Extra-adrenal glucocorticoids and mineralocorticoids: evidence for local synthesis, regulation, and function

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Departments of 1Psychology and 2Zoology and 3Graduate Program in Neuroscience, University of British Columbia, Vancouver, British Columbia, Canada; 4G. V. (Sonny) Montgomery Veterans Affairs Medical Center, and 5Department of Medicine, University of Mississippi Medical Center, Jackson, Mississippi

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Taves MD, Gomez-Sanchez CE, Soma KK. Extra-adrenal glucocorticoids and mineralocorticoids: evidence for local synthesis, regulation, and function. Am J Physiol Endocrinol Metab 301: E11–E24, 2011. First published May 3, 2011; doi:10.1152/ajpendo.00100.2011.—Glucocorticoids and mineralocorticoids are steroid hormones classically thought to be secreted exclusively by the adrenal glands. However, recent evidence has shown that corticosteroids can also be locally synthesized in various other tissues, including primary lymphoid organs, intestine, skin, brain, and possibly heart. Evidence for local synthesis includes detection of steroidogenic enzymes and high local corticosteroid levels, even after adrenalectomy. Local synthesis creates high corticosteroid concentrations in extra-adrenal organs, sometimes much higher than circulating concentrations. Interestingly, local corticosteroid synthesis can be regulated via locally expressed mediators of the hypothalamic-pituitary-adrenal (HPA) axis or renin-angiotensin system (RAS). In some tissues (e.g., skin), these local control pathways might form miniature analogs of the pathways that regulate adrenal corticosteroid production. Locally synthesized glucocorticoids regulate activation of immune cells, while locally synthesized mineralocorticoids regulate blood volume and pressure. The physiological importance of extra-adrenal glucocorticoids and mineralocorticoids has been shown, because inhibition of local synthesis has major effects even in adrenal-intact subjects. In sum, while adrenal secretion of glucocorticoids and mineralocorticoids into the blood coordinates multiple organ systems, local synthesis of corticosteroids results in high spatial specificity of steroid action. Taken together, studies of these five major organ systems challenge the conventional understanding of corticosteroid biosynthesis and function.

CORTICOSTEROIDS ARE STEROID HORMONES produced in the adrenal cortex and are of two types, glucocorticoids and mineralocorticoids. Glucocorticoids, such as cortisol and cortisone, have numerous effects and can act on nearly all cells in the body. For example, glucocorticoids regulate metabolic activity, immune function, and behavior (84). Circulating glucocorticoid levels increase in response to a variety of stressors under control of the hypothalamic-pituitary-adrenal (HPA) axis. Hypothalamic release of corticotropin-releasing hormone (CRH) triggers pituitary release of adrenocorticotropic hormone (ACTH), which stimulates glucocorticoid production by the zona fasciculata of the adrenals. The adrenals can secrete cortisol, corticosterone, or both, depending on the species.

Mineralocorticoids, such as aldosterone, promote sodium reabsorption in transporting epithelia of the kidneys, salivary glands, and large intestine. Sodium reabsorption is followed by passive reabsorption of water. Circulating aldosterone concentrations rise in response to low blood volume or sodium depletion under control of the renin-angiotensin system (RAS). The kidneys release renin, which converts angiotensinogen to angiotensin I. Angiotensin I is then cleaved by angiotensin-converting enzyme (ACE) to active angiotensin II. Angiotensin II stimulates mineralocorticoid production by the zona glomerulosa of the adrenals.

The classical corticosteroid biosynthetic pathway is shown in Fig. 1. Traditionally, it was thought that glucocorticoids and mineralocorticoids were synthesized solely in the adrenal cortex, and research has been focused overwhelmingly on measuring circulating levels of these steroids in plasma or serum. However, a growing body of evidence has demonstrated de novo synthesis of glucocorticoids and mineralocorticoids in other organs, such as primary lymphoid organs, intestine, skin, brain, and possibly the heart and vasculature (15, 87). Here, we review evidence of local de novo corticosteroid production, regulation of local corticosteroid production, and the potential functions of locally produced corticosteroids.

PRIMARY LYMPHOID ORGANS

Primary lymphoid organs are the sites of T and B cell (lymphocyte) development. In mammals, both cell lineages originate from the same early precursors in the bone marrow.
Evidence for Local Synthesis

Steroidogenic enzymes. The first demonstration of thymic steroid production was in the mouse, and using fetal thymic organ culture, Vacchio et al. (110) demonstrated conversion of a cholesterol analog into pregnenolone and 11-deoxycorticosterone. Steroidogenic capability was high in thymic epithelial cells and low in thymocytes (110). Murine thymic epithelial cells have since been shown to have mRNA, protein, and activities of the enzymes required for de novo glucocorticoid synthesis (47, 47, 69, 75, 110) (Fig. 1 and Table 1). The absence of CYP17 activity results in the formation of corticosterone (47), which is also the major adrenal glucocorticoid in mice. Thymic epithelial cells also activate GR-mediated transcription in cocultured cells (69). In addition to thymic epithelial cells, thymocytes themselves also express StAR, CYP11A1, 3β-HSD, CYP17, CYP21, and CYP11B1 mRNA and synthesize corticosterone (10, 75, 76). Moreover, the chicken thymus contains functional CYP11A1, 3β-HSD, CYP21, and CYP11B1 enzymes for glucocorticoid synthesis, but the additional presence of CYP17 activity directs synthesis toward cortisol rather than corticosterone, in contrast to the chicken adrenals (46). All the enzymes found in the chicken thymus are also present in the bursa of Fabricius (hereafter bursa) (46). The bursa is the site of avian B cell development, analogous to mammalian bone marrow. Together, these studies of corticosteroidogenic enzymes demonstrate that the murine and avian thymus, and also the avian bursa, contain the machinery for de novo glucocorticoid synthesis.

Local steroid levels. Endogenous glucocorticoid levels have not been measured in rodent thymus but have been measured in the avian thymus and bursa (Fig. 2 and Table 1). The major circulating glucocorticoid in birds is cortisol, but in zebra finch (Taeniopygia guttata) thymus and bursa, cortisol levels are higher than corticosterone levels. Also, cortisol (but not corticosterone) levels are higher in lymphoid tissue than in plasma, which provides evidence that cortisol is a locally synthesized “immunosteroid” (85, 88). High local levels could also result from accumulation of circulating glucocorticoids, but the local presence of cortisol-synthetic machinery and the low circulating levels of cortisol suggest local origin. Local synthesis of cortisol, rather than corticosterone, offers an opportunity to parse the regulation and functions of lymphoid glucocorticoids from those of adrenal glucocorticoids. Furthermore, glucocorticoid production in the avian bursa raises the possibility that similar production occurs in the mammalian bone marrow. The very high lymphoid cortisol concentrations
Table 1. Evidence for and against local synthesis of glucocorticoids and mineralocorticoids

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<th>Skin</th>
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Local steroid levels

| Glucocorticoids | Tissue > plasma | 85, 88 | 14, 48, 88 | 30 |                |
|                | Present postadrenalectomy | 14 | 30 | 29 |
| Synthesis in vitro | 10, 69, 75, 76 | 3, 11, 13, 57, 58, 61, 62 | 38, 95, 97, 98 | 90, 91, 102, 103 | 64 |

Mineralocorticoids

| Tissue > plasma | 30 | 29 |
| Present postadrenalectomy | 29 |
| Synthesis in vitro | 95 | 26 |

Nos. are references. +, detected; −, not detected. Note: this table does not account for important factors such as species, age, sex, season, etc.
Glucocorticoid levels. Proopiomelanocortin (POMC) mRNA is expressed in epithelial cells and thymocytes could help to maintain local glucocorticoid production by thymic epithelium. ACTH increases in vitro steroidogenesis by thymic epithelial cells, and ACTH decreases steroidogenic enzyme expression (7). The divergent effects of ACTH on glucocorticoid production by thymic epithelial cells and thymocytes help to maintain local glucocorticoid levels. Proopiomelanocortin (POMC) mRNA is present in rat thymus (7), and ACTH immunoreactivity has been detected in rat, bird, and human thymi (4, 65). CRH mRNA is also present in rat thymus (2, 79), and CRH immunoreactivity has been detected in rat and bird thymi (66). Thus, an exciting possibility is that a local analog of the HPA axis regulates local glucocorticoid production in the thymus. In bone marrow and bursa, less is known about regulatory factors. The avian bursa contains immunoreactive ACTH (22), but CRH has not been reported. The bursa also contains bursal antisteroidogenic peptide (BASP), which suppresses adrenal glucocorticoid production in vitro (7).

Function

Locally produced glucocorticoids have major effects on thymocyte development (Fig. 3A and Table 3). In general, thymocyte selection is thought to be driven largely by TCR affinity for antigen presented by self MHC molecules. Low affinity (weak or absent TCR signal) results in death; intermediate affinity (moderate signal) results in positive selection and survival; and high affinity (strong signal) results in negative selection and death. The discovery of thymic glucocorticoid synthesis suggests an alternative model ("mutual antagonism"), in which TCR signaling induces apoptotic signals in thymocytes with intermediate- or high-affinity TCR, and glucocorticoids antagonize these proapoptotic signals to allow survival of thymocytes with intermediate-affinity TCR (110). The mutual antagonism model is supported by in vitro observations that TCR signaling decreases glucocorticoid-induced thymocyte apoptosis (39) and that endogenous thymic glucocorticoids decrease TCR-dependent thymocyte apoptosis (109, 110). Furthermore, in thymi of fetal mice, thymocyte-specific underexpression of the GR (via antisense transgene) reduces thymocyte numbers, suggesting that GR signaling promotes survival (44).

The effects of glucocorticoids on thymocytes, however, change with age. At puberty, GR underexpression (as above) increases thymocyte numbers, whereas overexpression decreases thymocyte numbers (70). Thus, at puberty, endogenous glucocorticoids promote thymocyte apoptosis rather than survival. GR overexpression also delays thymus growth and involution (68). The necessity of GR function for thymocyte development has also been tested in knockout mice. Mutants with partial (74) or complete (6) abrogation of GR function retain functional thymocyte maturation. GR signaling is therefore not necessary for thymocyte maturation. Nonetheless, in intact adult mice, inhibition of thymic glucocorticoid production by low-dose metyrapone treatment (which did not significantly affect plasma glucocorticoid levels in this study) increased thymocyte numbers, showing that locally produced glucocorticoids have physiological effects even in the presence of functional adrenal glands (76).

In sum, GR signaling promotes thymocyte survival in fetal thymus, promotes apoptosis in postnatal (pubertal) thymus, but is not critical for thymocyte maturation. How glucocorticoids interact with TCR signaling is unclear, but this might involve membrane-associated receptors rather than cytosolic receptors. The unbound GR has been shown to associate at the cell membrane with TCR kinases and facilitate TCR signaling, while glucocorticoid binding causes dissociation of this complex and inhibition of TCR signaling (49, 50).
Far less work has been done on locally synthesized glucocorticoids and B cell development. Systemic glucocorticoid treatment depletes B lineage cells in murine bone marrow (24) and decreases bursa size in the chicken (60). In the zebra finch, lymphoid cortisol may act on different receptors than adrenal corticosterone, because cortisol (but not corticosterone) shows specific binding to bursa membranes (86). The accessibility of the avian bursa and the ability to remove it intact make this a

Fig. 3. Regulation and functions of local glucocorticoid synthesis. A: in the thymus, glucocorticoids and T cell receptor (TCR) signaling each function independently as proapoptotic stimuli in developing thymocytes. Glucocorticoids also antagonize TCR signaling and alter the threshold of TCR affinity for self peptide:MHC that results in survival and proliferation vs. apoptosis. B: in the intestine, activated macrophages and Th1 cells secrete the proinflammatory cytokine TNFα, which upregulates the transcription factor LRH-1 in epithelial cells of the intestinal crypt. LRH-1 induces local production of glucocorticoids, which downregulate immune cell activation and resulting inflammation. C: activation of keratinocytes (and other types of skin cells) results in production of CRH, which stimulates production of ACTH and proinflammatory cytokines such as IL-1β and TNFα. ACTH then stimulates local synthesis of glucocorticoids, which inhibit further production of CRH and proinflammatory cytokines, thus downregulating inflammation.

ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; GCs, glucocorticoids; IFNγ, interferon-γ; IGF-1, insulin-like growth factor 1; IL, interleukin; LRH-1, liver receptor homolog-1; MHC, major histocompatibility complex; Th1, type I helper T cell; TNFα, tumor necrosis factor-α.
useful model for investigating the effects of local glucocorticoid synthesis in B cell development.

**INTESTINE**

The intestine is a critical barrier between the internal environment (within the organism) and external environment (the lumen; outside the organism). The intestinal mucosa contains the largest number of immune cells in the body and protects the epithelial surface from pathogens as well as commensal bacteria. Intestinal immune cells are concentrated in distinct lymphoid tissues (Peyer’s patches, mesenteric lymph nodes, and appendix) and are also present as individual cells throughout the epithelium (40). Tight regulation of immune activation is necessary to maintain intestinal homeostasis.

**Evidence for Local Synthesis**

**Steroidogenic enzymes.** The de novo steroidogenic capacity of the gut was first suggested in a study using in situ hybridization, which detected CYP11A1 and 3β-HSD mRNA in the mouse gut during embryonic development (42) (Table 1). Subsequent studies have demonstrated expression of mRNA and protein for several glucocorticoid-synthetic enzymes in murine small intestine and colon (11, 13). CYP11A1 mRNA is highest in the proximal small intestine, intermediate in the middle and distal small intestine, and lowest in the colon (11). The mRNAs for steroidogenic enzymes are restricted to the proliferating epithelial cells of the crypts (3, 11), and differentiation of immature intestinal epithelial cells into mature non-proliferating cells results in a decrease of steroidogenic enzyme expression (3). Also, CYP11A1 and CYP11B1 mRNAs are detectable in a murine intestinal epithelial cell line (57). Murine small intestine contains CYP11A1 protein, and the activity of this and other steroidogenic enzymes is demonstrated by ex vivo synthesis of corticosterone (11, 13). Corticosterone synthesis is blocked by metyrapone (11, 13). In humans, CYP11A1 and CYP11B2 mRNAs are present in colon biopsies.

Table 3. **Possible functions of locally synthesized glucocorticoids and mineralocorticoids**

<table>
<thead>
<tr>
<th>Lymphoid</th>
<th>Intestine</th>
<th>Skin</th>
<th>Brain</th>
<th>Cardiovascular</th>
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<td>Glucocorticoids</td>
<td>Glucocorticoids</td>
<td>Glucocorticoids</td>
<td>Mineralocorticoids</td>
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<tr>
<td>Antagonism of T cell receptor signal</td>
<td>Downregulate TH1 immune response and inflammation</td>
<td>Local stress response to different environmental stimuli</td>
<td>Regulation of inflammatory response</td>
<td>Collagen deposition</td>
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<td>Promote thymocyte apoptosis</td>
<td>Allow tissue repair</td>
<td>Regulation of inflammatory response</td>
<td>Drug-seeking behavior</td>
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<td>B cell development?</td>
<td>Prevent induction of autoimmune response to commensal gut bacteria?</td>
<td>Regulation of skin immune response?</td>
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(13), suggesting that the human intestine, like that of the mouse, is capable of glucocorticoid synthesis.

Local steroid levels. To our knowledge, local corticosteroid levels within the intestinal epithelium have not been measured in vivo.

Regulation

In the murine intestine, expression of specific steroidogenic enzymes increases in response to immune activation and inflammation (Fig. 3B). In vivo treatment of mice with anti-CD3 (to activate T cells) profoundly increases ex vivo corticosterone production by the small intestine (11) (Table 2). Mouse small intestine and colon constitutively express mRNA of most enzymes required for corticosterone synthesis (11, 13), but CYP11A1 and CYP11B1 expression reach high levels only after T cell activation (11) or inflammation (61, 62). The inflammation-induced increases in enzyme expression and corticosterone synthesis require the secretion of tumor necrosis factor-α (TNFα), a proinflammatory cytokine (61, 62). Immune activation, possibly via TNFα induction of NF-κB (nuclear factor-κ light-chain enhancer of activated B cells, a proinflammatory transcription factor) (61), increases intestinal expression of the transcription factor liver receptor homolog-1 (LRH-1) (58). LRH-1 is closely related to steroidogenic factor-1 (SF-1), which regulates the expression of most steroidogenic enzymes in the adrenal (57). LRH-1 is important in cell cycle progression, and LRH-1 expression in intestine is limited to proliferating crypt cells (3). In a murine intestinal epithelial cell line, overexpression of LRH-1 increases CYP11A1 and CYP11B1 mRNA levels and corticosterone production (58). In vivo, LRH-1 haploinsufficiency prevents T cell activation-induced upregulation of CYP11A1 mRNA and corticosterone production in small intestine and attenuates upregulation of CYP11A1 and CYP11B1 mRNA levels in large intestine (58). The requirement for both TNFα and LRH-1 suggests that during immune activation TNFα might (in addition to its proinflammatory effects) induce LRH-1 and subsequently increase glucocorticoid production and epithelial cell proliferation (63).

The expression of steroidogenic enzymes is differentially regulated in adrenal and intestinal cells. For example, cAMP increases CYP11A1 and CYP11B1 mRNA levels in an adrenal cell line but has the opposite effects in an intestinal epithelial cell line (57). Also, cAMP increases adrenal corticosterone release but decreases intestinal corticosterone release in culture. Similarly, ACTH administration increases adrenal-derived serum corticosterone levels but has no effect on intestine corticosterone production (57). Local production of ACTH in the intestine is unlikely, as POMC mRNA is undetectable in the gut (21). In contrast to the effects of ACTH and cAMP, protein kinase activation has no effect on enzyme transcription in adrenal cells but increases CYP11B1 transcription in intestinal epithelial cells (57). These findings show that regulatory signals have differing or opposite effects on adrenal and intestinal glucocorticoid production.

Function

Stimulation of T cells or macrophages results in secretion of TNFα and inflammation-mediated damage to the intestinal epithelium (Fig. 3B). TNFα also induces expression of LRH-1, which results in increased glucocorticoid production by and increased proliferation of epithelial cells (61, 62). Together, these processes may downregulate the inflammatory immune response and mediate repair of inflammatory damage (Table 3). Consistent with this hypothesis, intestinal glucocorticoids decrease damage resulting from inflammatory bowel disease in both mice and humans, and glucocorticoid-synthetic enzyme expression in the colon is decreased in patients with Crohn’s disease and inflammatory bowel disease (13). Interestingly, endogenous glucocorticoid synthesis may regulate only cell-mediated (T₃₁,1-polarized) and not humoral (T₃₂,2-polarized) immune responses, as the latter does not stimulate glucocorticoid production in mouse intestine (61). Thus, glucocorticoid synthesis by the intestine is specifically stimulated by activation of a cell-mediated T₃₁ immune response, and acts to suppress and repair the damaging effects of this response. A cell-mediated T₃₁ inflammatory response also seems likely to stimulate local glucocorticoid synthesis, but this possibility remains to be tested.

SKIN

The skin, like the intestine, provides a boundary between the internal and external environments and is critical as a physical and immunological barrier. The epidermis is the outermost skin layer, which consists of keratinocytes that are continuously produced. Underneath the epidermis is the dermis, which contains connective tissues, nerve endings, sweat glands, hair follicles, and sebaceous glands. Under the dermis is the subcutaneous layer, which is composed of adipose tissue. The skin is continuously exposed to solar, thermal, mechanical, and immune stressors and responds rapidly to varying stressors to maintain its physical and functional integrity. The discovery of CRH and ACTH expression in skin, along with the presence of steroid-metabolizing enzymes, suggested that the skin might also synthesize glucocorticoids (93).

Evidence for Local Synthesis

Steroidogenic enzymes. The de novo steroidogenic capacity of human skin was first demonstrated by the conversion of cholesterol into pregnenolone and by the expression of CYP11A1 mRNA and protein (106). Human skin expresses a functional homolog of StAR, StAR-related lipid transfer protein (MLN64, or STARD3) (99), and steroidogenic enzyme mRNA, protein, and activities needed for glucocorticoid synthesis have been shown (19, 81, 94, 112) (Table 1). Localization of steroidogenic enzyme mRNA and protein suggests glucocorticoid production in sebaceous cells (106), keratinocytes (112), fibroblasts (99) and potentially melanocytes (97). Cortisol appears to be the major corticosteroid product in human skin (98).

Mouse skin expresses CYP11A1, but further metabolism from pregnenolone into other steroids has not been reported (99). Glucocorticoid synthesis is likely to occur in rat skin, as most glucocorticoid-synthetic enzymes have been detected by measurements of mRNA (92) or activity (95), but CYP11A1 has not specifically been shown in rat skin.

Local steroid levels. To our knowledge, local corticosteroid levels within the skin have not been measured.
Regulation

Human skin expresses CRH and ACTH proteins (38, 93, 97, 105). Incubation of melanocytes with CRH increases ACTH and cAMP levels, and incubation with ACTH increases cortisol levels in conditioned media (97) (Fig. 3C and Table 2). Remarkably, these data suggest that melanocytes may contain a miniature “analog” of the HPA axis. A similar local HPA axis also regulates glucocorticoid production in human hair follicles and fibroblasts (38, 96), complete with negative feedback of cortisol on CRH expression (38).

Mouse skin also contains CRH protein (82). The absence of CRH mRNA in mouse skin suggests that this CRH protein originates elsewhere (82). CRH levels in murine skin correspond with hair growth: CRH levels are high during the growth phase and low during the regression and resting phases (82).

Glucocorticoid synthesis in skin is induced by inflammation. Insults such as tissue damage, UV radiation, or pathogens result in local production of CRH (93, 94), which promotes inflammation. CRH-induced proinflammatory cytokines IL-1β and TNFα then increase ACTH and glucocorticoid production in the skin (105, 112).

Function

Glucocorticoid synthesis in the skin functions as a rapid and localized stress-response system (93) (Table 3). It is well known that topical treatment with high doses of glucocorticoids is anti-inflammatory and immunosuppressive. Locally produced glucocorticoids play a similar role, inhibiting production of proinflammatory signal molecules such as CRH and IL-1β (38, 112) (Fig. 3C). In contrast, adrenal glucocorticoids have rapid, short-term enhancing effects on cutaneous immunity by stimulating immune cell migration from circulating blood into skin tissues (16). Localized synthesis of glucocorticoids, by downregulating production of CRH and inflammatory cytokines, aids in preventing subsequent overshoot of the inflammatory immune response and further tissue damage. The physiological functions of skin-derived glucocorticoids, especially in vivo, require further study.

CENTRAL NERVOUS SYSTEM

Steroids play numerous important roles in the development and function of the central nervous system. Robel and Baulieu (80) first observed that steroids (e.g., pregnenolone, dehydroepiandrosterone) were present in the rat brain at high concentrations long after castration and adrenalectomy. Since then, these brain-derived steroids (“neurosteroids”) have been widely studied, but with an overwhelming focus on progestins, androgens, and estrogens (17). As the synthesis of sex steroids in the central nervous system has been extensively reviewed (12, 17, 78, 108), we focus here on the final steps of glucocorticoid or mineralocorticoid production.

Evidence for Local Synthesis

Steroidogenic enzymes. The rat brain expresses the mRNAs of all the steroidogenic enzymes required for de novo synthesis of glucocorticoids and mineralocorticoids from cholesterol (26, 27, 45, 51, 54, 101) (Table 1). CYP11B1 and CYP11B2 proteins and activities have also been detected, and activity can be blocked by specific enzyme inhibitors (26, 51). The mouse brain contains mRNAs for most corticosteroid-synthetic enzymes, but mRNA levels for CYP11B1 and CYP11B2 are minimal (101). In the human brain, several regions also express enzymes for glucocorticoid and mineralocorticoid synthesis (5, 121). Interestingly, in both rat and human brain, CYP21 mRNA is very low or nondetectable (53, 101), but the same 21-hydroxylase function is performed by an alternate enzyme, CYP2D (CYP2D4 in the rat and CYP2D6 in the human) (45). This example highlights the possibility that corticosteroid synthesis in the brain (and other organs) can differ from corticosteroid synthesis in the adrenals.

Local steroid levels. In intact rats, corticosterone and aldosterone levels are lower in brain than in plasma, but in adrenalectomized rats, aldosterone (but not corticosterone) levels are higher in brain than in plasma (30). These data suggest that aldosterone is synthesized in the rat brain. In contrast, in intact mice, corticosterone levels are similar or lower in brain compared with plasma, but in adrenalectomized mice, corticosterone levels are higher in brain than in plasma (14). This suggests that corticosterone is synthesized in the mouse brain (aldosterone was not quantified). There is also evidence for glucocorticoid synthesis in the developing and adult songbird brain. In newly hatched zebra finches, cortisol levels are higher in caudal telencephalon than in plasma (88), and in adult song sparrows (Melospiza melodia), corticosterone levels can be higher in plasma from the jugular vein (exit ing the brain) than in plasma from the brachial vein (59).

Regulation

Expression of steroidogenic enzymes in the brain during early life is tightly controlled, as sex steroids are critical in neural development (43). Although glucocorticoids are also important in neural development, little is known regarding ontogenetic patterns of corticosteroid synthesis in brain. In the zebra finch, brain glucocorticoid levels during development are low except in the caudal telencephalon immediately after hatching (88). Glucocorticoids have potentially harmful effects during neural development, and altricial species such as finches, rodents, and humans may avoid such effects by maintaining low brain and circulating glucocorticoid levels.

In adult rats and mice, brain corticosterone production is triggered by an acute injection of alcohol (14) or by withdrawal from chronic alcohol consumption (48) (Table 2). Alcohol withdrawal causes dramatic and region-specific increases in brain corticosterone levels, with no change in plasma corticosterone levels. In some cases, corticosterone concentrations are much higher in brain than in plasma (48). Similar effects are seen with a social stressor, social defeat (14). The effects of ethanol and social defeat could be mediated by ACTH, which increases brain CYP11B1 mRNA (119).

In the adult song sparrow, data suggest that acute restraint stimulates brain corticosterone synthesis and secretion during the molt, a life history stage in which corticosterone production by the adrenals is dramatically reduced (to allow feather growth) (59). In the zebra finch, saline perfusion was used to remove blood contamination from the brain prior to measurement of brain steroid levels. Surprisingly, saline perfusion caused a rapid and region-specific increase in corticosterone levels in the brain, suggesting that hypoxia or ischemia could stimulate brain glucocorticoid synthesis (104).
In adult rats, the regulation of brain mineralocorticoid synthesis is well studied. Aldosterone synthesis in the rat brain is regulated by sodium intake. Low sodium intake increases expression of CYP11B2 (but not CYP11B1) mRNA in brain and in adrenals (118). However, high sodium intake or systemic angiotensin II administration does not affect CYP11B2 expression in brain (118). The lack of a response to systemic angiotensin II could be due to its limited crossing of the blood-brain barrier. Interestingly, the brain contains all the components of the RAS, including production of angiotensin II (52). These components could thus function as a miniature analog of the classical RAS.

Function

Systemic glucocorticoids influence behavior and neurophysiology (84). Locally produced brain glucocorticoids could have similar, and possibly additive, effects. In birds, during the molt, when adrenal glucocorticoid production is low, local synthesis in the brain during capture and restraint might facilitate escape behavior or learning (e.g., to avoid capture in the future). Alterations in behavior are also important after social defeat (e.g., to prevent further physical aggression) (Table 3). In contrast, chronic elevation of brain glucocorticoid levels may contribute to cognitive and memory deficits that can result from alcohol withdrawal (48).

Aldosterone acts on mineralocorticoid receptors in the brain to regulate blood pressure and salt consumption (28, 83). Central production of aldosterone is involved in the development of hypertension in the Dahl salt-sensitive rat model, in which hypothalamic aldosterone synthesis is increased relative to a control rat strain (31, 37). Brain-derived aldosterone is critical in driving sodium-induced hypertension. Infusion of a 3β-HSD inhibitor into the lateral ventricle prevents development of systemic hypertension in the adrenal-intact Dahl salt-sensitive rat (32). In addition, infusion of a CYP11B2 inhibitor into the lateral ventricle of adrenal-intact rats dramatically decreases blood pressure induced by salt consumption (31, 36). This is not due to an effect on adrenal aldosterone synthesis, as no decrease in blood pressure is seen with systemic administration of the same dose (Fig. 4). The effect of CYP11B2 inhibition is also reversible, as replacement with control vehicle results in blood pressure elevation (31). These studies demonstrate that, even in the presence of adrenal aldosterone synthesis, brain-derived aldosterone is critical for the regulation of blood pressure and the development of hypertension.

Even low levels of corticosteroid synthesis in the brain could have physiological significance, as the blood-brain barrier excludes several corticosteroids. The uptake of cortisol and aldosterone from the systemic circulation into the brain is especially low due to active removal by the transporter mdr1 (p-glycoprotein) (25).

CARDIOVASCULAR SYSTEM

Aldosterone plays an important role in the physiopathology of congestive heart failure, which prompted researchers to examine local synthesis of aldosterone in the heart and vasculature. The possibility of cardiovascular synthesis of aldosterone took on added importance after it was found that mineralocorticoid receptor blockade had beneficial effects in heart failure patients, even when plasma aldosterone levels were normal or low (71).

Evidence for Local Synthesis

Steroidogenic enzymes. Cardiovascular corticosteroid production was first investigated in human blood vessels and later in the heart itself. In human vascular cells or tissue, local synthesis was suggested by PCR detection of CYP11A1, 3β-HSD, and CYP21 mRNA (35, 41, 120) (Table 1). However, reports are divided on whether CYP11B1 mRNA is detectable (41) or not (35, 120) and whether CYP11B2 mRNA is detectable (34, 35, 41) or not (1, 120). Similarly, in the adult human heart, steroidogenic enzyme mRNAs have been detected (9, 41, 120). Reports are again divided on whether CYP11B1 mRNA is present, but CYP11B2 mRNA is not detectable (9, 41, 120) except in subjects with heart failure (120).

In rats, CYP11A1, CYP11B1, and CYP11B2 mRNAs have been detected in blood vessels (102, 103), and StAR, CYP11B1, and CYP11B2 have been detected in the heart (8, 90). However, subsequent studies found expression of CYP11B1 and CYP11B2 mRNA to be extremely low or nondetectable in the heart of various rat strains (29, 64, 117). Ex vivo perfused rat blood vessels convert labeled pregnenolone to labeled corticosterone (103), and perfused blood vessels from adrenalectomized rats release corticosterone and aldosterone into the perfusate (102). Ex vivo perfused rat heart also contains corticosterone and aldosterone and releases them into the perfusate (90). Studies incubating rat heart homogenate with radiolabeled deoxy corticosterone disagree on the presence of CYP11B1 and CYP11B2 activity (64, 90). In mice, the heart contains the mRNAs for some steroidogenic enzymes, but not the mRNAs for CYP11B1 and CYP11B2, arguing against corticosteroid production (120). The chicken heart appears not to have steroidogenic capacity, because several steroidogenic enzyme activities are not detectable (46).
**Locally Synthesized Mineralocorticoids**

Aldosterone synthesis by the heart is minimal or absent in healthy individuals. Under normal conditions, these low levels of local aldosterone synthesis are probably insufficient to affect sodium and water reabsorption by the kidney and thus would not affect blood volume and blood pressure by this mechanism. In cases of pathophysiology, when local aldosterone synthesis might increase, chronic production of local aldosterone could paradoxically exacerbate heart problems (Table 3). Aldosterone acts directly upon the heart to stimulate fibrosis and left ventricular hypertrophy (52), which increase the risk of heart failure. This effect is independent of any change in blood pressure. A pathological role of local aldosterone synthesis is consistent with the observation that mineralocorticoid receptor antagonist treatment dramatically lowers human mortality from heart failure, even when circulating aldosterone levels are normal (71). Other mechanisms can explain this finding without the need to invoke local aldosterone synthesis, such as retention of circulating adrenal-derived aldosterone in cardiac tissue (23), but this possibility is unlikely (9).

### COMMON THEMES

**Locally Synthesized Glucocorticoids**

One emerging theme is that local glucocorticoid synthesis occurs in immunologically important tissues. The thymus and bursa are critical sites of lymphocyte development, and the intestine and skin contain large numbers of immune cells. All of these areas are sites of exposure to antigen and lymphocyte activation. Locally produced glucocorticoids in lymphoid organs, intestine, and skin antagonize signals that promote lymphocyte activation or proliferation, thus acting to prevent lymphocyte overresponsiveness. This role of locally synthesized glucocorticoids is similar to a key role of circulating glucocorticoids in response to an immune challenge (16, 84). The lung, another immunologically important barrier, may also synthesize glucocorticoids (69, 73).

Local glucocorticoid synthesis appears to be independent of, or even in contrast to, patterns of adrenal glucocorticoid synthesis. For example, lymphoid glucocorticoid synthesis is high in early development and decreases over time, whereas adrenal glucocorticoid synthesis is low in early development and increases over time (75, 88, 110). Similarly, in the intestine, ACTH suppresses local glucocorticoid synthesis, although ACTH stimulates adrenal glucocorticoid synthesis (57). In the skin, local glucocorticoid synthesis is regulated by a local HPA axis analog (38) and is induced by local tissue damage (112).

Local glucocorticoid synthesis in primary lymphoid organs, intestine, and skin might have evolved as an adaptive mechanism to allow for localized action of glucocorticoids where and when they are needed, without incurring the costs of exposing all tissues to high glucocorticoid levels. Similar compartmentalization, or “Balkanization,” of steroid synthesis is seen in seasonally breeding birds (87). Breeding male song sparrows (in spring) have high systemic testosterone levels, while nonbreeding males (in winter) have low systemic testosterone levels (114). Both breeding and nonbreeding males must aggressively defend a territory, which is critical for survival and reproduction. In nonbreeding males, local synthesis of sex steroids in the brain is upregulated to support the expression of aggression while avoiding the costs of high systemic testosterone levels during this season (72, 100).
sterone remains controversial. Studies in lymphoid organs and intestine have not examined aldosterone or its synthetic enzyme CYP11B2, and this would be a useful goal for future research. Lymphoid organs contain few mineralocorticoid receptors, making aldosterone an unlikely product (55, 86), but the possibility has not been ruled out.

In the brain and heart, local aldosterone synthesis may be regulated by local expression of upstream RAS mediators. In brain, aldosterone synthesis is increased in response to low salt intake, in parallel with adrenal aldosterone synthesis. However, systemic treatment with angiotensin II has no effect on brain aldosterone synthesis, suggesting that brain aldosterone synthesis is independent of systemic RAS mediators and may depend on local expression of RAS mediators. Brain aldosterone synthesis plays an important role in systemic blood pressure regulation, as brain-specific inhibition of aldosterone synthesis reversibly decreases blood pressure (31).

While locally synthesized glucocorticoids have anti-inflammatory functions and serve to minimize tissue damage, local aldosterone synthesis in the brain and heart drives hypertension and heart failure, exacerbating tissue damage. Local aldosterone synthesis in healthy individuals may occur at low levels that allow beneficial effects of locally elevated mineralocorticoid levels while avoiding the costs of high systemic aldosterone levels (e.g., high systemic blood pressure). For the brain in particular, the blood-brain barrier allows only minimal uptake of circulating aldosterone (25), suggesting that it is important to minimize the effects of systemic aldosterone on the brain or that it is important for brain aldosterone levels to be regulated independently of systemic aldosterone levels.

CONCLUSIONS

The accumulated mass of evidence (Table 1) demonstrates that de novo corticosteroid synthesis occurs outside the adrenal cortex. The evidence is heavily weighted toward PCR studies of steroidogenic enzyme mRNA and corticosteroid synthesis in vitro. However, some studies have also measured local endogenous corticosteroid levels in adrenal-intact as well as adrenal-ectomized subjects (14, 29, 30, 48, 85, 88). Together, these different lines of evidence show that locally elevated steroid concentrations cannot simply be accounted for by the sequestration of circulating adrenal steroids. Furthermore, recent studies have used steroidogenic enzyme inhibitors in vivo to decrease local steroid synthesis in adrenal-intact subjects (31, 36, 76). These studies have convincingly demonstrated that local corticosteroid synthesis has functional consequences and is physiologically relevant. The effects of locally synthesized steroids likely depend on high local steroid concentrations either within an entire target organ (Fig. 2) or on a smaller scale (such as at the neuronal synapse). Thus, at level of the receptors, local corticosteroid concentrations can be far higher than those of systemic corticosteroids synthesized by distant adrenal cortices. Furthermore, most circulating glucocorticoids (90–95%) are bound with high affinity to corticosteroid-binding globulin and are unavailable to bind receptors (84).

In addition to the tissues discussed above, corticosteroid synthesis de novo from cholesterol might also occur in the lung (69, 73), retina (122), and kidney (115). In addition to de novo synthesis, bones, joints, liver, muscle, and fat express 11β-HSD type I and can convert circulating inactive glucocorticoid metabolites (e.g., cortisone) into active glucocorticoids (77) (Fig. 1). Also, tissues expressing only CYP11B1 or CYP11B2 can convert circulating precursors (e.g., 11-deoxycorticosterone) into glucocorticoids or mineralocorticoids, respectively. Overall, it is clear that the circulating systemic levels of cortisol, corticosterone, and aldosterone need not (and very often do not) reflect the local levels of these steroids at crucial target tissues.

Clinically, the local administration of glucocorticoids (e.g., via inhaler, topical application, injection into joints) can be advantageous over systemic glucocorticoid administration, which has numerous side effects throughout the body. This practice mimics the endogenous local synthesis of corticosteroids by various organs. Knowledge about these natural physiological processes may inform therapies that target corticosteroids to specific tissues or stimulate endogenous local corticosteroid synthesis at specific sites.

Compared with adrenal corticosteroids, locally produced corticosteroids can be synthesized by different enzymes (45), can differ in identity (46, 88), can bind differentially to receptors (86), can be differentially regulated (57), and have greater spatial specificity. These differences allow locally synthesized corticosteroids to complement the functions of systemic, adrenal-synthesized corticosteroids.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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LOCAL CORTICOSTEROID SYNTHESIS


