Sex and Salt Hormones: Rapid Effects in Epithelia
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Recent evidence points to protein kinase C isoforms as highly specific receptors for aldosterone and estradiol in epithelia. The end targets of the kinase activation are Na+/H+ exchange and K+ and Ca2+ channels. The physiological role of the nongenomic response is to increase electrolyte absorption and inhibit secretion in pluripotential epithelia.

Over the past decade, the number of reports dealing with the cell signaling aspects of fast actions of steroid hormones has multiplied without an accompanying validation of their physiological meaning. The reasons for this mismatch between signal transduction and a physiological role are, in the main, due to difficulties in identification of the "nongenomic" hormone receptor and the apparent lack of rapid in vivo effects. The understanding of the relationship between rapid cell signaling and nongenomic responses to steroid hormones requires an integrative approach in which both the measurement of the cytosolic transduction processes and the macroscopic effects are determined simultaneously in whole tissue under physiological conditions.

The classical pathway for steroid action involves the binding of hormone to a specific intracellular receptor [e.g., aldosterone binding to the cytosolic mineralocorticoid type I receptor]. The receptor-steroid complex interacts with hormone-responsive elements located within target genes, initiating transcription and de novo protein synthesis. The steroid-induced proteins influence various cell functions (Fig. 1). Genomic effects of steroid hormones are characterized by a latency of onset of 2–8 h and a sensitivity to inhibitors of transcription and translation, such as actinomycin D or cycloheximide. The genomic pathway can also be inhibited by specific antagonists of the various intracellular receptors.

In contrast to the classic genomic pathway, many members of the steroid hormone receptor superfamily produce rapid in vitro effects on various second messenger systems and ion channels (Fig. 1). These fast responses are incompatible with the involvement of the genomic steroid hormone pathway, and the unique characteristics of the new pathway include its rapid time course and insensitivity to inhibitors of transcription and translation as well as antagonists of the classic intracellular steroid receptors.

Nongenomic targets of aldosterone
Mineralocorticoid hormones increase Na+ reabsorption and promote K+ and H+ secretion in high-resistance epithelia. Aldosterone is the most important mineralocorticoid released from the adrenal glands. It regulates solute reabsorption in epithelia such as distal colon and kidney, acting in both a rapid and a delayed manner to influence transport (16). The classic mechanism of aldosterone stimulation of Na+ reabsorption involves the activation of a genomic pathway. Over the past decade, studies have described rapid actions of aldosterone on ion transport that precede the genomic events by 2–4 h. These rapid effects consistently show a high selectivity for aldosterone over cortisol and other steroids. The rapid, nongenomic pathway offers an additional explanation for the differential effects of glucocorticoids and mineralocorticoids in Na+ homeostasis.

In extrarenal, nonepithelial cells, aldosterone has been reported to produce rapid in vitro effects (onset within 1–2 min) on intracellular electrolyte concentrations, cell volume, Na+/H+ exchange, and inositol 1,4,5-trisphosphate production in human mononuclear leukocytes (17). Rapid effects of aldosterone on free intracellular Ca2+ concentration ([Ca2+]i), phospholipase C, diacylglycerol, and protein kinase C (PKC) activity, specifically by mineralocorticoids (glucocorticoids were without effect) in human and rat distal colon cells. The primary ion transport target of the nongenomic signal transduction cascades elicited by aldosterone in epithelia is the Na+/H+ exchanger at the basolateral cell membrane. In isolated colonic crypts, aldosterone produces an intracellular alkalinization within 1 min of hormone addition (17). Inhibition of PKC activity prevents the cellular alkalinization induced by aldosterone. Because serine kinases are important regulators of the Na+/H+ exchanger, it is likely that rapid activation of PKC by aldosterone mediates the nongenomic stimulation of the exchanger. The effect of aldosterone on Na+/H+ exchange appears to be ubiquitous in mineralocorticoid-receptive epithelia such as human sweat gland, renal collecting duct, and amphibian skin (8, 9, 15).

In pluripotential epithelia (tissues capable of simultaneous secretion and absorption), the rapid activation of Na+/H+ exchange and the resulting intracellular alkalinization have important consequences for transepithelial ion transport. An increase in intracellular pH (pHi) produces opposite effects on two classes of basolateral K+ channels: an ATP-sensitive K+ (KATP) channel and a Ca2+-sensitive K+ (KCa) channel. The KATP channel is stimulated by increases in pHi, and this channel is involved in K+ recycling to maintain the electrical driving force.
for amiloride-sensitive Na\(^+\) absorption. In contrast, the K\(\text{Ca}\) channels are inhibited by cellular alkalinization, and these channels generate the charge balance for Cl\(^-/G\(10\) secretion (11). At physiological concentrations (0.1\(\text{nM}\) \(\sim 1\text{nM}\)), aldosterone activates the KATP conductance and inhibits the KCa conductance within 5 min in human distal colon (11). Stimulation of PKC and Na\(^+\)/H\(^+\) exchanger activity are required for these rapid effects. Thus it would appear that the nongenomic response to aldosterone in pluripotential epithelia is to enhance the capacity for absorption while downregulating the potential for secretion (Fig. 2).

A rapid activation of the basolateral KATP conductance in Na\(^+\)-absorbing epithelia should increase the driving force for Na\(^+\) entry and therefore increase transepithelial Na\(^+\) absorption. However, the nongenomic effect is apparent within 5 min, yet the stimulation of transepithelial Na\(^+\) absorption does not occur until 1–2 h after the hormone. This paradox is understood when we consider that the membrane potential of the Na\(^+\)-absorbing cells, under physiological conditions, is already close to the equilibrium potential for K\(^+\). Therefore, activation of basolateral KATP channels would not be expected to greatly influence the electrical driving force for Na\(^+\) absorption but will increase the basolateral membrane conductance. An increased K\(^+\) conductance will serve as a “physiological voltage clamp” to counteract membrane depolarization produced by increased Na\(^+\) entry into the cell during the genomic phase of enhanced Na\(^+\) absorption. The physiological role, therefore, of a rapid and maintained nongenomic response to aldosterone is to preserve the driving force for chronic Na\(^+\) absorption.

### Cell signaling responses to aldosterone

Aldosterone directly stimulates the activity of PKC-\(\alpha\) in a cell-free assay system containing only purified commercially available enzyme, appropriate substrate peptide, cofactors, and lipid vesicles (3). Aldosterone did not stimulate the activity of the other PKC isoforms known to be present in distal colonic epithelium: PKC-\(\delta\), -\(\varepsilon\), and -\(\zeta\) (2). In addition, aldosterone stimulates PKC-dependent Ca\(^2+\) entry through an L-type channel in rat and human distal colonic crypts (4, 5).

These results demonstrate a direct and specific interaction of aldosterone with the Ca\(^2+\)-sensitive PKC isoform PKC-\(\alpha\). The PKC-\(\alpha\) is a candidate nongenomic receptor for aldosterone (Fig. 3). There is also evidence that more than one receptor is involved in the nongenomic aldosterone response. Arachidonic acid release via the phospholipase A\(_2\) pathway is also an important step in transducing rapid responses to aldosterone. Pretreatment with the G protein inhibitor pertussis toxin and the phospholipase A\(_2\) antagonist quinacrine significantly inhibited aldosterone-induced stimulation of the Na\(^+\)/H\(^+\) exchange and KATP current in colonic cells. Inhibition of prostaglandin synthesis via the cyclooxygenase pathway abolished the stimulatory response to the steroid hormone. Conversely, inhibition of the lipoxygenase pathway did not prevent the increase in KATP channel activity or activation of the Na\(^+\)/H\(^+\) exchange induced by aldosterone (18). The results of these studies indicate that the rapid signaling responses to aldosterone involve at least two separate pathways: a G protein-coupled receptor (GPCR) and a direct interaction with the Ca\(^2+\)-sensitive PKC-\(\alpha\).

*FIGURE 1.* Summary of the key differences between genomic and nongenomic mechanisms of steroid hormone action. The classic genomic pathway is characterized by an increase in protein synthesis, which mediates the in vivo response to the hormone some hours after hormone-receptor binding. In contrast, the nongenomic pathway is characterized by rapid second messenger activation, which leads to acute (within seconds) cellular responses. The precise nature of the receptor proteins that initiate the nongenomic pathway is unclear.

*FIGURE 2.* The role of salt-retaining steroid hormones in transepithelial ion transport in a pluripotential epithelium. In surface cells of the colonic epithelium, basolateral K\(_{\text{ATP}}\) channels maintain the driving force for Na\(^+\) absorption. Basolateral Ca\(^{2+}\)-sensitive K\(^+\) (K\(_{\text{Ca}}\)) channels in secretory cells at the base of the crypt generate charge balance during Cl\(^-\) efflux. Estradiol rapidly inhibits Cl\(^-\) secretion by downregulating K\(_{\text{Ca}}\) channels. PKC, protein kinase C.
cate that, in addition to PKC, there may be a role for membrane-associated signaling in the transduction of rapid steroid hormone responses on ion transport and pH regulation in pluripotential epithelia.

**Rapid nongenomic targets of estradiol**

Several reports suggest that estrogens may be active in previously unsuspected tissues. The presence of functional estrogen receptors has been demonstrated in colon, ileum, jejunum, and duodenum by RT-PCR (14). The physiological significance of estrogen receptors in nonclassic target tissues remains to be determined.

A possible physiological function of estradiol may be to alter transepithelial ion transport. Plasma estrogen concentrations increase dramatically during gestation and in other high-estrogen states such as certain phases of the menstrual cycle and following use of the combined oral contraceptive pill. High estrogen states are associated with salt and water retention, resulting in edema and hypertension (cf. Ref. 3). This observation supports a role for estradiol in whole body salt and water homeostasis.

The transcellular movement of Cl⁻ drives the efflux of salt and water in many secretory epithelia. Basolateral K<sub>ATP</sub> channels play an important role in this process, because blockade of these channels reduces both basal Cl⁻ secretion and secretagogue-induced Cl⁻ secretion prestimulated by secretagogues (10). Estradiol has been shown to rapidly inhibit basolateral K<sub>ATP</sub> channel activity, which would be expected to result in an acute inhibition of Cl⁻ secretion (Fig. 2). Recently, we have shown that physiological concentrations of estradiol (0.1–10 nM) reduce both basal and secretagogue-induced Cl⁻ secretion (1). The antiserective effect of estradiol is sensitive to PKC inhibition or intracellular Ca<sup>2+</sup> chelation and is gender specific, occurring only in tissues of female origin. These observations link nongenomic second messengers with a rapid, gender-specific effect in the whole tissue. The nongenomic effect of estradiol to decrease Cl⁻ secretion occurs only in epithelia of female origin and may underlie the increased salt and water retention observed clinically in high-estrogen states.

**Cell signaling responses to estradiol**

One of the earliest reports of rapid effects of 17β-estradiol was made in 1969 by Szego and Davis (13). They showed that estrogen treatment of rats in vitro resulted in an acute elevation of cAMP in uterine smooth muscle. In female rat distal colon, estradiol produces rapid nongenomic effects on PKC and

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**FIGURE 3.** Common and divergent nongenomic signaling pathways for aldosterone and estradiol. Aldosterone binds directly to PKC-α, which stimulates Ca<sup>2+</sup> influx via L-type Ca<sup>2+</sup> channels. The resulting increase in intracellular Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>) amplifies the activity of the Ca<sup>2+</sup>-dependent PKC-α, enabling upregulation of Na<sup>+</sup>/H<sup>+</sup> exchange. This causes an intracellular alkalinization that stimulates basolateral K<sub>ATP</sub> channels. Estradiol directly activates both PKC-α and PKC-δ. PKC-δ stimulates adenylyl cyclase (AC), causing activation of protein kinase A (PKA), which phosphorylates L-type Ca<sup>2+</sup> channels causing Ca<sup>2+</sup> influx and increased [Ca<sup>2+</sup>]<sub>i</sub>, permits PKC-dependent upregulation of Na<sup>+</sup>/H<sup>+</sup> exchange, and the subsequent increase in intracellular pH (pHi) inhibits K<sub>ATP</sub> channel activity. The estradiol-induced PKC-δ signaling step (*) is absent in tissues of male origin.
cAMP-dependent protein kinase (PKA) (3, 4). Fast effects on [Ca^{2+}], are also observed via PKA- and PKC-sensitive pathways. These rapid signaling responses are sex-steroid selective, insensitive to estrogen receptor antagonists, and gender specific.

Because the only known physiological regulator of PKA is cAMP, the stimulatory effect of estradiol on PKA activity must be indirect since the nongenomic response is sensitive to adenyl cyclase inhibition and is mimicked by forskolin. Also, the hormone does not cause a direct stimulation of PKA activity in either type I or type II commercially available PKA holoenzymes (3). The most obvious conclusion therefore is that estradiol stimulates adenyl cyclase activity indirectly to produce the nongenomic effect on PKA.

It has previously been demonstrated (19) that certain isoforms of adenyl cyclase are activated by PKC. Estradiol produces a rapid stimulation of PKC activity in rat distal colonic epithelium (4), and it is possible that the estradiol-stimulated PKA activity is mediated via this pathway. This conclusion is reinforced by the observation that specific PKC antagonists inhibit the estradiol-induced stimulation of PKA activity. In contrast, however, PKC inhibition has no inhibitory effect on cAMP-stimulated PKA activity. Estradiol stimulation of PKA involves an initial activation of PKC before upregulation of adenyl cyclase activity (3). This effect is PKC isoform specific (Fig. 3).

Estradiol directly and specifically stimulates PKC-δ activity in a cell-free assay system. Other steroid hormones such as 17α-estradiol, progesterone, testosterone, aldosterone, hydrocortisone, and dexamethasone are without effect on PKC-δ activity. Estradiol also activates PKC-α. However, the involvement of this Ca^{2+}- and phospholipid-dependent PKC isoform in the estradiol-induced stimulation of PKA activity can be ruled out. Both aldosterone and the stereoisomer 17α-estradiol, which activate PKC-α, have no stimulatory effect on PKA (3). We propose, therefore, that the cytosolic protein responsible for the rapid nongenomic PKA response to estradiol is the Ca^{2+}-independent, phospholipid-dependent PKC-δ isoform. The PKC-δ isoform may be a nongenomic receptor for estradiol (Fig. 3).

Common and divergent signaling pathways for rapid effects of aldosterone and estradiol

Aldosterone and estradiol differ in their protein kinase signal transduction, and both hormones stimulate specific PKC isoforms. Isozyme-specific properties such as substrate specificities, subcellular localization, activation requirements, and rates of downmodulation suggest that different PKC isoforms may perform unique cellular functions.

Initially, there appeared to be a common stimulatory signaling pathway for rapid nongenomic responses to aldosterone and estradiol, i.e., PKC. Our recent results (3) now suggest that these steroid hormones stimulate different PKC isozymes, with aldosterone stimulating PKC-α selectively and estradiol stimulating both PKC-α and PKC-δ. These results indicate both common and divergent signaling systems for salt-retaining steroid hormones. These properties could offer an explanation for common transporter targeting and yet confer gender specificity.

Conclusion

Current evidence points to different PKC isoforms as nongenomic receptors for rapid responses to steroids (3, 12). The physiological function of nongenomic effects of aldosterone and estradiol is to shift the balance from net secretion to net absorption in a pluripotential epithelium.

References