INHIBITION OF THE EFFECTS OF ANGIOTENSIN II ON ADRENAL STEROID PRODUCTION BY DIETARY SODIUM

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Communicated by Robert W. Berliner, April 24, 1969

Abstract.—The pressor octapeptide, angiotensin II, can stimulate the production of aldosterone by the adrenal cortex. The present results show that in the dog a high-sodium diet can eliminate the steroidogenic action of angiotensin II, which is thus dissociated from the pressor action which remains.

Angiotensin II was infused intravenously for 48 hours into conscious, undisturbed hypophysectomized dogs that were receiving each day either 60 or 200 mEq of dietary sodium. Blood pressure and secretion of aldosterone, corticosterone, and cortisol were measured (1) throughout the infusion in some dogs, and (2) at the end of the infusion in all dogs. In those dogs receiving 60 mEq of sodium, angiotensin II elevated the blood pressure and produced sustained increases of secretion of aldosterone, corticosterone, and cortisol. In those dogs receiving 200 mEq of sodium, angiotensin II, while retaining its pressor activity, had no effect on the production of aldosterone, corticosterone, or cortisol after 24 hours. Thus, if angiotensin II can produce hypertension clinically, there need not be secondary aldosteronism as well.

Introduction.—A large body of evidence has been interpreted to indicate that angiotensin II is the major humoral agent that stimulates the secretion of aldosterone during depletion of body sodium in the dog. Sodium depletion produces sustained increases in aldosterone secretion. If the concentration of angiotensin II in the plasma were the sole physiologic regulator of this hypersecretion of aldosterone, one would expect that infusion of angiotensin II would constitute a sustained stimulus to the secretion of aldosterone. Urquhart *et al.*[1] have reported, with measurements of urinary aldosterone, such a sustained stimulus of angiotensin II in studies on normal dogs receiving 60 mEq of dietary sodium each day. In the present studies we have found that angiotensin II will not elicit a sustained hypersecretion of aldosterone in sodium-loaded, hypophysectomized dogs even when sustained hypertension is produced by the infusion. Angiotensin II did produce a sustained hypersecretion of aldosterone when infused into hypophysectomized dogs receiving a diet containing 60 mEq of sodium each day. Thus, it appears that the effects of angiotensin II in producing hypertension and its effects on the biogenesis of aldosterone can be dissociated. This suggests that the effects of angiotensin II on the biogenesis of aldosterone are complex and can be abolished by other factors such as the dietary sodium intake.

Methods.—Mongrel dogs were used for all experiments. They were all fed the same synthetic diet, except that either 60 mEq or 200 mEq of sodium had been added to each daily portion as sodium chloride. Correct sodium intake was maintained by adding sodium chloride to the drinking water of the occasional animal that would not eat. Within

24 hr of the start of the angiotensin infusion, they were hypophysectomized by the buccal approach. Angiotensin II (CIBA) was administered in 5% dextrose in water through a polyethylene cannula inserted into a leg vein. The infusion was maintained for 48 hr at a rate of 0.75 ml/min with a Bowman constant-infusion pump. The solution of angiotensin II was replaced every 4 hr to minimize loss of angiotensin activity.

A polyethylene cannula was also placed in a femoral artery to measure arterial pressure with a strain-gauge transducer. Blood pressure was measured hourly during the infusion. In some, an adrenal venous catheter was inserted and led out through a stab wound. These catheters were carefully placed so that the dogs could have maximal comfort and mobility in their cages.

Samples of adrenal venous blood to be analyzed for aldosterone, corticosterone, and cortisol were drawn (1) periodically throughout the infusion in 8 dogs and (2) at the end of the infusion in the remaining 20 dogs. In a given experiment, the production of aldosterone by these dogs was compared to that of dogs receiving the same diet and hypophysectomized at the same time, but not given angiotensin. No heparin was given at any time during the infusion.

Outer slices from the adrenal cortex were prepared from dogs receiving 200 mEq of sodium each day, some of which had received angiotensin infusions and some of which had not. These were incubated *in vitro* as described previously,[3] and their production of aldosterone, corticosterone, and cortisol was measured.

In three dogs receiving the 200 mEq Na diet, blood volume was measured at the start and at the end of infusion with ^{131}I-labeled human serum albumin.

Aldosterone, corticosterone, and cortisol were measured in adrenal venous blood by the method of Kliman and Peterson,[2] modified by us as previously described.[3] Sodium and potassium in adrenal venous blood was measured by flame photometry, with lithium as internal standard.

Results.—Effect of infusion of angiotensin into hypophysectomized dogs with indwelling adrenal venous catheters: Figure 1 shows the results of infusion of angiotensin II at a rate of 40 μg/kg/day into eight dogs receiving either the 60-mEq or the 200-mEq sodium intake. In all dogs, angiotensin initially increased the secretion of aldosterone.[4-6] The dogs receiving the high-sodium intake showed a progressive loss of the effect of angiotensin on the secretion of aldosterone, whereas those receiving the 60-mEq sodium intake showed a sustained effect of angiotensin over the two days of the infusion. The sustained effect of angiotensin over several days of infusion in the dogs receiving the 60-mEq sodium intake is in agreement with results of previous studies.[1, 7] In all the dogs, regardless of intake, there was a marked, sustained pressor response to this dosage of angiotensin (Fig. 1). At the end of 48 hours, an *increase in the rate* of infusion of angiotensin to 120 μg/kg/day again produced an increase in the secretion of aldosterone in the dogs receiving 200 mEq of sodium per day. This rate of infusion was not prolonged to see whether this stimulus would become ineffective, because this dosage of angiotensin was not tolerated for long periods of time.

There was no evidence of sodium loss in any of the dogs receiving angiotensin. Serum sodium concentration remained stable in all dogs during the infusion. Body weight was either stable or increased during the infusion in all dogs. During the infusion of angiotensin, four dogs were on daily collections of urine. Over the two-day infusion, two dogs receiving 60 mEq of sodium retained 99 mEq and 30 mEq of sodium, respectively; two dogs receiving 200 mEq of sodium retained 6 mEq and 337 mEq of sodium, respectively.

Effect of 48 hours of infusion of angiotensin into hypophysectomized dogs receiving

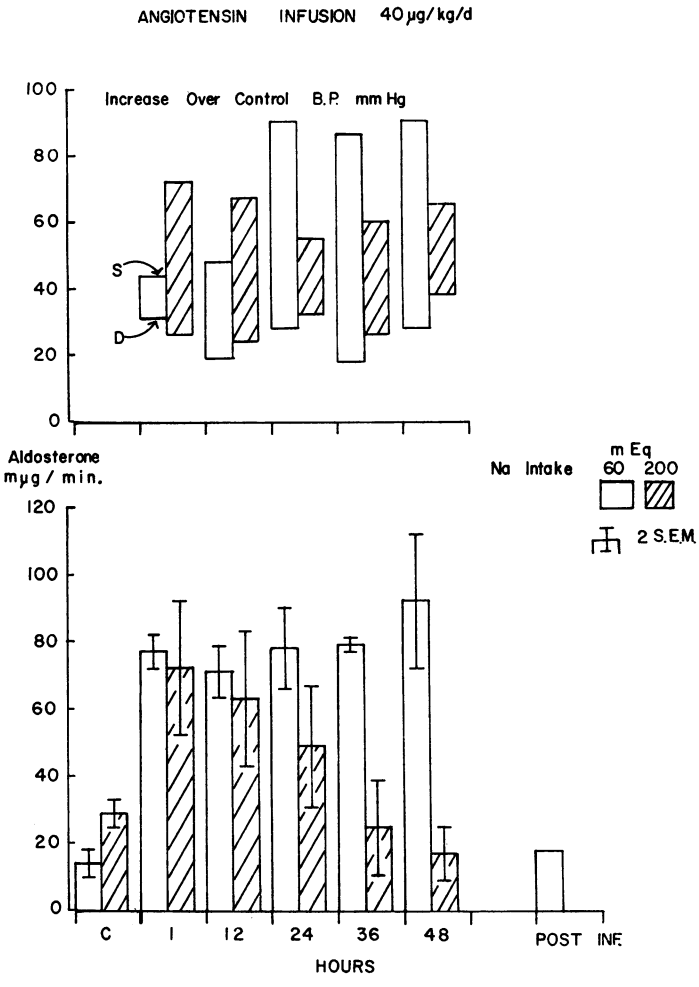

FIG. 1.—Effects of sustained infusion of angiotensin II on blood pressure and on secretion of aldosterone in conscious, hypophysectomized dogs.

TABLE 1. *Effect of 48-hour infusions of angiotensin II and of dietary sodium intake on blood pressure, serum sodium and potassium concentrations, hematocrit, adrenal blood flow, and adrenal steroid secretion in hypophysectomized dogs.*

	No. of dogs	Dietary sodium intake	Dose of angiotensin (µg/kg/day)	Average Increase in Blood Pressure during Infusion (mm Hg) Systolic	Diastolic	Serum Na (mEq/liter)
Control	7	60 mEq/day	0	...	...	...
Infusion	8	60 mEq/day	40	76 ± 5	33 ± 5†	146 ± 4
Control	9	200 mEq/day	0	...	...	...
Infusion	2	"	20	...	...	...
Infusion	11	"	40	55 ± 10	40 ± 5†	139 ± 4
Infusion	3	"	70	65 ± 8	45 ± 5	...

* $p < 0.025$.
† $p < 0.01$.

60 mEq or 200 mEq of sodium daily: Table 1 shows the tabulated results of 48-hour infusions of angiotensin into 24 dogs receiving daily 60 mEq or 200 mEq of sodium on blood pressure, serum electrolyte concentration, adrenal blood flow, and the secretion of aldosterone, corticosterone, and cortisol. The final adrenal venous specimen from the eight dogs with the indwelling venous catheters was included in this table. Angiotensin produced similar increases in blood pressure in both groups. The dogs receiving angiotensin and taking the 60 mEq-sodium diet had secretion rates of aldosterone, corticosterone, and cortisol significantly higher than those of control dogs not receiving angiotensin. The secretion rates of the dogs receiving angiotensin are in the same range as those of dogs given low-sodium diets for two days.[3]

In the sodium-loaded dogs receiving angiotensin at rates of 20, 40, or 70 μg/kg/day, the secretion rates of aldosterone, corticosterone, and cortisol *at 48 hours* were at or below the values found in dogs not receiving the angiotensin infusion. There were no consistent differences among groups as regards the serum sodium or potassium concentrations, the hematocrit, or the adrenal venous blood flow.

When adrenal slices from dogs taking the 200-mEq sodium intake and given the infusion of angiotensin were incubated *in vitro*, the production of aldosterone and corticosterone was lower than that of slices from dogs receiving a 200-mEq sodium intake alone (Table 2).

In contrast to these results, the adrenal cortex of dogs receiving a low-sodium intake for 48 hours shows a sustained increase of secretion of aldosterone *in vivo* and *in vitro*.

In three dogs receiving the 200-mEq sodium diet and angiotensin, 40 μg/kg/day, which had lost its effect on steroidogenesis, the blood volume was decreased by the end of the 48-hour infusion period (Fig. 2).

Discussion.—These results demonstrate that the responsiveness of the adrenal cortex to infused angiotensin II is variable and can be influenced by the dietary sodium. In the dogs receiving the high-sodium intake the angiotensin-induced increase in secretion of aldosterone was not maintained.

It is known that there can be variations in the pressor response to angiotensin II. In the sodium-depleted state and in conditions associated with hyperaldosteronism, increased plasma renin, and hypertension, the sensitivity to the

TABLE 1. (*continued*)

Serum K (mEq/liter)	Hct (%)	Adrenal flow (ml/min)	Steroid Secretion (mμg/min) Aldosterone secretion	Corticosterone secretion	Cortisol secretion
...	45 ± 2	4.0 ± 5	32 ± 2	33 ± 7	39 ± 13
4.6 ± 0.6	45 ± 1	5.3 ± 1	110* ± 30	73 ± 9†	116 ± 20*
...	43 ± 2	2.5 ± 0.9	22 ± 2	12 ± 2	23 ± 10
...	...	...	6	10	18
4.2 ± 0.3	48 ± 4	3.5 ± 0.9	14 ± 4	14 ± 6	27 ± 11
...	48 ± 3	6 ± 2	18 ± 4	16 ± 6	16 ± 5

TABLE 2. *In vitro secretion of aldosterone, corticosterone, and cortisol by outer slices of adrenal cortex of hypophysectomized dogs fed 200 mEq of sodium each day: Effect of 48-hour infusion of angiotensin II.*

	No.	Aldosterone (μg/gm/hr)	Corticosterone (μg/gm/hr)	Cortisol (μg/gm/hr)
Control	8	4.36 ± 0.08	6.5 ± 0.16	1.27 ± 0.2
Angiotensin II 48 hr	8	1.10 ± 0.08	1.7 ± 0.9	1.86 ± 0.5

pressor effect of angiotensin II is reduced.[8, 9] In addition, the infusion of angiotensin into sodium-replete subjects is characterized by a progressive increase in the sensitivity to the pressor effects of the drug.[9, 10] However, in short-term experiments, pressor doses of angiotensin II are always effective in stimulating the secretion of aldosterone.[4–6] A dissociation between a pressor dose of angiotensin and its steroidogenic effect has not been heretofore reported in the dog.

A similar dissociation between the pressor dose of angiotensin II and its steroidogenic effect has been found in the sodium-replete sheep.[11–13] In these studies, infusion of angiotensin II in dosages that produced mild pressor effects produced sustained increases in plasma angiotensin II concentrations as determined by radioimmunoassay.[13]

Angiotensin probably promotes steroidogenesis by potentiating the conversion of cholesterol to pregnenolone.[14] Sodium depletion increases the production of angiotensin[15, 16] but, in addition, promotes the conversion of corticosterone to aldosterone.[3] Sodium loading, as in these experiments, could thus limit the production of aldosterone in response to angiotensin by inhibiting conversion of corticosterone to aldosterone. Such inhibition would not explain the present results, as sodium loading also lowers the production of corticosterone in response to angiotensin. Clearly, it must affect biosynthetic pathways at an earlier site.

One explanation for the present results is suggested by a recent report that renin can increase adrenal cortical sensitivity to angiotensin as a stimulus to steroidogenesis.[17] Thus, sodium loading might act by depression of endogenous renin production. The present results neither support nor reject this hypothesis.

Expansion of blood volume could be one mechanism by which the adrenal

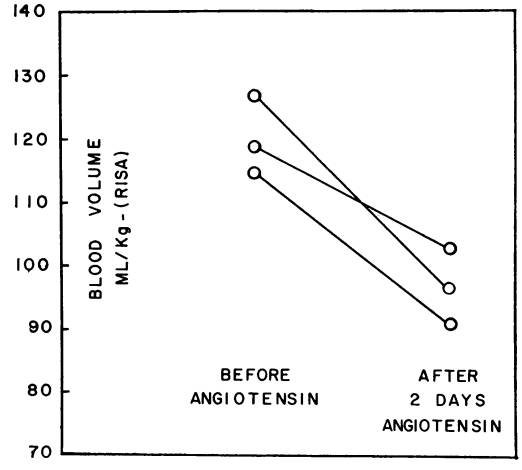

FIG. 2.—Effect of sustained infusion of angiotensin II on blood volume in three dogs receiving the high-sodium diet.

response to angiotensin II is diminished in the sodium-loaded dogs. However, in three dogs receiving 200 mEq of dietary sodium each day, the blood volume fell during the infusion of angiotensin. The cause of this fall in blood volume is not clear.

The present results do not reject the postulated role of the renin-angiotensin system in the regulation of the secretion of aldosterone. They do suggest, however, that the regulation of the secretion of aldosterone is complex and not necessarily determined by the plasma concentration of angiotensin II.

Whatever the mechanism for this "escape" from the steroidogenic effects of angiotensin, it has important clinical implications. If hypertension can result from sustained overproduction of angiotensin, it is clear from these experiments that patients with this type of hypertension need not manifest sustained overproduction of aldosterone. Sodium loading alone could inhibit production of aldosterone, even if it did not lower angiotensin production.

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[1] Urquhart, J., J. O. Davis, and J. T. Higgins, *Am. J. Physiol.*, **205**, 1241 (1963).
[2] Kliman, B., and R. E. Peterson, *J. Biol. Chem.*, **235**, 1639 (1960).
[3] Davis, W. W., L. R. Burwell, A. G. T. Casper, and F. C. Bartter, *J. Clin. Invest.*, **47**, 1425 (1968).
[4] Carpenter, C. C. J., J. O. Davis, and C. R. Ayers, *J. Clin. Invest.*, **40**, 2026 (1961).
[5] Mulrow, P. J., W. F. Ganong, G. Cera, and A. Kuljian, *J. Clin. Invest.*, **41**, 505 (1962).
[6] Slater, J. D. H., B. H. Barbour, H. H. Henderson, A. G. T. Casper, and F. C. Bartter, *J. Clin. Invest.*, **42**, 1504 (1963).
[7] Urquhart, J., in *Proceedings of the 47th Endocrine Society*, 62, (1965).
[8] Laragh, J. H., *Circulation*, **25**, 203 (1962).
[9] Ames, R. P., A. J. Borkowski, A. M. Sicinski, and J. H. Laragh, *J. Clin. Invest.*, **44**, 1171 (1965).
[10] Slater, J. D. H., C. S. Delea, and F. C. Bartter, in preparation.
[11] Blair-West, J. R., J. P. Coghlan, D. A. Denton, J. R. Goding, J. A. Munro, R. E. Peterson, and M. Wintour, *J. Clin. Invest.*, **41**, 1606 (1962).
[12] Blair-West, J. R., J. P. Coghlan, D. A. Denton, J. R. Goding, M. Wintour, and R. D. Wright, *Recent Progr. Hormone Res.*, **19**, 311 (1963).
[13] Blair-West, J. R., G. W. Boyd, J. P. Coghlan, D. A. Denton, J. R. Goding, M. Wintour, and R. D. Wright, *Proc. 23rd Int. Congr. Physiological Sciences*, Tokyo, (1965).
[14] Kaplan, N., and F. C. Bartter, *J. Clin. Invest.*, **41**, 715 (1962).
[15] Brown, J. J., D. L. Davis, A. F. Lever, and J. I. S. Robertson, *Lancet*, **2**, 278 (1963).
[16] Binion, P. F., J. O. Davis, T. C. Brown, and M. J. Olichney, *Am. J. Physiol.*, **208**, 655 (1965).
[17] Ganong, W. F., A. T. Boryczka, and R. Shackelford, *Endocrinology*, **80**, 703 (1967).