

Adrenocortical Responsiveness to Adrenocorticotropin: StAR Is Ascendant

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The circadian rhythm in glucocorticoid secretion by the adrenal cortex was long considered to be a product and direct reflection of the circadian rhythm of ACTH secretion by the anterior pituitary. However, data have accumulated for several years indicating that changes in circulating ACTH cannot fully explain the adrenal rhythm, and under many conditions there is a dissociation between ACTH and glucocorticoids (1). The circadian rhythm in plasma glucocorticoid concentration is extremely robust, and in many species has a much greater amplitude than the ACTH rhythm (2). In rats after hypophysectomy, the corticosteroid rhythm persists and exhibits 3-fold differences in response to exogenous ACTH, depending on the time of day (3, 4); and in capuchin monkeys, markedly rhythmical cortisol secretion continues after ACTH suppression with dexamethasone (5). Additional findings of nycthemeral differences in adrenal responsiveness to ACTH (6, 7), the ability of a restricted feeding schedule to alter those rhythms (8), and the demonstration that innervation of the adrenal cortex via the splanchnic nerve plays a major role in regulation of circadian glucocorticoid secretion (2, 9) have all contributed to our understanding that stimulation of adrenal steroidogenesis is subject to a number of potent influences in addition to ACTH.

Circadian clocks, both the central rhythm generator in the suprachiasmatic nuclei of the hypothalamus and the peripheral oscillators that have been discovered in multiple mammalian tissues, regulate temporal output via interlocking transcriptional/translational feedback loops involving the core clock genes *Per1-2*, *Cry1-2*, *Clock*, and *Bmal1*, and their protein products (10). In recent years the molecular machinery necessary for generation of circadian rhythms has been found in the adrenal cortex of rodents and primates, and the adrenal clock of capuchin monkeys maintains a circadian rhythm *in vitro* (11). These findings suggest that this molecular adrenal clock may also be among the modulators of the steroidogenic response to ACTH.

In this issue of *Endocrinology*, Torres-Farfan *et al.* (12) have demonstrated that in the capuchin monkey (*Cebus apella*) ad-

renal cortex, an antisense knockdown of the expression of the adrenal clock protein cryptochrome 2 (CRY2) blocks the adrenal's steroidogenic response to stimulation by ACTH. Another recent study showed that expression of a different canonical clock protein, BMAL1, is necessary for steroidogenesis in the Y-1 immortalized mouse adrenocortical cell line (13). In addition, a transgenic mouse line in which a part of the BMAL1 coding region was expressed in an antisense orientation under tissue-specific control failed to express rhythms of corticosteroid synthesis and secretion when the animals were maintained in constant darkness.

Because steroidogenic cells store very little steroid, acute increases in secretion in response to a stimulus require rapid synthesis of new steroid, and the rate-limiting step of transfer of cholesterol across the mitochondrial membranes is dependent on *de novo* protein synthesis (14). Acute steroidogenesis was found to be accompanied by the synthesis of a phosphoprotein (15) that occurs much more rapidly than ACTH-elicited synthesis of steroidogenic enzymes. The protein was cloned and named steroidogenic acute regulatory protein (StAR) (16). In the aforementioned studies in which adrenal steroidogenic responses to ACTH were found to be dependent on clock proteins and in several other investigations of factors observed to modulate steroidogenesis, the effects were achieved at least partly by alteration of StAR transcription.

StAR is expressed only in steroidogenic tissues [adrenal cortex, testes, ovaries, placenta, and brain (17)] and mediates the transfer of mobilized cholesterol from the outer to the inner mitochondrial membrane, the site of the first enzymatic step in steroidogenesis, side chain cleavage of cholesterol to form pregnenolone. The clinical consequence of dysfunctional StARs resulting from gene mutations is congenital lipid adrenal hyperplasia (18). The absence of functional StAR results in a roughly 85% loss of steroidogenesis that elicits compensatory ACTH secretion and subsequent cellular damage from massive accumulation in lipid droplets of esterified cholesterol that cannot be transported into mitochondria (14).

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Abbreviations: Ang II, Angiotensin II; CRY2, cryptochrome 2; StAR, steroidogenic acute regulatory protein.

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StAR seems an ideal site for simultaneous multifactorial modulation of steroidogenesis, not only because its synthesis represents the rate-limiting step, but because it has a transcription factor-rich promoter region that is under the regulation of a large number of transcription factors (19), and it can be regulated via multiple signaling pathways, including arachidonic acid metabolites, calcium messenger systems, chloride ion channels, and extracellular signal-related kinases, as well as by cAMP pathways (20). In addition, there is a circadian rhythm in the expression of StAR in the adrenal cortex, but not in the testes in which circadian regulation is much weaker (13).

The relationship of the adrenal circadian clock to StAR function in the adrenal cortex was demonstrated in the current study that showed that in capuchin monkey adrenal gland explants treated with CRY2 antisense probes, basal cortisol production was not affected, but ACTH-stimulated steroidogenesis was markedly inhibited (12). Control and CRY2 sense probes had no effect. Similarly, ACTH-induced increases in expression of StAR and 3β -hydroxysteroid dehydrogenase, which catalyzes the conversion of pregnenolone to progesterone, were blocked in CRY2 knockdowns.

In addition to these findings of the requirement of clock proteins for ACTH-induced steroidogenesis, several earlier studies have shown that factors related to sleep/wake cycles, energy balance, cardiovascular regulation, and immune function can also alter adrenal steroidogenic responses by influencing StAR transcription (Fig. 1). For example, Serón-Ferré and colleagues (21) demonstrated the presence of MT1 melatonin receptors in the capuchin adrenal cortex, and showed that melatonin inhibited ACTH- and dibutyryl-cAMP-stimulated cortisol production without affecting basal steroidogenesis. The cellular mechanism of the effect was not determined, but a similar action of melatonin on steroidogenesis in MA-10 mouse Leydig tumor cells stimulated by trophic hormones or dibutyryl-cAMP was due to the blockade of StAR expression, suggesting that a similar mechanism of action may occur in the adrenal cortex (22).

The adipocyte-derived hormone leptin has also been found to inhibit ACTH-induced steroidogenesis without affecting basal

glucocorticoid production, at least in part by attenuating the increase in StAR mRNA in response to stimulation (23, 24). In addition, IL-10 knockout mice (IL-10^{-/-}) have markedly greater glucocorticoid responses to acute immune and physiological stresses than wild-type mice (25); and in the Y-1 adrenocortical cell line, IL-10 inhibits ACTH-induced, but not basal, steroid production, at least in part by reducing the expression of StAR (26).

Additional factors have been shown to affect adrenal steroidogenesis via actions on StAR both in the presence and absence of ACTH. Adipocyte Wnt-signaling molecules (27) and orexins A and B (28), neuropeptides implicated in energy balance and sleep/wake control up-regulate StAR transcription in human H2952R adrenocortical carcinoma cells, whereas group X-secretory phospholipase A₂ has a negative effect in mouse Y-1 cells (29) (Fig. 1). In bovine adrenocortical cells, IGF-I stimulates and TGF- β and angiotensin II (Ang II) inhibit cortisol production and transcription of StAR (30, 31). Ang II appears to have a biphasic effect with short-lived stimulation, followed by a subsequent and virtually complete inhibition (31).

StAR may play a dominant role in regulating adrenal steroidogenesis by functionally integrating inputs from a variety of ligands activating several signaling pathways, but it is not the only mechanism for altering adrenocortical responsiveness to ACTH. Innervation of the rat adrenal cortex by the splanchnic nerve has been shown to be at least partially responsible for increases in rat adrenocortical responsiveness to ACTH at the time of the normal circadian peak of corticosterone synthesis and secretion. This effect occurs proximally to intracellular generation of cAMP, perhaps by alteration of blood flow to the adrenal or changes in ACTH receptor activity (2). Another locus at which adrenal responsiveness to ACTH appears to be modulated is the enzyme hormone sensitive lipase, which catalyzes the hydrolysis of cholesterol esters to make free cholesterol available to the steroidogenic pathway. The proopiomelanocortin product Lys- γ ₃-MSH has no steroidogenic activity alone, but it has a marked potentiating effect on ACTH-induced steroidogenesis by activating hormone sensitive lipase (32, 33).

Acute and circadian modulation of responsiveness to ACTH is clearly a prevalent and adaptable strategy for fine-tuning regulation of adrenal steroidogenesis, and glucocorticoid rhythms are potent in activating and synchronizing both peripheral and central oscillators (34). StAR, as the rate limiter of steroidogenesis receiving a multiplicity of inputs from a wide diversity of signaling pathways and transcription factors, plays a stellar role.

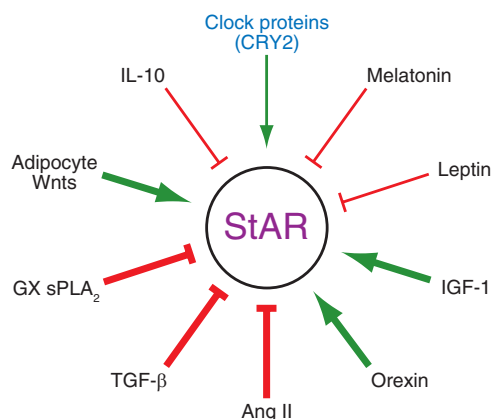


FIG. 1. Factors that have been reported to influence adrenocortical steroidogenesis by acting on StAR transcription. *Green arrows* indicate stimulation; *red T-bars* indicate inhibition. *Thin lines* denote effects demonstrated on ACTH-induced but not basal steroidogenesis. *Thick lines* denote actions in the presence and absence of ACTH. GX sPLA₂, Group X secretory phospholipase A₂; Wnts, Wnt-signaling molecules.

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