α-ketoglutarate dehydrogenase complex. *J. Biol. Chem.* **264** (1989) 12303–12312.
108. Dalziel K.: Isocitrate dehydrogenase and related oxidative decarboxylases. *FEBS Lett.* **117** (1980) K45–K55.
109. Stevens V. L., Aw T. Y., Jones D. P. and Lambeth J. D.: Oxygen dependence of adrenal cortex cholesterol side chain cleavage. *J. Biol. Chem.* **259** (1984) 1174–1179.
110. Mandella R. D. and Sauer L. A.: The mitochondrial malic enzymes. I. Submitochondrial localization and purification and properties of the $NAD(P)^+$-dependent enzyme from adrenal cortex. *J. Biol. Chem.* **250** (1975) 5877–5884.
111. Bar L. K., Barenholz Y. and Thompson T. E.: Dependence on phospholipid composition of the fraction of cholesterol undergoing spontaneous exchange between small unilamellar vesicles. *Biochemistry* **26** (1987) 5460–5465.
112. Pfanner N., Rassow J., Wienhues U., Hergersberg C., Sollner T., Becker K. and Neupert W.: Contact sites between inner and outer membranes: structure and role in protein translocation into the mitochondria. *Biochim. Biophys. Acta* **1018** (1990) 239–242.
113. Hwang S. T., Wachter C. and Schatz G.: Protein import into the yeast mitochondrial matrix: A new translocation intermediate between the two mitochondrial membranes. *J. Biol. Chem.* **266** (1991) 21083–21089.
114. Voelker D. R.: Reconstitution of phosphatidylserine import into rat liver mitochondria. *J. Biol. Chem.* **264** (1989) 8019–8025.
115. Van Venetie R. and Verkleij A. J.: Possible role of non-bilayer lipids in the structure of mitochondria: A freeze-fraction electron microscopy study. *Biochim. Biophys. Acta* **692** (1982) 397–405.
116. Kuznetsov A. V., Khuchua Z. A., Vassil'eva E. V., Medved'eva N. V. and Saks V. A.: Heart mitochondrial creatine kinase revisited: The outer mitochondrial membrane is not important for coupling of phosphocreatine production to oxidative phosphorylation. *Archs Biochem. Biophys.* **268** (1989) 176–190.
117. Biermans W., Bakker A. and Jacob W.: Contact sites between inner and outer mitochondrial membrane: a dynamic microcompartment for creatine kinase activity. *Biochim. Biophys. Acta* **1018** (1990) 225–228.
118. Schlegel J., Zurbriggen B., Wegmann G., Wyss M., Eppenberger H. M. and Wallimann T.: Native mitochondrial creatine kinase forms octameric structures. I. Isolation of two interconvertible mitochondrial creatine kinase forms, dimeric and octameric mitochondrial creatine kinase characterization, localization and structure-function relationships. *J. Biol. Chem.* **263** (1988) 16942–16962.
119. Kottke M., Adam V., Riesinger I., Bremm G., Bosch W., Brdiczka D., Sandri G. and Panfili E.: Mitochondrial boundary membrane contact sites in brain: points of hexokinase and creatine kinase location, and control of Ca^{2+} transport. *Biochim. Biophys. Acta* **935** (1988) 87–102.
120. Rojo M., Hovius R., Demel R. A., Nicolay K. and Wallimann T.: Mitochondrial creatine kinase mediates contact formation between mitochondrial membranes. *J. Biol. Chem.* **266** (1991) 20290–20295.
121. Muller M., Moser R., Cheneval D. and Carafoli E.: Cardiolipin is the membrane receptor for mitochondrial creatine phosphokinase. *J. Biol. Chem.* **260** (1985) 3839–3843.
122. Lambeth J. D.: Cytochrome $P450_{scc}$: Cardiolipin as an effector of activity of a mitochondrial cytochrome *P*450. *J. Biol. Chem.* **256** (1981) 4757–4762.
123. Killian J. A. and deKruijff B.: The influence of proteins and peptides on the phase properties of lipids. *Chem. Phys. Lipids* **40** (1986) 259–284.
124. Ardail D., Privat J.-P., Egret-Charlier M., Levrat C., Lerme F. and Louisot P.: Mitochondrial contact sites: Lipid composition and dynamics. *J. Biol. Chem.* **265** (1990) 18797–18802.
125. Krause J., Hay R., Kowollik C. and Brdiczka D.: Cross-linking analysis of yeast mitochondrial outer membrane. *Biochim. Biophys. Acta* **860** (1986) 690–698.
126. Benz R., Kottke M. and Brdiczka D.: The cationically selective state of the mitochondrial outer membrane pore: a study with intact mitochondria and reconstituted mitochondrial porin. *Biochim. Biophys. Acta* **1022** (1990) 311–318.
127. Brdiczka D., Knoll G., Riesinger I., Weiler U., Klug G., Benz R. and Krause J.: Microcompartmentation at the mitochondrial surface: its function in metabolic regulation. In *Myocardial and Muscle Bioenergetics* (Edited by N. Brantbar). Plenum Press, New York (1986) pp. 55–70.
128. Verma A. and Snyder S. H.: Peripheral type benzodiazepine receptors. *A. Rev. Pharmac. Toxic.* **29** (1989) 307–322.
129. Sprengel R., Werner P. and Seeburg P. H.: Molecular cloning and expression of cDNA encoding a peripheral-type benzodiazepine receptor. *J. Biol. Chem.* **264** (1989) 20415–20421.
130. Amsterdam A. and Suh B. S.: An inducible functional peripheral benzodiazepine receptor in mitochondria of steroidogenic granulosa cells. *Endocrinology* **129** (1991) 503–510.
131. Mukhin A. G., Papadopoulos V., Costa E. and Krueger K. E.: Mitochondrial benzodiazepine receptors regulate steroid biosynthesis. *Proc. Natn. Acad. Sci. U.S.A.* **86** (1989) 9813–9816.
132. Papadopoulos V., Mukhin A. G., Costa E. and Krueger K. E.: The peripheral-type benzodiazepine receptor is functionally-linked to Leydig cell steroidogenesis. *J. Biol. Chem.* **265** (1990) 3772–3779.
133. Papadopoulos V., Nowzari F. B. and Krueger V. E.: Hormone-stimulated steroidogenesis is coupled to mitochondrial benzodiazepine receptors. *J. Biol. Chem.* **266** (1991) 3682–3687.

134. Besman M. J., Yanagibashi K., Lee T. D., Kawamura M., Hall P. F. and Shively J. E.: Identification of des-(Gly-Ile)-endozepine as an effector of corticotropin-dependent adrenal steroidogenesis: Stimulation of cholesterol delivery is mediated by the peripheral benzodiazepine receptor. *Proc. Natn. Acad. Sci. U.S.A.* **86** (1989) 4897–4901.
135. Yanagibashi K., Ohno Y., Nakamichi N., Matsui T., Hayashida K., Takamura M., Yamada K., Tou S. and Kawamura M.: Peripheral-type benzodiazepine receptors are involved in the regulation of cholesterol side chain cleavage in adrenocortical mitochondria. *J. Biochem.* **106** (1989) 1026–1029.
136. Guidotti A., Berkovich A., Muhkin A. and Costa E.: Diazepam-binding inhibitor: response to Knudsen and Nielsen. *Biochem. J.* **265** (1990) 928–929.
137. Knudsen J. and Nielsen M.: Diazepam-binding inhibitor: a neuropeptide and/or an acyl-CoA ester binding protein? *Biochem. J.* **265** (1990) 927–928.
138. Brown A. S., Hall P. F., Shoyab M. and Papadopoulos V.: Endozepine/diazepam binding inhibitor in adrenocortical and Leydig cell lines: Absence of hormonal regulation. *Molec. Cell. Endocr.* **83** (1992) 1–9.
139. Brdiczka D.: Contact sites between mitochondrial envelope membranes, structure, and function in energy- and protein-transfer. *Biochim. Biophys. Acta* **1071** (1991) 291–312.
140. Alberta J. A., Epstein L. F., Pon L. A. and Orme-Johnson N. R.: Mitochondrial localization of a phosphoprotein that rapidly accumulates in adrenal cortex cells exposed to adrenocorticotropic hormone or to cAMP. *J. Biol. Chem.* **264** (1989) 2368–2372.
141. Epstein L. F. and Orme-Johnson N. R.: Regulation of steroid hormone biosynthesis: Identification of precursors of a phosphoprotein targeted to the mitochondrion in stimulated rat adrenal cortex cells. *J. Biol. Chem.* **266** (1991) 19739–19745.
142. Stocco D. M. and Sodeman T. C.: The 30-kDa mitochondrial proteins induced by hormone stimulation in MA-10 mouse Leydig tumor cells are processed from larger precursors. *J. Biol. Chem.* **266** (1991) 19731–19738.
143. Stocco D. M. and Chen W.: Presence of identical mitochondrial proteins in unstimulated constitutive steroid-producing R_2C rat Leydig tumor and stimulated nonconstitutive steroid-producing MA-10 mouse Leydig tumor cells. *Endocrinology* **128** (1991) 1918–1926.
144. Pon L., Moll T., Vestweber D., Marshallsay B. and Schatz G.: Protein import into mitochondria ATP-dependent protein translocation activity in submitochondrial fraction enriched in contact sites and specific proteins. *J. Cell Biol.* **109** (1989) 2603–2616.
145. Cozza E. N., del Carmen Vila M., Acevedo-Duncan M., Gomez-Sanchez C. E. and Farese R. V.: ACTH increases *de novo* synthesis of diacylglyercol and translocates protein kinase C in primary cultures of calf adrenal glomerulosa cells. *J. Steroid Biochem.* **35** (1990) 343–351.
146. Igarashi Y. and Kimura T.: Adrenocorticotropic hormone-mediated changes in rat adrenal mitochondrial phospholipids. *J. Biol. Chem.* **259** (1984) 10745–10753.
147. Privalle C. T., McNamara B. C., Dhariwal M. S. and Jefcoate C. R.: ACTH control of cholesterol side-chain cleavage at adrenal mitochondrial cytochrome $P450_{scc}$. Regulation of intramitochondrial cholesterol transfer. *Molec. Cell. Endocr.* **53** (1987) 87–101.
148. Igarashi Y. and Kimura T.: Adrenic acid content in rat adrenal mitochondrial phosphatidylethanolamine and its relation to ACTH-mediated stimulation of cholesterol side chain cleavage reaction. *J. Biol. Chem.* **261** (1986) 14118–14124.
149. Hotta M. and Baird A.: The inhibition of low density lipoprotein metabolism by transforming growth factor-β mediates its effects on steroidogenesis in bovine adrenocortical cells *in vitro*. *Endocrinology* **121** (1987) 150–159.
150. Perrin A., Pascal O., Defaye G., Feige J.-J. and Chambaz E. M.: Transforming growth factor β_1 is a negative regulator of steroid 17α-hydroxylase expression in bovine adrenocortical cells. *Endocrinology* **128** (1991) 357–362.
151. Elliott M. E. and Goodfriend T. L.: Inhibition of aldosterone synthesis by atrial natriuretic factor. *Fedn. Proc.* **45** (1986) 2376–2381.
152. Hurwitz A., Payne D. W., Packmann J. N., Andreani C. L., Resnick C. E., Hernandez E. R. and Adashi E. Y.: Cytokine-mediated regulation of ovarian function. *Endocrinology* **129** (1991) 1250–1256.
153. Lin T., Wang D., Nagpal M. L., Calkins J. H., Chang W. and Chi R.: Interleukin-1 inhibits cytochrome $P450_{scc}$ expression in primary cultures of Leydig cells. *Endocrinology* **129** (1991) 1305–1311.