FRONTIERS IN NEUROENDOCRINOLOGY **18,** 440–462 (1997)
ARTICLE NO. FN970157

Central Hypertensive Effects of Aldosterone

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The soluble mineralocorticoid receptor bound to an agonist acts as a transcription factor for several genes relevant to ion transport by kidney and colon epithelial cells and is a major regulator of electrolyte and fluid homeostasis. Mineralocorticoids, the most prominent of which is aldosterone, also influence the activity of nonepithelial target cells, including vascular smooth muscle cells, by altering intracellular ion transport and content. Evidence is summarized for mineralocorticoid modulation of neuronal activity in a center or centers within the brain, probably in the periventricular area of the anterior hypothalamus, where information on electrolyte, fluid, and cardiovascular status is received and integrated, resulting in alterations in central sympathetic efferent activity. These functions are distinct from central aldosterone effects on salt appetite and peripheral trophic effects on cardiovascular tissue. The isolated mineralocorticoid receptor binds several adrenal steroids, including aldosterone and the major glucocorticoids, with equal affinity. Ligand specificity for the mineralocorticoid receptor differs between tissues, including different organs in the brain. Specificity is conferred extrinsically by the 11-β-hydroxysteroid dehydrogenase enzymes in transport epithelia, but mechanisms for mineralocorticoid ligand specificity have not been completely defined in the brain. The functional interaction between the mineralocorticoid receptor bound to different ligands and between the mineralocorticoid and glucocorticoid receptors is complex and as yet unresolved. Evidence is presented for the *de novo* synthesis of adrenal corticosteroids in the brain which may, by paracrine regulation of central control mechanisms, be relevant for certain clinical and experimental forms of hypertension characterized by low circulating levels of mineralocorticoids which respond to mineralocorticoid receptor antagonists. KEY WORDS: Hypertension; steroids; mineralocorticoids; adrenal regeneration hypertension; Dahl Salt-Sensitive rat; neurosteroids.

INTRODUCTION

Mineralocorticoid steroids, aldosterone being the most important, are produced in the adrenal cortex and are primary regulators of water, sodium, and potassium homeostasis. Bound to the soluble mineralocorticoid receptor (MR), they act as gene transcription factors for the expression of several proteins involved in the vectorial transport of sodium and potassium across transport epithelia, such as that in the kidney, colon, lung, salivary gland, and amphibian

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0091-3022/97 $25.00

urinary bladder (103, 118). Mineralocorticoids also alter ion movement across the plasma membrane of cells in nonepithelial tissues, including brain, B lymphocytes, and cardiac and vascular smooth muscle cells (8, 18, 20, 107, 129, 156). Among mineralocorticoid-induced proteins are amiloride-sensitive sodium channel subunits (23, 36, 102) and proteins which directly or indirectly enhance channel activity on the luminal side of the transport epithelia cells (5, 53, 127, 132, 143), components of the Na^+/K^+ATPase pump on the basolateral side of sodium transport cells, and citrate synthase, a crucial enzyme in the Krebs cycle (103). Because these "classical" steroid actions involve gene transcription and mRNA translation, there is an obligatory delay of at least 30 min, often several hours, in their appearance. In addition to genomic effects, aldosterone also produces direct membrane effects occurring within 15 min through the modulation of the inositol-1,4,5-trisphosphate messenger system, resulting in the alteration of cellular Na^+ transport and intracellular free Ca^{2+} ion stores in lymphocytes, vascular smooth muscle, and endothelial cells (24, 158–160). Rapid membrane receptor effects of aldosterone in the central nervous system have not been reported. This review is limited to phenomena which are compatible with classical mineralocorticoid receptor mediated effects in the CNS.

The majority of human patients with hypertension suffer from Primary, or Essential, hypertension, for which there is no known definitive cause. While these patients eventually suffer end-organ damage as a result of their hypertension, they regulate their moment to moment blood pressures normally, albeit around a higher set point. The association between mineralocorticoids and hypertension was recognized 60 years ago (99), but was assumed to be of renal origin. Excessive mineralocorticoids produce sodium and water retention and potassium excretion by the kidneys, from which there is an eventual "escape," with maintenance of the new volume, along with hypertension and hypokalemia. However, it became apparent that the kidney was not the sole effector of mineralocorticoid modulation of the blood pressure (9). Acting through MR in vascular smooth muscle cells, mineralocorticoids alter ion transport and content in these cells and increase vascular responsiveness to pressor agents, including norepinephrine, angiotensin II, serotonin, and tyramine, even before systemic blood pressure increases (7, 54). Two types of evidence implicated the brain: MR were found in the brain and ablation of specific regions prevented mineralocorticoid hypertension.

By tissue weight there are as many or more MR in several regions of the brain than in the kidney (162). This is particularly true in the hippocampus, where the MR are now known to be occupied primarily by glucocorticoids and to be crucial for serotonin-mediated functions and synaptic plasticity (31, 89, 117). Hypothalamic periventricular structures and the amygdala were also found to have a high density of MR and to be associated with the regulation of ACTH release, arousal, water and electrolyte equilibrium, salt appetite, and blood pressure (13, 15, 84, 133).

The increase in blood pressure in experimental models of hypertension in rats associated with derangements of sodium and water homeostasis, including

mineralocorticoid and renovascular hypertension and salt-induced hypertension in the Dahl Salt-Sensitive rat, was attenuated or prevented by the destruction of the tissue anteroventral to the third ventricle of the brain (the AV3V area) or the area postrema, or by chemical ablation of the central sympathetic system (13, 17, 75, 98, 126). The AV3V area comprises several anterior hypothalamic structures, including the organum vasculosum lamina terminalis and the periventricular, preoptic, and median preoptic nuclei and is involved in the integration of the neurohumoral control of volume, osmolality, and blood pressure (15, 91, 109, 151). In addition to preventing mineralocorticoid hypertension, AV3V lesions prevent the pressor and dipsogenic responses to the intracerebroventricular (icv) administration of angiotension II and hypertonic saline and attenuate the hypertension of the intravenous (iv) infusion of angiotensin II, in part due to a failure in argenine vasopressin (AVP) release (14, 90). Lesions limited to the paraventricular nuclei attenuate the hypertension produced by mineralocorticoid–salt excess, without preventing it (114). The involvement of catecholamines, angiotensin II, and vasopressin in the development of mineralocorticoid–salt hypertension has been studied extensively (43). While the sympathetic nervous system is the efferent mediator of central effects of mineralocorticoid upon blood pressure (75, 98, 126), central angiotensin II and vasopressin interactions in this form of hypertension are important or permissive (6, 43, 55, 85). Experimental and clinical observations led Bohr to propose that, in addition to well-documented renal and vascular effects, aldosterone regulates ion transport, particularly Ca^{2+} thereby altering the "set point" or threshold for activation of cells within those areas of the brain which modulate the multiple homeostatic mechanisms controlling blood pressure (9).

CONTINUOUS INTRACEREBROVENTRICULAR INFUSION STUDIES OF MINERALOCORTICOID RECEPTOR AGONISTS AND ANTAGONISTS

Essential hypertension is a chronic insidious disease in which the blood pressure increases over a relatively long period of time. To provide further evidence for a central role for mineralocorticoids in tonic blood pressure control, we conducted a series of experiments comparing the effects upon the basal blood pressure in rats of the continuous subcutaneous (sc) or icv infusion of aldosterone, corticosterone, a "pure" glucocorticoid receptor (GR) agonist, RU29688, and the mineralocorticoid receptor (MR) antagonists prorenone and RU28318. The amounts of steroids were chosen so that the rats continued to grow and behave normally; icv doses were small enough not to cause changes in blood pressure when infused sc. Agents were delivered by indwelling miniosmotic pumps or continuous release pellets, allowing the rats to live in normal social groups, and tail cuff blood pressures were taken two to three times a week in unheated well-trained rats to minimize stress-related changes in blood pressure (64). The continuous icv infusion of aldosterone produced a significant increase in resting blood pressure which was blocked by the concomitant icv infusion of prorenone, an MR antagonist (62). A high salt intake causes

hypertension by itself in some strains of rats and individuals of other species, including humans, and is commonly used to accelerate and exacerbate mineralocorticoid hypertension. The hypertension induced by the continuous icv infusion of aldosterone was dose responsive and enhanced, but not dependent upon sensitization to the effects of mineralocorticoids by renal mass reduction and excess salt consumption (63, 121). Similar continuous icv infusions of aldosterone also produced hypertension in dogs associated with an increase in total peripheral resistance (93).

Bilateral adrenalectomy prevented the increase in blood pressure produced by the continuous icv infusion of aldosterone in rats; replacement by continuous release pellet designed to deliver 0.83 mg corticosterone/day permitted the response (71). Similarly, corticosterone replacement was necessary for the blood pressure response to bolus injections of agonists and antagonists in adrenalectomized rats (155).

The icv infusion of RU28318, a selective MR antagonist, blocked the development of hypertension produced by the sc infusion of aldosterone at doses which had no effect when infused icv alone or sc with the aldosterone (67, 86). The icv dose of RU28318 which effectively blocks mineralocorticoid hypertension is less than that required to inhibit the increased appetite for saline associated with systemic mineralocorticoid excess (67, 86, 108) or salt depletion (134). Deoxycorticosterone acetate (DOCA) is a potent, relatively inexpensive mineralocorticoid traditionally used to produce experimental hypertension. Dietary salt plus subcutaneous DOCA–salt treatment attenuates baroreflex responses before hypertension becomes evident (106, 148). The icv infusion of the MR antagonist RU28318 had no effect on baroreceptor sensitivity, vasomotor tone, or vascular reactivity in normotensive rats (86). However in DOCA–salt-treated rats the icv infusion of the MR antagonist normalized baroreflexes, reduced neurogenic vasomotor tone, and prevented hypertension without altering the mineralocorticoid excess-induced increase in the pressor response to iv phenylephrine, AVP, or angiotensin II infusions. Though an increase in central sympathetic activity is associated with systemic mineralocorticoid hypertension, the reduction of DOCA–salt hypertension by the icv infusion of the MR antagonist was associated with only a partial reduction in sympathetic tone (86). The depressor effect of a single large acute icv bolus of RU28318 was attributed by one author to the blocking of the sympathoexcitatory response to heating and handling of the animals for tail cuff plethysmography, rather than blocking mineralocorticoid-induced hypertension (154). However, studies in another laboratory demonstrated that the continuous icv infusion of the same MR antagonist blocked DOCA–salt hypertension whether blood pressure was measured indirectly or directly (86).

The prehypertensive stage of icv aldosterone-induced hypertension, in contrast to that of systemic mineralocorticoid excess states, is not associated with a decrease in baroreceptor reactivity, nor is there an increase in vascular reactivity to the iv infusion of angiotensin II, norepinephrine, or argenine vasopressin (AVP) (84, 86). The dissociation between baroreceptor function and icv mineralocorticoid hypertension is surprising, as depression in baroreceptor sensitivity

had been considered a major mechanism, along with increased central sympathetic activity, for mineralocorticoid excess hypertension. Normal vascular reactivity in icv aldosterone hypertension, like that of renal handling of Na^+ and K^+, can be attributed to normal circulating levels of aldosterone interacting directly with the vascular and renal MR.

In addition to an intact central sympathetic nervous system and AV3V area (13, 17, 75, 90, 98, 126), AVP is also necessary for the development of systemic mineralocorticoid hypertension (6, 43). The central administration of angiotensin II, carbachol, or hypertonic saline in normotensive rats increases the blood pressure both by activating the sympathetic nervous system and by causing the release of AVP into the circulation. The icv administration of AVP in the normotensive rat increases blood pressure and sympathetic nervous system activity without increasing circulating AVP (15, 86). This response to the icv injection of AVP is increased in systemic mineralocorticoid excess states. The icv infusion of aldosterone did not alter the pressor or dipsogenic responses to the central administration of angiotensin II, carbachol, or hypertonic saline; however, the pressor response to icv administered AVP was paradoxically decreased by the icv infusion of aldosterone, even though the response to iv AVP was unaltered (84, 86).

Pressor Effects of Aldosterone Are Distinct from Other MR-Mediated Effects

Pressor effects of aldosterone are distinct from mineralocorticoid-mediated changes in fluid and electrolyte balance, increase in salt appetite, increase in vascular reactivity, and trophic effects on the vessles and heart. Dose–response studies allowed the selection of sc and icv doses of aldosterone, 1 µg/h and 10 ng/h, respectively, that produce consistent moderate rises in blood pressure of the same amplitude and temporal progression (Fig. 1). These icv doses are small in comparison to normal aldosterone production by the adrenal gland and do not increase circulating levels of aldosterone (86), so direct effects of mineralocorticoid excess upon peripheral target organs are separated from those of hypertension.

The increases in renal Na^+ retention and K^+ excretion and in drinking and Na^+-seeking behavior produced by systemic mineralocorticoid excess were not associated with the hypertension produced by the icv infusion of 5, 10, or 15 ng/h of aldosterone (3, 62, 63, 121). The normal Na^+/K^+ and creatinine ratios found with these icv infusion doses indicate that the kidney MR are exposed to normally regulated circulating levels of aldosterone (62). The increased salt appetite in mineralocorticoid excess is mediated by MR of the amygdala where aldosterone and angiotensin II act synergistically, as has been confirmed by the injection of MR antagonists and antisense oligonucleotides (133, 134). Prevention of the hypertension of systemic mineralocorticoid excess by the central infusion of small amounts of either a MR or a Na^+ channel antagonist did not prevent the associated saline polydipsia (67, 68, 86). While the icv infusion of 15 ng/h aldosterone did not cause an increase in saline drinking, icv 45 ng/h did,

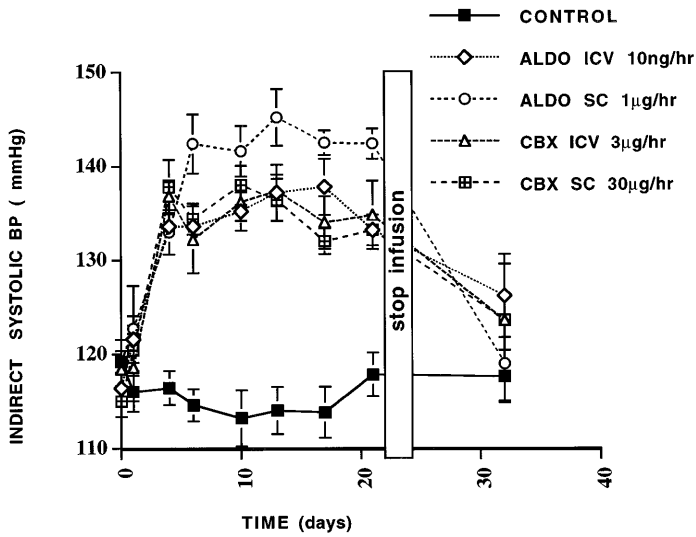

FIG. 1. The systolic blood pressure measured by tail cuff in unheated pregnant Sprague–Dawley rats drinking 0.45% saline and receiving similarly hypertensinogenic doses of aldosterone (ALDO) or the 11β-HSD inhibitor carbenoxolone (CBX) delivered either icv or sc by miniosmotic pump. Control rats and those receiving sc agents have vehicle filled icv cannulae. Bars indicate standard error of the mean.

suggesting that the dissociation of the centrally mediated effects on blood pressure in circumventricular tissue and those on salt appetite in the more lateral amygdala at the lower doses might reflect a diffusion concentration gradient across the brain parenchyma (34).

Aldosterone–salt excess in rats produces cardiac hypertrophy and fibrosis, which are prevented by the systemic administration of the MR antagonist spironolactone (12). Preventing the hypertension of systemic mineralocorticoid excess by the icv infusion of the MR antagonist RU28318 does not prevent the development of cardiac hypertrophy and fibrosis (164), clearly supporting other *in vitro* and *in vivo* evidence of a specific trophic effect of aldosterone on the heart which is separate from its pressor effects (12, 78, 135). Induction of hypertension of similar magnitude and temporal pattern was produced by the sc and icv infusion of aldosterone at 1 μg/h and 10 ng/h, respectively, but an increase in cardiac mass was produced only in rats receiving the large sc dose which exposed their hearts to elevated levels of aldosterone (65).

Role for the Amiloride-Sensitive Na^+ Channel

Mineralocorticoids act through MR to enhance the transcription of specific mRNAs for proteins which promote vectorial ion transport across epithelia. Among these are the amiloride-sensitive Na^+ channel subunits and/or proteins which directly or indirectly enhance Na^+ channel activity on the luminal side of

the transport epithelia cells (5, 23, 36, 53, 68, 102, 103, 127, 132, 143). Amiloride is a pyrazinoylguanidine diuretic developed for its saluretic and K^+ sparing properties used in the treatment of essential hypertension. Benzamil is a Na^+ channel-selective amiloride analog. To define a cellular mechanism for the central effect of aldosterone, benzamil was infused icv in amounts which did not alter growth, behavior, or blood pressure in normotensive rats and which were smaller than those required for systemic effects in rats made hypertensive by the chronic icv or sc infusion of aldosterone (65, 68). The central administration of the Na^+ channel antagonist prevented the hypertension in both of these models. As with the central administration of the MR antagonist, the icv infusion of the Na^+ channel blocker prevented hypertension without decreasing the mineralocorticoid-induced saline polydipsia or increase in urine volume (68). These studies indicate that mineralocorticoids act in the brain through at least one mechanism, enhancement of ion transport through the Na^+ channel, in common with other mineralocorticoid target tissues. The icv infusion of aldosterone in hypertensinogenic amounts that do not alter serum Na^+ or K^+, plasma renin, serum norepinephrine, or atrial natriuretic factor do elevate the Na^+/K^+ ratio of the cerebrospinal fluid, suggesting that aldosterone modulates vectorial transport of Na^+ in the epithelium producing CSF as it does in the kidney and colon (3). Alteration of CSF electrolytes may be instrumental in the pressor effect of mineralocorticoids. However, it seems more likely that increased entry of Na^+ into neurons with cardiovascular modulatory function, with subsequent changes in concentrations and subcellular distribution of other ions, particularly Ca^{2+} alters membrane potential and excitability of these cells (9, 37, 72).

The Na^+ channel comprises four subunits, the α, β, γ, and the recently described δ subunits (21, 22, 131, 157). The δ subunit is found in significant amounts in the brain, but not the kidney, where the α, β, and γ subunits abound (157). Liddle's syndrome, or pseudohyperaldosteronism, is an autosomal inherited disease of humans presenting with severe hypertension and hypokalemia, suggesting primary aldosteronism, but aldosterone levels are low. For most of these patients a renal tubular defect resulting in uncontrolled sodium reabsorption is due to mutations in the intracellular domain of the C-terminal area of either the β or the γ subunit, rendering the Na^+ channel constitutively open, perhaps by protecting it from degradation (76, 141, 146). There are a significant number of patients with essential hypertension who are salt sensitive and whose hypertension is mitigated by amilorides, but who do not suffer severe hypokalemia or have elevated serum aldosterone (51). A mutation in the newly described δ subunit similar to that of the β or γ subunits in Liddle's syndrome might produce a centrally mediated hypertension with no or minimal renal involvement.

Role for a Ouabain-like Factor

Efficient sodium transport occurs across mineralocorticoid-responsive epithelia when, in addition to increasing Na^+ channel activity on the luminal cell

membrane, moving Na^+ into the cell, mineralocorticoids enhance the transcription of several factors which increase the activity of the Na^+/K^+-ATPase pump on the basolateral side, moving Na^+ out (103). Inhibition of the pump by cardenolides blocks the active arm of this dual transport mechanism, causing the accumulation of Na^+ within the cell, resulting in the perturbation of other ion transport systems linked to the Na^+ gradient. Such alterations, particularly in Ca^{2+} concentration, have profound effects on cell function, including excitability and contractility in nontransport epithelial cells. Inhibition of the Na^+/K^+-ATPase by a circulating endogenous digitalis- or ouabain-like factor (OLF), thought to be made in the hypothalamus and/or adrenal gland, has been implicated in the pathogenesis of several forms of clinical and experimental hypertension involving volume expansion, including that induced by mineralocorticoid–salt excess (33, 77, 96, 104, 105).

Ouabain has been found to amplify DOCA hypertension in rats (138). The experimental hypertension produced by the continuous sc or icv administration of ouabain, like that of mineralocorticoids, has a crucial central component mediated through the central sympathetic nervous system (80). The pressor effect of infused ouabain was prevented or attenuated by the concomitant icv infusion of digoxin-specific antibody Fab fragments which cross-react with ouabain, as well as by a sympatholytic agent. The continuous icv administration of digoxin-specific antibody Fab fragments also mitigated the hypertension in two genetic models of high blood pressure, the Spontaneously Hypertensive (SHR) and Dahl Salt-Sensitive (SS) rats (42, 82). It is interesting to note that the component of blood pressure elevation in the SHR which was independent of a high salt diet was not abrogated by the digoxin-specific antibody Fab fragments.

Induction of endogenous anti-ouabain antibodies in inbred Dahl SS rats, the SS/jr, by the inoculation of ouabain–urease conjugates significantly attenuated the pressor response to salt-loading in comparison to SS/jr inoculated with urease alone (70). Central sympathoexcitation, as in ouabain-induced and mineralocorticoid excess hypertension, is the effector of the increased blood pressure in both the SHR and the Dahl SS (80, 82). An endogenous pump inhibitor may also be involved in the hypertension of the Milan Hypertensive rat, in which adrenal and hypothalamic levels of OLF were found to be greater than those in the Milan Normotensive rat (42).

Cardenolide treatment in congestive heart failure leads to diuresis by increasing the efficiency of the heart, leading to better blood flow to the kidneys, and by decreasing renal tubular sodium reabsorption. While a OLF has been implicated in mineralocorticoid-induced hypertension, this has been difficult to reconcile with the "kidney based" model of mineralocorticoid-induced hypertension (retention of sodium, followed by water, increased blood volume leading to increased cardiac output and, finally, vascular autoregulation producing an increase in total peripheral resistance). An inhibitor of the Na^+/K^+-ATPase pump would reduce the mineralocorticoid-enhanced vectorial transport of sodium and subvert this mechanism, but it would also alter membrane poten-

tial and excitability in mineralocorticoid target cells in the as yet unidentified brain cells crucial for providing the "set point" for "normal" blood pressure.

Similarities between the Central Component of the Hypertension in the Dahl Salt-Sensitive Rat and the Mineralocorticoid–Salt Hypertensive Rat

The Dahl Salt-Sensitive and Salt-Resistant rats were developed by selective breeding of Sprague–Dawley rats for sensitivity or resistance to the hypertensive effects of a high salt diet (28, 122, 124). The etiology of the hypertension in this genetic model involves several genetic loci (123–125). Similarities between the SS rat and mineralocorticoid hypertension include the prevention of hypertension by the destruction of the AV3V area or central sympathetic system (14), and by the icv infusion of antagonists of the MR and Na^+ channel at doses devoid of effects when infused peripherally (66, 69). This is particularly significant because aldosterone and other known mineralocorticoids circulate at normal to low levels in the SS rats (27, 161). Low aldosterone levels in the SS may be due, at least in part, to a difference in their gene for the aldosterone synthase enzyme, rendering it less efficient than that of the Salt-Resistant (SR) rat (72). In this regard, the SS rats resemble those hypertensive patients in which blood pressure is lowered by therapies reducing mineralocorticoid effects even though there are no significant increases in circulating mineralocorticoid levels. We have not found a difference between the SS/jr and SR/jr α, β, γ sodium channel subunit sequence (E. Gómez Sánchez, unpublished data); the δ subunit and Nedd4 protein remain to be examined in this strain. Salt-induced hypertension in the SS rat is also significantly attenuated by the icv infusion of digoxin-specific antibody Fab fragments (81, 82). These data suggest that the SS/jr brain MR may be more fully occupied or are more effective as transcription factors for Na^+ channel proteins compared to those of normotensive rats. Alternatively, there there may be an excessive amount of OLF amplifying the action of aldosterone at the level of the aldosterone-sensitive blood pressure regulatory centers. SS/jr rats with induced antibodies to ouabain–urease had significantly lower blood pressures than those with urease antibodies (70).

Adrenal Regeneration Hypertension

Adrenal regeneration hypertension (ARH) is produced by removing the right adrenal gland and kidney, enucleating the left adrenal gland (removing the medulla along with most of the zonas fasciculata and reticularis), and providing 0.9% NaCl to drink. ACTH levels are very high during the first week of ARH, followed by a gradual decline, while blood pressure steadily increases after the first week. The initial rise in ACTH is required for both adrenal regeneration and hypertension in this model; adrenal steroidogensis is necessary for the hypertension (19, 44). While systemic administration of a MR antagonist prevents hypertension, the circulating levels of known mineralocor-

ticoids are not commensurate with the degree of hypertension seen, so an effort has been made over the years to isolate an as yet undiscovered mineralocorticoid responsible for ARH (44, 52, 56–59, 142). In this regard the ARH hypertension model resembles that of the SS rat and the hypertension of ectopic ACTH production in humans. Adrenal regeneration hypertension, like that of mineralocorticoid excess, is prevented by the icv infusion of a selective MR antagonist at a dose far below that which is required to alter the blood pressure when infused systemically (65). While it can be assumed that circulating ligand is binding the brain MR in systemic mineralocorticoid excess states, the relatively moderate amounts of known circulating mineralocorticoids in ARH, as in the SS/jr, do not produce hypertension in intact rats.

Receptor Specificity for Central Effects of Aldosterone on the Blood Pressure

Aldosterone binds the glucocorticoid receptor (GR), albeit with low affinity relative to the MR or compared to natural glucocorticoids binding to the GR (48). The continuous icv infusion of the selective GR agonist RU26988 at comparable molar amounts to that of aldosterone had no effect on the blood pressure by itself or in combination with aldosterone. However, the continuous icv infusion of an MR antagonist at several orders of magnitude less than that required for an effect when infused sc blocked the hypertensive effect of both the icv and the sc infusion of aldosterone. This demonstrates that the central hypertensive effect of aldosterone is mediated by MR (62, 67, 71).

Mineralocorticoid Receptor Ligand Selectivity

Only one mineralocorticoid receptor gene has been identified (2). MR isolated from different tissues have the same physicochemical properties (162), although subtle differences in molecular weight between rat hippocampal and kidney MR have been reported, possibly resulting from differences in transcription or posttranslational processing of the same gene product (35). Isolated MR, whether from the kidney or the brain or expressed from cDNA, have similar affinities for aldosterone, corticosterone, and deoxycorticosterone and have slightly lower affinity for cortisol, raising the problem of receptor specificity, especially since glucocorticoids normally circulate at 100–1000 times the levels of aldosterone (97). Circulating corticosteroid binding globulin (CBG) reduces free circulating glucocorticoid levels. Because CBG does not cross the blood–brain barrier it is not a determinant of extrinsic specificity for brain MR. The 11β-hydroxysteroid dehydrogenase (11β-HSD) enzymes catalyze the oxidation–reduction reaction between cortisol and cortisone, and corticosterone and 11-dehydocorticosterone. 11β-HSD1 is bidirectional, depending on the stoicheometry of the microenvironment; in the liver it primarily functions as a reductase, converting cortisone and 11-dehydocorticosterone to the active steroids cortisol and corticosterone. MR ligand specificity in transport epithelia is

conferred extrinsically by the unidirectional activity of the 11β-HSD2, which oxidizes cortisol and corticosterone, but not aldosterone, to produce inactive 11-keto metabolites. This limits access to the MR to aldosterone even though aldosterone circulates at concentrations several orders of magnitude less than that of the glucocorticoids (39, 40, 46, 50). Defects in the gene coding for the human 11β-HSD2 enzyme leave the kidney MR unprotected from occupation by normal circulating levels of cortisol and are responsible for the syndrome of apparent mineralocorticoid excess (AME). AME is characterized by hypokalemia and hypertension, but low aldosterone and plasma renin activity (112, 140, 153). In some tissues, including placenta and specific nuclei of the brain, the 11β HSD enzymes also limit access of glucocorticoids to the GR (16, 38, 49, 137, 144). 11β-HSD2 regulation of corticosterone levels in the paraventricular nucleus of the hypothalamus modulates feedback activity to the CRF-41 neurons responsible for the synthesis and release of CRF to the portal circulation (137).

The licorice derivative glycyrrhizic acid, its hemisuccinate, carbenoxolone, and 11α-hydroxyprogesterone are inhibitors of the 11β-HSD enzymes (145). Excessive ingestion of licorice or carbenoxolone, an antiinflammatory treatment for gastric and duodenal ulcers, causes hypertension and hypokalemia (74). The icv infusion of the 11β-HSD inhibitors carbenoxolone or glycyrrhizic acid, at doses which are ineffective when infused sc, produces hypertension (71) (Fig. 1). Further, the hypertension produced by the oral administration of carbenoxolone or glycyrrhizic acid is blocked by the icv administration of the selective MR antagonist RU28318. However, we have also found that the continuous icv infusion of corticosterone at molar amounts comparable to that of aldosterone had no effect on the blood pressure by itself, but attenuated the increase in pressure produced by the concomitant icv infusion of aldosterone in a dose-dependent manner (71). If the 11β-HSD enzyme inhibitors were acting solely by allowing corticosterone access to MR in brain blood pressure control centers, our icv aldosterone + corticosterone results would lead us to expect a decrease blood pressure (71). These data suggest a much more complex explanation for MR-ligand specificity, particularly in different parts of the brain.

There are distinct regional distributions of both 11β-HSD1 and 2 mRNA as well as oxidase and reductase activity in the brain (60, 73, 100, 111, 120, 130, 144, 165). At physiologic serum levels of corticosterone, the hippocampal MR are almost completely occupied by corticosterone (25, 29, 100, 128, 163), so hippocampal 11β-HSD 2 activity is presumed to be minimal (30, 100, 128). However, minces of various areas of the rat brain, including the hippocampus, hypothalamus, and cerebellum, effectively convert tritiated corticosterone to 11-dehydrocorticosterone, indicating that 11β-HSD2 mRNA is translated into functional protein (60). The degree of corticosterone inactivation is insufficient to limit hippocampal MR binding to aldosterone, but may be important in regulating corticosterone occupation of these MR.

Corticosterone and aldosterone binding patterns to MR in whole brains of adrenalectomized rats are similar (1, 147), but excess corticosterone antagonizes [^{3}H]aldosterone binding in the periventricular regions of the hypothala-

mus of the adrenalectomized rat less effectively than in other regions, and periventricular hypothalamic MR are occupied by endogenous aldosterone to a greater extent than are MR in the hippocampus (39, 87). The sensitivity of hypothalamic 11β-HSD activity to the icv administration of its antagonist was reported to be significantly greater than that of the hippocampus, pituitary, and amygdala, suggesting different 11β-HSD isozymes (87). 11β-HSD2 activity has been reported in the rat ventrolateral portion of the ventromedial nucleus of the hypothalamus, but its levels in the rest of the anterior hypothalamus appear rather low to explain the preferential binding of aldosterone over corticosterone in these periventricular areas, given the much greater availability of the glucocorticoid (45, 130).

A similar situation exists in the heart, where 11β-HSD2 is expressed in very low amounts (165). While most of the heart MR are normally occupied by glucocorticoids, a crucial small number of MR are "spared" for aldosterone binding by an as yet unclear mechanism which is probably not 11β-HSD2 protection (45).

Mineralocorticoid Receptor Functional Selectivity: Ligand Specificity

When the MR of classic mineralocorticoid transport epithelia such as the kidney or colon are occupied by glucocorticoids, sodium retention and potassium loss are the same as when these MR are occupied by aldosterone. Moreover, there is evidence that the genomic effects of MR and GR binding in epithelial mineralocorticoid target tissues are mediated by the same nuclear hormone response element (47, 118, 119). The administration of corticosterone or a "pure" GR agonist after blocking both the 11β-HSD and the MR in the kidney results in mineralocorticoid-type patterns of Na^+ and K^+, presumably via glucocorticoid:GR interaction with same hormone response element with which an aldosterone:MR complex interacts (47). This occurs even though under more physiologic conditions GR- and MR-mediated functions are generally in opposition in the renal tubule (94).

In contrast to epithelial MR which mediate the same transcription effects regardless of whether the ligand is aldosterone, corticosterone, or cortisol, some nonepithelial MR appear to subserve functions which are ligand specific, and aldosterone and corticosterone appear to have different, antagonistic functions while binding to the same receptor. The continuous icv infusion of corticosterone or the "pure" GR agonist RU29688 had no effect on the blood pressure of rats. However, corticosterone, but not the GR agonist, antagonized the pressor effect of the concomitant icv infusion of aldosterone (71). An analogous situation occurs with hippocampal MR. Adrenalectomy produces deficits in learning and response to stress and changes in serotonin metabolism in the hippocampus which are normalized by corticosterone replacement. These effects are mediated by the MR within the hippocampus, since dexamethasone, a GR agonist with low affinity for the MR, did not restore these parameters. The effect appears to be specific for corticosterone binding to the hippocampal MR; not

only is aldosterone replacement ineffective in restoring the deficits produced by adrenalectomy, but also aldosterone blocks the normalizing effects of corticosterone replacement (10, 32). Differences in steroid receptor transcriptional factor specificities may be the mechanism by which corticosterone and aldosterone produce opposite effects on pressure and serotonin metabolism while binding the same receptor (118).

Functional Interactions between MR and GR

The functional interaction of glucocorticoids and mineralocorticoids with the hippocampus and hypothalamo–pituitary–adrenal axis has been extensively studied in relation to stress responses, behavior, and learning in the hippocampal serotonergic system, and opposing and balancing effects have been observed (29). The interaction of central GR and MR in the modulation of systemic blood pressure was studied by single bolus injections of supraphysiologic amounts of GR and MR agonists and antagonists into the lateral ventricles of intact normotensive rats (155). Results from these studies suggest that the brain MR mediate pressor effects and the GR mediate depressor effects. Different latent times and duration of blood pressure changes suggest different mechanisms for the opposing GR and MR mediated actions upon the central control of the blood pressure.

The synthetic glucocorticoid dexamethasone has a low affinity for the MR. Dogs, like rats, develop hypertension upon the chronic icv infusion of aldosterone at doses which are not effective when infused iv (93) and upon the systemic administration of large amounts of dexamethasone. However, the icv infusion of dexamethasone in normotensive dogs (113) and rats (150) produces a dose-related decrease in resting blood pressure. While the icv infusion of the GR antagonist RU38486 by itself had no significant effect on the blood pressure in the dog, its concommitant icv infusion blocked the hypotensive effect of the icv infusion of dexamethasone, suggesting that the central depressor effect was through the GR (113). In addition, the icv infusion of a GR antagonist potentiated the increase in blood pressure produced by oral dexamethasone administration (113). These results suggest that GR in the brain exert a mitigating influence on the pressor effect of systemic GR occupation.

Sheep, which are resistant to systemic mineralocorticoid hypertension, develop hypertension upon the infusion of ACTH associated with the increase of several corticosteroid hormones, including both MR and GR agonists. The iv infusion of a steroid cocktail designed to reproduce the elevation of hormones levels found during ACTH-induced hypertension mimics the hypertension (136). The icv infusion of this cocktail did not elevate the blood pressure, leading to the conclusion that there was no central pressor effect of adrenocortical steroids in sheep (152). The interpretation of these results, however, is complicated by the concommitant icv infusion of both GR and MR agonists, particularly in light of the opposite effects of systemic and icv infusions of dexamethasone in dogs and rats (113, 150).

EXTRA-ADRENAL SYNTHESIS OF CORTICOSTEROIDS

Neurosteroids, steroids which, in addition to being synthesized in the adrenal glands and gonads, are synthesized and act within the central nervous system, were recognized over 50 years ago (60, 110, 116, 139). Crucial enzymes for each step in the synthesis of adrenal cortical steroids from cholesterol to aldosterone have now been identified in the CNS. Cytochrome P450 side chain cleavage ($P450_{scc}$) enzyme activity is found in white matter and oligodendrocyte mitochondria throughout the brain (79, 101). Brain $P450_{scc}$ mRNA and its expression follow a distinct ontogenic pattern, suggesting a role in development and maturation (26). Conversion of pregnenolone to progesterone by the 3β-hydroxysteroid dehydrogenase/isomerase occurs in CNS glia and peripheral Schwann cells (95). The cytochrome $P450_{21}$ activity necessary for the conversion of progesterone and 17-hydroxyprogesterone to 11-deoxycorticosterone and 11-deoxycortisol is most abundant in the myelinated tracts of the ascending reticulothalamic fibers (83).

The cytochrome P450 11β-hydroxylase converts 11-deoxycorticosterone and 11-deoxycortisol to corticosterone and cortisol, respectively. The 11β-hydroxylase has been found in the myelinated tracts in the same general areas of the brain where the $P450_{scc}$ has been located (115). However, unlike the $P450_{scc}$, the 11β-hydroxylase was not found in cultured glia, suggesting that it is neuronal (110). Evidence for functional relevance of corticosterone synthesis in the brain has been obtained from studies of the TGR(mRen2)27 rat (41).

We have demonstrated expression of the mRNA for aldosterone synthase by RT-PCR/Southern blot in adrenal, aorta, hypothalamus, hippocampus, amygdala, and cerebellum (61). Both corticosterone and aldosterone were found in the incubation media of brain minces from intact and adrenalectomized rats, demonstrating synthesis from endogenous precursors. The incubation of brain minces with [1,2^{3}H]deoxycorticosterone followed by extraction and three different successive TLCs resulted in the demonstration of labeled aldosterone, corticosterone, and 18-hydroxydeoxycorticosterone. Similar incubations in the presence of 10 μ*M* cortisol, a competetive inhibitor of aldosterone synthase, or metyrapone, an inhibitor of both 11β-hydroxylase and aldosterone synthase, showed that these compounds inhibited the synthesis of aldosterone or both aldosterone and corticosterone, respectively, as they do in the adrenal gland.

These studies indicate that the rat brain has the enzymatic machinery for the synthesis of adrenal corticosteroids, including aldosterone. Extra-adrenal synthesis of aldosterone has also been demonstrated in human pulmonary arterial endothelial and smooth muscle cells (11, 78) and by perfused rat mesenteric arteries (149). As in the adrenal, angiotensin II stimulates aldosterone synthesis in these tissues. How aldosterone synthesis in the brain might be regulated, assuming that enough is produced to warrant regulation, is unknown. However, gene expression for the components of the brain renin–angiotensin system responds to dietary sodium manipulations in the same way as for the kidney and adrenal gland (88). The implications of even a very small amount of local aldosterone production acting in a paracrine fashion at blood pressure regula-

tory areas of the brain are intriguing, particularly in view of those experimental and clinical forms of hypertension with normal or low renin and mineralocorticoids which respond to antimineralocorticoid maneuvers.

19-Ethynyldeoxycorticosterone is a mechanism-based inhibitor of various 11β-hydroxylases (92) which decreased salt-induced blood pressure in the SS/jr rat when administered as a subcutaneous implant (4). To study the possibility that aldosterone or corticosterone synthesis in the brain might mediate the hypertension of the SS/jr rat, low doses of 19-ethynyldeoxycorticosterone were infused icv in SS/jr rats given saline to drink. The increase in blood pressure was attenuated by an icv dose far below that which is effective systemically (61).

SYNOPSIS

Essential or primary hypertension is an important disease in humans for which, by definition, no cause is known. In a significant number of these patients the blood pressure can be lowered by therapies which mitigate aldosterone action even though renin and aldosterone are low or normal. In addition to effects on vectorial transport of sodium and water and upon vascular smooth muscle reactivity, evidence from ablation and selective infusion studies indicates that mineralocorticoids act through the mineralocorticoid receptor in the floor of the anterior hypothalamus to modulate the response of cardiovascular centers in the brain to information about blood pressure and/or to alter central sympathetic nerve outflow regulating cardiovascular functions. The complex interactions between brain "aldo-preferring" MR and MR that are normally occupied by the more abundant corticosterone and between GR in the brain are still being sorted out. The discovery that occupation of brain MR by aldosterone elevates the blood pressure even when circulating levels of mineralocorticoids remain low led to the study of other experimental models of low renin–low aldosterone hypertension and the finding that the blood pressure is lowered in these models by the central infusion of agents which either block binding to the MR or subvert cellular sequelae of mineralocorticoid action. The physiologic relevance of the intriguing discovery that brain tissue has the ability to synthesize aldosterone, corticosterone, and several other adrenal steroids *de novo* has yet to be determined.

ACKNOWLEDGMENTS

These studies were supported by medical research funds from the Department of Veterans Affairs, American Heart Association, Missouri and Florida Affiliates, and NIH Grants HL27255 and HL27737.

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