ICV infusion of corticosterone antagonizes ICV-aldosterone hypertension

GÓMEZ-SÁNCHEZ, ELISE P., MYTHILI T. VENKATARAMAN, DWAYNE THWAITES, AND CHRISTINE FORT. ICV infusion of corticosterone antagonizes ICV-aldosterone hypertension. Am. J. Physiol. 258 (Endocrinol. Metab. 21): E649-E653, 1990.— There is evidence of crucial central nervous system involvement in the pathogenesis of mineralocorticoid-excess salt hypertension, as well as data indicating that corticosterone is the predominant ligand for the type I adrenocorticoid receptor in the brain. Miniosmotic pumps were used to deliver artificial cerebrospinal fluid (CSF), aldosterone (10 ng/h), corticosterone (10 or 20 ng/h), aldosterone (10 ng/h) plus corticosterone [10 ng/h intracerebroventricularly (icv)], or aldosterone (10 ng/h) plus corticosterone (20 ng/h icv). All animals were sensitized to mineralocorticoid hypertension by removing the right kidney and offering saline to drink. Indirect blood pressure by the unheated tail-cuff method and weights were measured twice weekly; 24-h urine volumes were measured once a week. The blood pressures of the four groups did not differ statistically before infusion. The blood pressures of those animals receiving CSF or corticosterone were not significantly elevated after 4-5 wk of intracerebroventricular infusion, whereas the aldosterone group had become significantly elevated within 2 wk. A similar study was done comparing the effects of intracerebroventricular infusion of aldosterone (10 ng/h), aldosterone (10 ng/h) and RU26988 (20 ng/h), and RU26988 (20 ng/h). RU26988, a selective type II receptor agonist, had no effect on the blood pressure, nor did it alter the pressor effect of intracerebroventricular aldosterone. The concomitant infusion of corticosterone antagonized the increase in blood pressure in a dose dependent manner. Neither steroid nor their combinations produced significant differences in daily urine volume or body weight gain compared with the CSF group.

THE IMPORTANCE of the central nervous system in the development of mineralocorticoid hypertension has been well documented. In the rat, ablation of the anteroventral third ventricle (AV3V) area (6, 7) and central, but not peripheral, sympathectomy prevent the development of deoxycorticosterone acetate (DOCA)-salt hypertension (21, 25, 30). In addition, mineralocorticoids are thought to act centrally to modulate salt appetite in adrenalectomized rats (15, 26, 27) as well as in mineralocorticoid excess (4, 15, 35). Previous studies in this laboratory have demonstrated that the hypertensinogenic effect of the intracerebroventricular infusion of minute amounts of aldosterone is dose dependent, blocked by the concomitant intracerebroventricular infusion of prorenone, an aldosterone antagonist, and enhanced, but not completely dependent on renal mass reduction and excess salt consumption.

There are distinct patterns of distribution of the type I mineralocorticoid and type II glucocorticoid receptors in the rat brain (1, 31). The affinity of the isolated type I receptor for aldosterone, corticosterone, or deoxycorticosterone is similar regardless of the source (2, 24, 29). Corticosterone production in the rat is generally about 100 times that of aldosterone; however, free circulating corticosterone is reduced to very low levels in normal unstressed animals by binding to plasma corticosterone binding globulin (CBG). However, the remaining free corticosterone should still suffice to favorably compete with aldosterone. Further specificity is thought to be conferred by the conversion of cortisol and corticosterone to inactive products, cortisone and 11-dehydrocorticosterone, by 11-β-hydroxysteroid dehydrogenase. This enzyme is present in tissues, such as the kidney, where aldosterone rather than corticosterone is the physiological ligand for the type I receptor (16). Recent immunochemical evidence showing that the enzyme and type I receptors are not necessarily found in the same cell in the kidney suggests a more complex explanation for type I receptor-ligand specificity (34). These modulators of corticosterone binding to the type I receptor have not been reported in the brain (8, 13, 10), where corticosterone binding to the type I receptor occurs at low physiological serum levels (9, 24, 32). Because corticosterone is less polar than aldosterone, one might also speculate that it traverses the blood-brain barrier with greater ease. There is also functional evidence of type I ligand specificity in the brain. The administration of corticosterone reverses some changes in behavior (5, 28, 33), brain serotonin (3, 10), and adrenergic receptor levels (23) produced in rats by adrenalectomy. Neither dexamethasone, which binds primarily the type II receptor and has low affinity for the type I receptor, nor aldosterone is effective in restoring these parameters. In fact, aldosterone blocks the normalizing effects of corticosterone in some systems (11). It has been argued that the mineralocorticoid or type I receptor to which the aldosterone in this model probably is binding is a corticosterone receptor in the brain and that aldosterone may serve little or
MATERIALS

Methods

Male out-bred Sprague-Dawley rats (Charles River), weighing 240–260 g at the onset, were sensitized for the development of mineralocorticoid hypertension by removing the right kidney and increasing salt consumption. Surgical procedures for right nephrectomy and intracerebroventricular cannulation, as well as the general procedures for handling the rats, measuring indirect blood pressure by the tail-cuff method, and collecting urine are described in detail elsewhere (18, 19). Surgery was performed under a combination of fentanyl and droperidol (Innovar-Vet, Pitman-Moore, NJ; 0.01 ml/100 g body wt subcutaneously) as preanesthetic and halothane plus nitrous oxide as anesthetic, with standard precautions to maintain sterility.

Five groups of 12 rats received intracerebroventricular infusions of artificial cerebrospinal fluid (CSF), aldosterone (10 ng/h), corticosterone (10 ng/h), aldosterone (10 ng/h) plus corticosterone (10 ng/h), or aldosterone (10 ng/h) plus corticosterone (20 ng/h). In a similar experiment, groups of 11 or 12 rats received intracerebroventricular infusions of artificial CSF, aldosterone (10 ng/h), corticosterone (20 ng/h), or aldosterone (10 ng/h) plus corticosterone (20 ng/h). Another study was done comparing the effects of intracerebroventricular infusion of aldosterone (10 ng/h), aldosterone (10 ng/h) plus RU26988 [a selective type II receptor agonist (17); 20 ng/h], and RU29688 (20 ng/h). The RU26988 was kindly provided by Roussel Uclaf, France; steroids (Sigma, St. Louis, MO) were infused with implanted miniosmotic pumps (Alzet 2002, Alza, Palo Alto, CA) that deliver 0.49 μl/h for 14 days. Pumps were changed on day 14 under halothane or isoflurane anesthesia, and pumps of the same lot were used throughout the experiment to ensure consistency. All solutions contained 2% propylene glycol and were made and sterilized by filtering through 0.2-μm filters (Acrodisc, Gelman Scientific) immediately before filling and implanting the pumps. The animals received standard chow (Purina 5001, 0.3% NaCl) and 0.9% NaCl plus 0.15% KCl to drink ad libitum. Blood pressures and weights were measured twice weekly; 24-h urine volumes were measured once a week.

Autopsies, including dye infusions to check cannula placement, were done at the conclusion of the study, and data from any animal, in which there was doubt about the delivery of the solutions or that had evidence of illness causing undue stress, were eliminated from the experiment. At the time of the biweekly pump changes, if the catheter was found to be disconnected from the pump or cannula, the data from the preceding 2 wk were discarded. The groups were thus reduced to no fewer than six animals by the end of the experiments. Data were compared by analysis of variance and the Dunnett t and Fisher PLSD tests (StatView 512+, BrainPower, Calabazas, CA).

RESULTS

The effects of the concomitant intracerebroventricular infusion of 10 ng/h aldosterone and 10 and 20 ng/h corticosterone on indirect systolic blood pressure are shown in Fig. 1. The mean indirect systolic blood pressures of the five groups did not differ statistically before infusion. By the end of the experiment on day 38, the mean pressures were unchanged for the CSF and corticosterone 10 ng/h groups and had increased from 123.1–140.7 mmHg for the aldosterone group. By day 17, the mean blood pressure of the aldosterone group was significantly greater than those of all other groups (P < 0.01). By day 31 the pressures of the aldosterone alone group were significantly greater than those of the two groups receiving corticosterone with the aldosterone. The pressures of both combination groups became significantly elevated compared with the CSF group by day 24, with the difference being greatest for the aldosterone 10 ng/h plus corticosterone 10 ng/h (P < 0.01) compared with the aldosterone 10 ng/h plus corticosterone 20 ng/h (P < 0.05) group. Body weight increased from a mean of 182 to 367 g during the experiment, but at no time were the weights significantly different between the groups. There was no clear effect on urine output by any of the infusions. The second experiment was actually done three separate times. In all three trials, the intracerebroventricular infusion of 20 ng/h of corticosterone alone produced no effect on the blood pressure but mitigated the increase produced by the concurrent infusion of aldosterone.

Figure 2 is a representative graph of mean blood pressures. The indirect systolic blood pressures of the four groups did not differ statistically before infusion. After 27 days of intracerebroventricular infusion, the blood pressures of those animals receiving CSF and 20 ng/h

![Graph showing indirect systolic blood pressure over time](http://ajpendo.physiology.org/)

**FIG. 1.** Indirect systolic blood pressure (BP) in uninephrectomized rats drinking a saline solution and receiving intracerebroventricular infusions of artificial cerebrospinal fluid (CSF), aldosterone (aldo), corticosterone (cortico), aldosterone 10 ng/h + corticosterone 10 ng/h (A10 + C10), or aldosterone 10 ng/h + corticosterone 20 ng/h (A10 + C20). *P < 0.01; + P < 0.05.
corticosterone were not significantly elevated, whereas the aldosterone group had increased from 120 to 147 mmHg, and the aldosterone plus corticosterone group had increased from 117 to 131 mmHg. The blood pressure of the aldosterone group had become significantly elevated ($P < 0.05$) compared with the other three groups by day 13. By day 16, the blood pressure of both the aldosterone and aldosterone plus corticosterone groups were significantly elevated ($P < 0.05$) compared with the CSF and corticosterone groups. By day 23 the aldosterone and aldosterone plus corticosterone groups were significantly different from each other ($P < 0.05$) and from the intracerebroventricular CSF and corticosterone groups ($P < 0.01$). There was no effect on weight gain or 24-h urine volumes. RU26988, a selective type II receptor agonist, had no effect on the blood pressure nor did it alter the pressor effect of intracerebroventricular aldosterone (Fig. 3). There was no difference in urine output or body weight.

**DISCUSSION**

The present study reconfirms that the chronic intracerebroventricular administration of a very small amount of aldosterone, 10 ng/h in this case, a dose that is not hypertensinogenic when delivered subcutaneously (18, 19), produces a significant elevation in systolic blood pressure. The intracerebroventricular infusion of corticosterone at 10 and 20 ng/h not only failed to produce an increase in blood pressure but it antagonized the effects of aldosterone in a graded manner. Aldosterone probably is acting through type I receptors, because the concomitant intracerebroventricular infusion of prorenone, a mineralocorticoid antagonist, blocks its pressor effect (18) and the intracerebroventricular infusion of RU29688, a selective type II agonist, had no effect on the blood pressure alone or in combination with aldosterone. We have found that the intracerebroventricular infusion of RU28318, a selective mineralocorticoid antagonist, at doses that have no effect when infused subcutaneously, blocks the hypertensinogenic effect of the subcutaneous infusion of aldosterone (20). Janiak and Brody (22) have also reported that DOCA-salt hypertension in rats can be blocked with the intracerebroventricular infusion of RU28318. The antagonism by corticosterone of the pressor effect of aldosterone in the present studies may have been caused by competitive binding to the type I receptor and/or to undefined counteractive effects resulting from binding to the type II receptor that are not duplicated by the "pure" type II agonist RU26988.

This experiment does not address where the intracerebroventricularly infused aldosterone is binding. Type I receptors in adrenalectomized rats are concentrated in the hippocampus, particularly the dorsal subiculum, CA1, areas, and dentate gyrus, and in the amygdala, lateral septum, and hypothalamus. The binding patterns of corticosterone and also aldosterone to the type I receptors in the brains of adrenalectomized animals is similar. However, in intact rats, Yongue and Roy (37) have shown that the regional distribution of endogenous aldosterone receptor binding within cell nuclei in the brain differs from that of corticosterone, most notably in the preponderance of aldosterone binding in the hypothalamus rather than in the hippocampus. Aldosterone binding within hippocampal cell nuclei appeared to be stable and was not greatly affected by altering endogenous aldosterone and corticosterone levels by varying salt consumption or by stressing the rats. McEwen et al. (27) found that corticosterone antagonized [3H]aldosterone binding in the periventricular regions of the hypothalamus of the adrenalectomized rat less effectively than in other regions. The mechanism of this selectivity is not yet clear. Aldosterone in this model of hypertension may be acting through these periventricular areas, as it is known that AV3V lesions block the development of DOCA-salt hypertension, a model of systemic mineralocorticoid excess (6). The relevance of aldosterone binding to hippocampal type I receptors has also been studied by Magariños et al. (26), who found that the effects of aldosterone on salt appetite in adrenalectomized rats was unaffected by hippocampectomy.

The type I receptor in the brain is thought to be almost 90% occupied by corticosterone under basal conditions (9, 31). Thus aldosterone received as a continuous infusion by adrenal intact animals in these studies may only
attain significant binding at the nadir of the normal circadian cycle of corticosterone secretion. Yongue and Roy (37) found that the only remarkable change in the amount of endogenous and aldosterone bound to the nuclei of cells in the hippocampus of intact rats occurred in the morning, when corticosterone levels are lowest. The reversal of certain adenoreceptor-induced behavioral and central biochemical changes by the administration of corticosterone is apparently mediated through binding to the type I receptor, because dexamethasone, which binds the type II receptor but has a very low affinity for the type I receptor, is ineffective in restoring these parameters (6, 29). Aldosterone not only fails to normalize these derangements produced by adrenalectomy, it apparently blocks the normalizing effects of corticosterone in some systems (11). Although they appear to be binding the same receptor in the brain, it is not known how corticosterone and aldosterone selectively produce different biological effects or antagonize each other.

The first step in steroid hormone action is the binding of the steroid to a specific receptor protein. This is followed by binding of the steroid-receptor complex to chromatin, resulting in mRNA transcription and protein translation. In the case of the type I adrenocorticoid receptor, tissue-dependent steroid specificity for aldosterone over corticosterone is conferred extrinsically by the preferential sequestration or catabolism of corticosterone. This certainly occurs in the periphery and may be happening in the hypothalamic areas, where aldosterone over corticosterone is conferred extrinsically by the preferential sequestration or catabolism of corticosterone. One explanation would be intrinsic tissue specificity at the level of steroid receptor complex binding to the chromatin. Evans (14) has described a “superfamily” of hormone receptors, sequence-specific DNA binding proteins, which function as ligand-dependent nuclear transcription factors to alter gene expression. Although most evidence suggests that the receptor is identical in all tissues from which it has been isolated (24, 36), subtle differences in molecular weight between rat hippocampal and renal type I receptors have been found, possibly resulting from a difference in posttranslational processing of the same gene product (12). Such functional preference of the mineralocorticoid receptor for aldosterone would be very important in the brain where neither CBG nor 11-β-hydroxysteroid dehydrogenase are reported to be present as determinants of ligand specificity. The existence of two such ligand-dependent transcription factors, one recognizing the type I-aldosterone complex, another the type I-corticosterone complex, could explain how these two steroids, which are presumed to bind the same receptor, could have different functions. If the “wrong” steroid-receptor complex could occupy the ligand-dependent transcription factor without inducing a given DNA transcriptional product, it would explain the antagonism by corticosterone of the elevation in blood pressure produced by the intracerebroventricular infusion of aldosterone or the antagonism by aldosterone of the central effects of corticosterone in adrenalectomized rats.

We are most grateful to R. Dersadt, Director of Pharmaceutical Research, Roussel Uclaf, for providing the RU26988.

This work was supported by medical research funds from the Department of Veterans Affairs and National Heart, Lung, and Blood Institute Grant HL-33997.

Address for reprint requests: F. P. Gómez-Sánchez, J. A. Haley VA Hospital (111-M), 13000 Bruce B. Downs Blvd., Tampa, FL 33612-4798.

Received 15 May 1989; accepted in final form 13 December 1989.

REFERENCES


20. GÓMEZ-SÁNCHEZ, E. P., C. M. PORT, AND C. E. GÓMEZ-SÁNCHEZ.


