Steroid-Producing Cells Regulate Arterial Tone of Adrenal Cortical Arteries

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Adrenal blood flow is coupled to adrenal hormone secretion. ACTH increases adrenal blood flow and stimulates the secretion of aldosterone and cortisol in vivo. However, ACTH does not alter vascular tone of isolated adrenal cortical arteries. Mechanisms underlying this discrepancy remain unsolved. The present study examined the effect of zona glomerulosa (ZG) cells on cortical arterial tone. ZG cells (10^5 to 10^7 cells) and ZG cell-conditioned medium relaxed preconstricted adrenal cortical arteries (15), and histamine relaxes the adrenal arteries through endothelium-derived nitric oxide and prostaglandins (16). In intact rat or canine adrenals, ACTH-induced dilation does not involve NO or prostaglandins (6, 13). Although these discrepancies could be explained by species variability, it is also possible that other mechanisms are involved in ACTH-induced vasodilation.

Previous studies have demonstrated a functional interaction between vascular and steroidogenic cells in the adrenal gland. Endothelial cells, in response to various vasoactive agents and shear stress, release a variety of soluble factors that regulate adrenal steroidogenesis. For example, prostacyclin and endothelin stimulate aldosterone release, whereas NO inhibits aldosterone production (17–20). In addition, endothelial cells release a transferable steroidogenic peptide other than endothelin that functions as a paracrine regulator of aldosterone secretion (21). Alternatively, it remains unknown whether adrenal steroidogenic cells can modulate vascular tone and adrenal blood flow. In the present study, we hypothesize that ACTH stimulates adrenal steroidogenic cells to release vasoactive factors, which diffuse to vascular cells to mediate relaxation. To test this hypothesis, we examined the effect of zona glomerulosa (ZG) cells and ZG-conditioned medium on adrenal arterial tone, and the vascular activity of ACTH with and without coincubation of ZG cells on isolated small adrenal cortical arteries. Furthermore, compound 48/80, a mast cell degranulator, mimics ACTH-induced increase in adrenal blood flow and steroidogenesis in the rat adrenal gland. Sodium cromoglycate, a mast cell stabilizer, blocks the effects of ACTH. Histamine and serotonin antagonists were not tested in these studies. Recently we found that serotonin constricts isolated bovine adrenal cortical arteries (15), and histamine relaxes the adrenal arteries through endothelium-derived nitric oxide and prostaglandins (16). In intact rat or canine adrenals, ACTH-induced dilation does not involve NO or prostaglandins (6, 13). Although these discrepancies could be explained by species variability, it is also possible that other mechanisms are involved in ACTH-induced vasodilation.
we isolated and identified vasoactive factors released by ZG cells.

**Materials and Methods**

**Isometric tension recording**

Fresh bovine adrenal glands were obtained from a local abattoir. Small cortical arteries closely attached to the adrenal surface (200–300 μm) were dissected and mounted on a four-chamber wire myograph as described (15). Arteries were maintained at 37 C in physiological saline solution (PSS) containing (in mM): NaCl 119, KC1 4.7, CaCl2 2.5, MgSO4 1.17, NaHCO3 24, KH2PO4 1.18, EDTA 0.026, and glucose 5.5, gassed with 98% O2-5% CO2. Arteries were contracted with submaximal concentra-
tions of the thromboxane mimetic U46619 (100–300 nM). Where indicated, the endothelium was removed by gently rubbing the intimal surface of the artery with a human hair. The endothelium was consid-
ered intact if acetylcholine (1 μM) caused more than 80% relaxation and effectively removed if acetylcholine induced less than 10% relaxation.

**Arterial diameter measurement**

Slices of adrenal glands (5–8 mm thickness) were pinned to a Silastic layer of a perfusion dish. A single cortical artery (250–350 μm outer diameter) was cannulated with heat-pulled PE-10 tubing and secured to the cortical surface with 6–0 suture. The adrenal slice was perfused and superfused with PSS equilibrated with 21% O2-5% CO2-balanced N2 and pressurized to 60 mm Hg at 37 C. In separate experiments, arteries were dissected, freed of adherent tissue, cannulated in a perfusion-superfu-
sion chamber, and pressurized to 60 mm Hg at 37 C (22). The arteries were equilibrated for 30–45 min and contracted with serotonin (50–200 nM) before the addition of ACTH. Digital images were captured and arterial diameters were measured using MetaVue software (Universal Imaging Corp., Downingtown, PA). At the end of the experiments, arteries were perfused and superfused with calcium-free PSS to deter-
mine maximum passive diameter. For adrenal slices, India ink (3% in calcium free buffer) was added to the perfusate to determine perfused cortical area.

**Preparation of ZG, zona fasciculata (ZF), and fibroblast cells**

ZG and ZF cells were prepared by enzymatic dissociation of adrenal cortical slices as previously described (23). Briefly, adrenal glands (8–12) were bisected and a 500-μm slices were obtained from the outer surface of the gland (first slice for ZG cell isolation and second slice for ZF cell isolation). The tissue was finely minced and digested for 30–45 min at 37 C in Earle’s balanced salt solution buffer containing dissociating enzymes collagenase and protease. After five digestion cycles were performed, rinsed in HEPES buffer, and stored on ice until use. ZG cells were 95–98% ZG cells and ZF cells were 2–5% ZF cells. ZF cells were 98% pure. Adrenal fibroblasts were selectively isolated from mixed cultures of adrenal capillary endothelial cells and fibroblasts by replating the cells under conditions that favored fibroblast but not endothelial cell growth (21). Adrenal fibroblasts were maintained in DMEM medium and passages of 3–5 were used.

**Identification of arachidonic acid metabolites**

ZG cells were incubated with [14C] arachidonic acid (0.05 mCi, 100 nm), and the buffers were extracted on C-18 solid-phase extraction columns and extracts dried under a stream of nitrogen gas. The extracts were redissolved in acetonitrile and analyzed by reverse-phase HPLC with a Nucleosil C-18 reverse-phase column (5 mm, 4.6 × 250 mm) as described (24, 25). Solvent A was distilled water and solvent B contained acetonitrile; glacial acetic acid (999:1). A linear gradient from 50% solvent B in A to 100% B over 40 min was used with a flow rate of 1 ml/min. Column effluent was collected in 0.2-ml fractions and analyzed for radioactivity by liquid scintillation spectrometry. Normal-phase HPLC with a Nucleosil silica column (5 mm, 4.6 × 250 mm) was used to separate EET regioisomers. Solvents were hexane containing 0.4% iso-
propanol and 0.1% glacial acetic acid. The flow rate was 2 ml/min and column eluate was collected in 0.4-ml fractions. An additional set of samples was analyzed by liquid chromatography/mass spectrom-
etry (LC/MS; 1100 LC/MSD, SL model; Agilent Technologies, Palo Alto, CA) as described (26). Arachidonic acid metabolites were resolved using a C18 (Kromasil, 250 × 2 mm) column using water/acetonitrile with 0.005% acetic acid at a flow rate of 0.2 ml/min. The separation used a 60–80% acetonitrile in water linear gradient over 30 min followed by an increase to 100% over 5 min and held at 100% for 5 min. Drying gas flow was 12 liters/min at 350 C, nebulizer pressure was 35 psig, vaporizer temperature was 325 C, and capillary voltage was 3000 V. Detection was made in the negative ion mode.

**Data analysis**

Relaxations and dilations are expressed as a percentage relative to U46619- or serotonin-preconstriction with 100% representing basal tension or maximum passive diameter in calcium-free PSS. Data are pre-
sented as mean ± SEM. Significance of difference between mean values was evaluated by Student’s t test or ANOVA followed by the Student-
Newman-Keuls multiple comparison test. A value of P < 0.05 was considered statistically significant.

**Drugs and chemicals**

ACTH, serotonin, N-nitro-l-arginine (L-NA), indomethacin, iberio-
toxin, SKF 525A, and miconazole were purchased from Sigma (St. Louis, MO). U46619 was obtained from Cayman Chemical Co. (Ann Arbor, MI). 14,15-EET and 14,15-EEZE (27) were synthesized as previously described (28). All solvents were HPLC grade and purchased from Burdick and Jackson (Morristown, NJ). [14C]-U-arachidonic acid (920 mCi/mmol) was obtained from NEN Life Science Products (Boston, MA).

**Results**

**ZG cell-induced relaxations**

Because the cortical, subcapsular arterioles are embedded in ZG cells rather than ZF cells, most studies used ZG cells. Isolated ZG cells (10⁵ to 10⁷ cells) added to preconstricted adrenal arteri-
es with or without intact endothelium caused relaxation responses that were related to the number of ZG cells added (maximal relaxations of 79 ± 4%, with endothelium, and 70 ± 8%, without endothelium; Fig. 1A). ZF cells relaxed adrenal arteries (data not shown). ZG cell-induced relaxations were not altered by indomethacin (10 μM; max-
imal relaxation of 64 ± 4%) or L-NA (30 μM; maximal relaxation of 64 ± 7%) but were blocked by the large-conduc-
tance, Ca²⁺-activated potassium (BKca) channel inhibitor,iberiotoxin (100 nm) and the EET antagonist-14,15-EEZE (2 μM).

**Effect of ZG cells on vascular responses to ACTH**

We tested whether ZG cells influenced the vascular re-
sponses to ACTH. ACTH did not relax adrenal arteries pre-
constricted with U46619 (maximal relaxation of 5 ± 2%, Fig. 1B). In the presence of a small number ZG cells (0.5–1 × 10⁶ cells), ACTH (10⁻¹⁰ to 10⁻⁷ M) caused a concentration-related relaxation (maximal relaxation of 67 ± 4%). Similar to ZG cells alone, relaxations to ACTH in the presence of ZG cells were not affected by endothelium removal (maximal relaxation of 73 ± 3%) or L-NA plus indomethacin (maximal relaxation of 72 ± 3%) but were blocked by iberiotoxin and high extracellular K⁺ ([K⁺]o) (60 mM). As a control, similar experiments were performed with isolated adrenal fibro-
blasts. In the presence of adrenal fibroblasts (10⁶ cells), ACTH did not cause significant relaxations (maximal relaxation of 6 ± 8%, data not shown).
Role of arachidonic acid metabolites in ZG-induced relaxations

Relaxations to ACTH in the presence of ZG cells were inhibited by the cytochrome P450 inhibitors SKF 525A (10 μM; maximal relaxation of 42 ± 4%, Fig. 2A) or miconazole (10 μM; maximal relaxation of 41 ± 4%) and also inhibited by the EET antagonist, 14,15-EEZE (2 μM; maximal relaxation of 30 ± 2%). SKF 525A did not inhibit aldosterone synthesis, and 14,15-EEZE did not alter ACTH- or angiotensin II-induced aldosterone synthesis by bovine ZG cells (data not shown). In U46619-precontracted arteries, 14,15-EET caused concentration-dependent relaxations with a maximal relaxation of 86 ± 1% (Fig. 2B). The relaxations were blocked by iberiotoxin (100 nM), high [K+]o (60 mM), and endothelium removal (data not shown). Arteries were precontracted with U46619 (n = 4–18). *, P < 0.05 vs. control.

ZG cells release a transferable relaxing factor(s)

To determine whether ZG cell-dependent relaxation involves a transferable factor, ZG cells (2 × 10⁶ cells/ml) were incubated for 5–10 min in PSS at 37 C. The cells were removed

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**Fig. 1.** ZG cell-dependent relaxations of adrenal arteries. A, Tracings, ZG cell-induced relaxations in a control artery (top) and an artery pretreated with iberiotoxin (bottom). ZG cell-induced relaxations were not altered by L-NA (30 μM), indomethacin (10 μM), and endothelium removal but were blocked by iberiotoxin (IbTX, 100 nM), and the EET antagonist 14,15-EEZE (2 μM). B, Tracings, effect of ACTH in an artery without ZG cells (top) and an artery with ZG cells (bottom). ACTH-induced relaxations in the presence of ZG cells were inhibited by iberiotoxin (100 nM) and high [K+]o (60 mM) but not affected by L-NA plus indomethacin or endothelium removal. Arteries were precontracted with U46619 (n = 4–18). *, P < 0.05 vs. control.

**Fig. 2.** Role of arachidonic acid metabolites in ZG cell-dependent relaxations to ACTH. A, ACTH-induced relaxations after the addition of ZG cells were inhibited by 14,15-EEZE (2 μM), and the cytochrome P450 inhibitors, SKF 525A (10 μM) and miconazole (10 μM). B, 14,15-EET-induced relaxations were inhibited by 14,15-EEZE (2 μM), and blocked by high [K+]o (60 mM), and iberiotoxin (IbTX, 100 nM). C, Arachidonic acid-induced relaxations were inhibited by 14,15-EEZE and blocked by iberiotoxin or endothelium removal. Arteries were precontracted with U46619 (n = 4–18). *, P < 0.05 vs. control.
by centrifugation and cell conditioned medium tested for activity on U46619-preconstricted arteries. ZG-conditioned medium relieved relaxed adrenal arteries in a concentration-dependent manner (maximal relaxation of 66 ± 4% at 100% medium; Fig. 3). Relaxations to ZG-conditioned medium were blocked by iberiotoxin and 14,15-EEZE.

The ZG conditioned medium was extracted with ethyl acetate. This extract also relaxed the adrenal arteries (data not shown), indicating that the relaxing factor is extractable by ethyl acetate. Because ZG cells release steroids that can be extracted by ethyl acetate, we tested several adrenal steroids for vascular activity. Aldosterone and dexamethasone were without effect on U46619-contracted arteries (Fig. 4). Progesterone relaxed the arteries at 10⁻⁵ and 10⁻⁴ M; however, the relaxations were not blocked by high [K⁺].

Metabolism of arachidonic acid by ZG cells

To provide further evidence that EETs mediate ZG cell-dependent relaxation, isolated bovine ZG cells were incubated with [14C]-arachidonic acid and metabolites were extracted and resolved by reverse-phase HPLC. The major metabolites (fraction 110–120) comigrated with the EETs (Fig. 5A). Other metabolites comigrated with the prostaglandins (fractions 20–50), and dihydroxyeicosatrienoic acids (DHETs), hydration products of EETs. EET fractions were collected and further resolved by normal-phase HPLC and [14C]-peaks comigrating with 14,15-, 11,12-, 8,9-, and 5,6-EETs were detected (Fig. 5B). LC/MS analysis indicated a major M-1 ion of 319 m/z for EET (Fig. 5C) and 337 m/z for DHET (Fig. 5D). These mass spectra are consistent with EET and DHET structures. In another set of experiments, ZG cells (10⁶ cells/ml) were incubated in the absence and presence of ACTH for 10 min in PSS at 37°C and the cells removed by centrifugation. The supernatant was extracted and analyzed by LC/MS. Basal production of 14,15-, 11,12-, and 8,9-EET by ZG cells was 31 ± 7, 28 ± 8, and 21 ± 7 pg/ml, respectively. Treatment of the ZG cells with ACTH significantly (P < 0.05) increased the production of these EETs to 56 ± 6, 68 ± 12, and 39 ± 6 pg/ml, respectively (n = 9). EETs were not detected in media without ZG cells.

**Effect of ACTH on small adrenal arterial diameter in situ**

As a more physiological approach, an artery in an adrenal cortical slice was cannulated and perfused in situ (Fig. 6A). Diameters of adrenal arteries embedded in the ZG layer (100–300 μm) were measured by videomicroscopy. Serotonin (50–200 nM) was added to the perfusate to precontract the arteries. Further addition of ACTH (10⁻⁹ M) to the perfusate caused a significant dilation (55 ± 10%, Fig. 6B) and supplemental Fig. 1, published as supplemental data on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org). Dilations were similar across the length of the arterial tree. At the end of the experiment, India ink (3%) was infused into the cannulated artery to demonstrate the area of vascular perfusion. Perfused arteries and

**Fig. 3**. ZG conditioned medium-induced relaxations. A, Tracings, relaxations of a control artery (top), and an iberiotoxin-treated artery (bottom) to ZG-conditioned medium. B, ZG-conditioned medium-induced relaxations were blocked by iberiotoxin (IbTX, 100 nM) and 14,15-EEZE (2 μM). Experiments were performed on endothelium-intact arteries in the presence of L-NA (30 μM) and indomethacin (10 μM). Arteries were precontracted with U46619 (n = 4–18). *, P < 0.05 vs. control.

**Fig. 4**. Effect of adrenal steroids on vascular tone of adrenal arteries. A, In U46619-preconstricted arteries, aldosterone and dexamethasone had no vasomotor activity. B, Progesterone relaxed U46619-contracted arteries at high concentrations. These relaxations were not altered by high [K⁺]o (60 mM) (n = 8–10).

**Fig. 5**. Arachidonic acid metabolism in ZG cells. A, ZG cells were incubated with [14C] arachidonic acid (AA; 0.05 mCi, 100 nM), and metabolites were extracted and resolved by reverse-phase HPLC. [14C]metabolites (fractions 110–120) comigrated with EETs. B, EET fractions were collected and further resolved by normal-phase HPLC. [14C]metabolites comigrated with 14,15-, 11,12-, 8,9-, and 5,6-EETs. Migration times of known standards are noted on the chromatogram. C and D, LC/MS analysis confirmed the molecular identity of 14,15-EET and 14,15-DHET. 14,15-EET and 14,15-DHET produced major ions of 319 m/z and 337 m/z (M-1), respectively.
and contracted with serotonin. ACTH (10 nM) was added to the per-
terities embedded in adrenal cortical slices were cannulated, perfused
and constricted with serotonin. ACTH failed
arteries dissected and freed of adherent tissue were cannu-
fused cortical arteries and ZG cells. In addition, adrenal
dicating the close anatomical association between the per-
the ZG cell layer surrounding the arteries were stained, in-
activating BKCa channels to cause relaxation. IbTX, Iberiotoxin.
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that include EETs, act in a paracrine manner. They are pro-
tacyclin, and endothelium-derived hyperpolarizing factors
arachidonic acid to a number of oxygenated metabolites such as prostaglandins,
hydroxyeicosatetraenoic acid, and EETs (34). In several blood
vessels including bovine small adrenal arteries, EETs are
vasoactive and mediate agonist-induced relaxation re-
sponses via the activation of smooth muscle BKCa channels (10, 24). A recent study by our laboratory of bovine coronary
arteries demonstrated that endothelium-derived EETs are
transferred to smooth muscle and cause dilations (35). Sim-
ilarly, we found that ZG cell-induced relaxations were trans-
ferable, and blocked by the BKCa channel blocker and the EET
antagonist. Using HPLC and LC/MS analysis, EETs were
identified in ZG cell-conditioned medium. Therefore, EETs
represent the transferable relaxing factor(s) released by ZG
cells.
Extracellular release of arachidonic acid provides for the
exchange of free arachidonic acid between vascular and other
cells, which could contribute to vascular tone regula-
For example, platelet microparticles transfer arachi-
donic acid to vascular endothelial cells, resulting in increased
production of the vasodilator prostacyclin (36). Another re-
cent study showed that arachidonic acid released from astro-
cytes diffuses to adjacent vascular smooth muscle cells, is
converted to 20-hydroxyeicosatetraenoic acid, and induces
cerebrovascular constrictions (37). However, the exchange
of arachidonic acid does not mediate ZG cell-induced relax-
ations of adrenal arteries because arachidonic acid did not
relax endothelium-denuded adrenal arteries, smooth muscle
cells do not synthesize EETs (data not shown), and ZG cells
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oids such as aldosterone, cortisol, and progesterone are
transferable and vasoactive in some vascular beds (38–40).
In adrenal arteries, aldosterone and cortisol were not active.
Progesterone relaxed adrenal arteries, but the relaxation was
not inhibited by the BKCa channel blocker, iberiotoxin, or
high [K+]. Therefore, these steroids could not mediate the
ZG cell-induced relaxations.

The pituitary hormone ACTH is a primary regulator of
adrenal cortical function. ACTH stimulates cortisol and al-
dosterone secretion and increases adrenal blood flow in vivo
and in perfused adrenal glands (6, 11–14). In conscious
calves, ACTH stimulated the secretion of cortisol and in-
creased adrenal blood flow in the same range of concentra-
tions (41). However, the threshold concentration of ACTH
that increased adrenal blood flow was higher than the thresh-
rectly acts on vascular smooth muscle cells to cause relax-
atation (Fig. 6C). The relaxing factor(s) is not NO and prosta-
cyclin because ZG cell-induced relaxations were not altered by
L-NA and indomethacin. In addition, the short half-life of
NO and prostacyclin in solution excludes their role as trans-
ferable factors under our experimental conditions. The lack
of NO involvement is also consistent with a previous report
of our laboratory that bovine ZG cells do not express NO
synthase (NOS) isoforms by the use of Western blot or en-
zymatic activity assay (31). The expression of NOS has been
reported in adrenal cells of other species. For example, both
neuronal and endothelial NOS mRNA and immunoreactiv-
ity have been detected in rat adrenal zona fasciculata cells
and endothelial NOS expression in rat and human ZG cells
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old concentration that increased cortisol secretion. ACTH increased adrenal cortical blood flow without changing adrenal medullary blood flow (14). In the perfused rat adrenal gland, ACTH increased perfusate flow and corticosterone secretion in similar concentrations (42). ACTH also increased adrenal blood flow in anesthetized rats (43). In contrast, the effect of ACTH is variable in some other species such as the dog. ACTH was either without effect or required high concentrations to increase adrenal blood flow (13, 14, 44, 45). The reason for this variability between species is not apparent.

In the ovine fetus, endogenous ACTH regulates adrenal blood flow. Hypoxia in the fetus increased the plasma concentrations of ACTH and cortisol and increased adrenal cortical blood flow (46, 47). Cortisol was infused during hypoxia to inhibit the pituitary release of ACTH. Cortisol blocked the increase in plasma ACTH concentration and inhibited the increase in adrenal cortical blood flow. These studies suggest that ACTH mediates the increase in adrenal cortical blood flow caused by fetal hypoxia.

Whereas ACTH dilates adrenal arteries and increases adrenal blood flow in vivo, it had no effect on isolated adrenal arteries under basal conditions (15). ACTH also did not relax preconstricted adrenal arteries, indicating that ACTH has no direct vasomotor effect on these arteries. The action of ACTH on its target tissues or cells depends on the activation of specific receptors. Cloning of the melanocortin receptor family has identified five distinct G protein- and adenylate cyclase-coupled receptors that have a wide distribution and diverse functions (48). The melanocortin-2 receptor preferentially binds ACTH. It is mainly expressed in the ZG and zona fasciculata of the adrenal cortex and is hence considered to be the main ACTH receptor (49, 50). The expression of the ACTH receptor is limited in extraadrenal tissues, although Northern blot analysis has demonstrated its presence in rodent adipocytes, human skin, and human aortic and umbilical vein endothelial cells (51, 52). To our knowledge, the ACTH receptor has not been identified in adrenal vascular cells. The lack of a direct vasomotor effect of ACTH on adrenal arteries is consistent with these findings.

ACTH-induced relaxations of adrenal arteries were revealed only with the presence of ZG cells, as indicated by our in vitro isolated arteries and in situ perfused adrenal slices. Similar to relaxations induced by ZG cells or ZG-conditioned medium, relaxations were not affected by endothelium removal, L-NA, or indomethacin but were inhibited by high concentrations to increase adrenal blood flow (13, 14, 44, 45). The reason for this variability between species is not apparent.

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ACTH-induced relaxations of adrenal arteries were revealed only with the presence of ZG cells, as indicated by our in vitro isolated arteries and in situ perfused adrenal slices. Similar to relaxations induced by ZG cells or ZG-conditioned medium, relaxations were not affected by endothelium removal, L-NA, or indomethacin but were inhibited by high K+ , iberiotoxin, cytochrome P450 inhibitors, and an EET antagonist. LC/MS analysis showed that ACTH stimulated the release of EETs from adrenal ZG cells. Together, these results provide strong evidence that ACTH stimulates the release of a relaxing factor(s) (probably EET) from ZG cells, which causes relaxations of adrenal arteries. Interestingly, this indirect effect of ACTH in the adrenal gland is not limited to the regulation of adrenal vascular tone. A recent study suggested that ACTH regulates vascular endothelial-cadherin transcription in mouse adrenal endothelial secondary to its indirect effect on steroidogenic cells (53). Similarly, ACTH stimulates expression and secretion of vascular endothelial growth factor from adrenocortical cells, which is responsible for the remodeling of adrenal vasculature and adrenal cortex mass (54).

Hinson et al. (11, 12) previously reported in perfused rat adrenal glands that stimulation of histamine and serotonin release with compound 48/80, a mast cell degranulator, mimics the ACTH-induced increase in adrenal perfusate flow and steroidogenesis. Additionally, sodium cromoglycate, a mast cell stabilizer, blocked ACTH-induced increase in perfusate flow (2, 11). Thus, the release of histamine and serotonin from mast cells may mediate ACTH-induced increase in adrenal blood flow. We recently reported that compound 48/80 relaxes isolated bovine small adrenal arteries, independent of histamine activation (16).

In the adrenal cortex, there are two cellular sources of the EETs, i.e., endothelial cells and ZG cells. We recently reported that acetylcholine and endothelin B receptor agonists cause relaxation of isolated small adrenal arteries that are partially mediated by the activation of the endothelial EET pathway (10, 15). In the present study, ACTH activates the ZG cell EET pathway. These results indicate that activation of the specific EET pathway may depend on the specific agonist involved.

Blood flow to the adrenal cortex delivers stimulants (i.e., ACTH and angiotensin) and substrates (i.e., oxygen and cholesterol) to steroidogenic cells and exports secretory products (i.e., cortisol and aldosterone) to the systemic circulation. Thus, it would be advantageous to tightly regulate adrenal blood flow to steroid hormone synthesis (1, 2). Our results on the regulation of vascular tone by adrenal ZG cells provide a functional link and mechanism for this close association of adrenal blood flow and hormone synthesis. The regulation of adrenal vascular tone by adrenal ZG cell EET production may have broader implications. This may represent a general mechanism for regulating vascular tone in other endocrine glands, in which blood flow is matched with hormone synthesis and secretion. EET synthesis has been documented in the pancreas, hypothalamus, pituitary gland, ovary, and placenta (55–59). Therefore, EET production by glandular tissue may represent a paracrine mechanism of blood flow regulation.

In summary, our data reveal a previously unknown mechanism by which ACTH increases blood flow in the adrenal gland. Specifically, ACTH acts on adrenal ZG cells to stimulate the release of EETs. EETs diffuse to adjacent adrenal arteries, activate vascular smooth muscle BKCa channels, and cause relaxation (Fig. 6C). ACTH-induced increase in adrenal blood flow is explained by this indirect effect on adrenal ZG cells. This functional interaction of adrenal steroidogenic cells and vascular cells may contribute to the close coupling of blood flow and hormone synthesis in the adrenal gland.

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