9. H. A. Krebs and R. L. Veech, in *The Energy Level and Metabolic Control in Mitochondria*, S. Papa, J. M. Tager, E. Quagliariello, E. C. Slater, Eds. (Adriatica Editrice, Bari, Italy, 1969), pp. 329–382.

10. Metabolite concentrations (μmole/g) in brains from control groups of six to ten adult rats and ten suckling rats were, respectively: glucose, 1.39 and 1.47; glucose 6-phosphate, 0.146 and 0.141; fructose 1,6-diphosphate, 0.019 and 0.012; dihydroxyacetone phosphate, 0.017 and 0.015; 2-phosphoglyceric acid, 0.0033 and 0.0025; phosphoenolpyruvate, 0.0048 and 0.0035; pyruvate, 0.086 and 0.111; lactate, 1.29 and 1.36; citrate, 0.275 and 0.321; α-oxoglutarate, 0.193 and 0.165; malate, 0.264 and 0.272; adenosine triphosphate, 2.38 and 2.51; adenosine diphosphate, 0.563 and 0.553; creatine phosphate, 3.55 and 3.54.

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Angiotensin II: Important Role in the Maintenance of Arterial Blood Pressure

Abstract. *An angiotensin II antagonist, [1-sarcosine, 8-alanine]-angiotensin II, was given intravenously to anesthetized dogs with thoracic caval constriction and ascites to investigate the role of angiotensin II in the control of arterial pressure. The antagonist produced a striking fall in arterial pressure and in aldosterone secretion and an accompanying increase in plasma renin activity. In a control experiment, normal anesthetized dogs were given the angiotensin analog, but it failed to reduce arterial pressure or to influence plasma renin activity. In conscious dogs with caval constriction, the antagonist produced essentially the same drop in arterial pressure as observed in anesthetized animals. These results suggest an important role for angiotensin II in the maintenance of arterial pressure by its action on specific receptor sites in arteriolar smooth muscle and in the adrenal cortex.*

In 1962, it was discovered that dogs with caval constriction (*1*) or sodium depletion (*1*) and patients with decompensated cirrhosis of the liver (*2*) are less sensitive to synthetic angiotensin II in their pressor response than are normal dogs or normal humans. This phenomenon has never been explained. The experiments reported here were designed to study this problem again and were based on the hypothesis that angiotensin II acts on the peripheral arterioles and plays a role in maintaining blood pressure in these pathophysiological states. It was reasoned that if angiotensin II is displaced from its receptor sites in the smooth muscle of the peripheral arterioles by a competitive antagonist, the arterial pressure will fall. This hypothesis was examined by infusing intravenously an analog of angiotensin II, [1-sarcosine, 8-alanine]-angiotensin II ([Sar^1, Ala^8]-A II), into dogs with thoracic inferior vena cava constriction and ascites. Pals *et al.* (*3*) demonstrated that this compound acts as a competitive antagonist of angiotensin II in the rat.

This angiotensin II antagonist also provides a unique opportunity for evaluating the relative importance of the renin-angiotensin system in the control of aldosterone secretion during caval constriction. Since the renin-angiotensin-aldosterone system is important in the control of sodium excretion and blood volume and thus, indirectly, of blood pressure, it was decided to study the effect of this angiotensin II analog on steroid secretion. A response of a marked decrease in aldosterone secretion would point to an indirect function via blood volume control for angiotensin II in the maintenance of blood pressure.

Under sterile conditions, nine female mongrel hounds were subjected to thoracic inferior vena cava constriction to produce ascites (*4*). Sodium balance studies were conducted while the dogs had a sodium intake of 65 meq/day; every dog showed marked sodium retention and ascites at the time of the experiment. Two days before the study, a catheter was placed in the left adrenolumbar vein (*5*). For the experiment, the dogs were anesthetized with pentobarbital and given 6 mg of dexamethasone (Decadron phosphate, Merck Sharp & Dohme) intramuscularly to depress anterior pituitary function. After control measurements of femoral arterial pressure for 30 minutes and after collection of adrenal venous blood for steroids and external jugular venous blood for plasma renin activity (PRA), [Sar^1, Ala^8]-A II was infused intravenously for 45 minutes at a rate of 6 μg kg^{-1} min^{-1}. Measurements were made at three 15-minute intervals for steroids, and PRA and blood pressure was recorded continuously; two recovery observations were made at 45 and 60

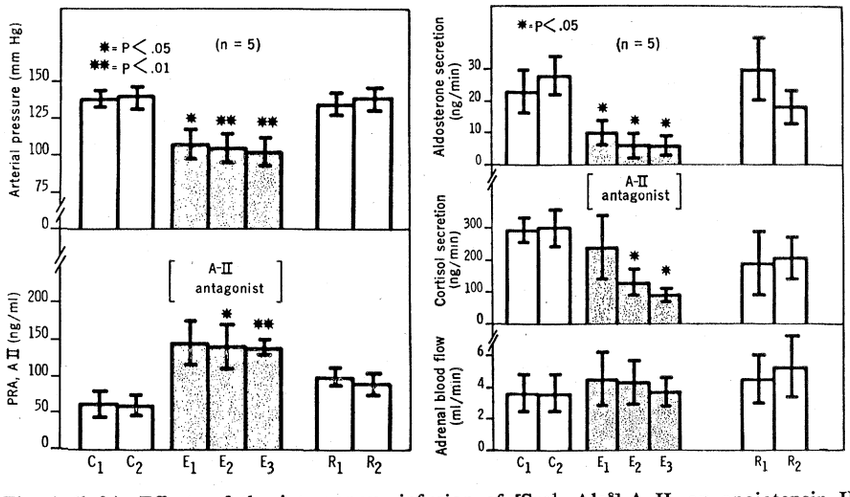

Fig. 1 (left). Effects of the intravenous infusion of [Sar^1, Ala^8]-A II, an angiotensin II (*A-II*) antagonist, on femoral arterial pressure and plasma renin activity (*PRA*) in five dogs with thoracic caval constriction. The abbreviations C_1, C_2, E_1, E_2, E_3, R_1, and R_2 represent control, experimental, and recovery periods. Fig. 2 (right). Effects of the A-II antagonist on aldosterone and corticol secretion and adrenal blood flow in the same dogs with thoracic caval constriction.

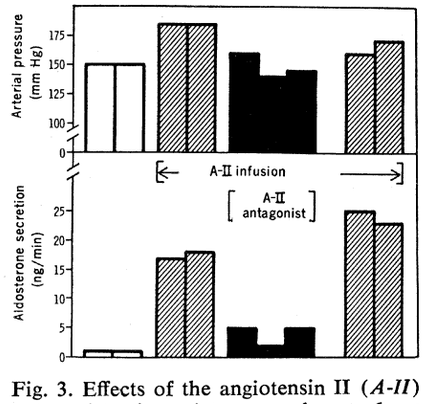

Fig. 3. Effects of the angiotensin II (*A-II*) antagonist given intravenously at 6 μg kg^{-1} min^{-1} on arterial pressure and aldosterone secretion during the simultaneous infusion of A-II at 1.5 μg min^{-1} in a normal dog.

minutes after stopping the infusion. All blood removed for analysis was replaced by fresh donor blood. Steroids were measured by the double isotope derivative assay (*6*), and PRA was determined by the method of Schneider *et al.* (*7*).

The angiotensin II antagonist produced a striking drop in arterial pressure and an associated increase in PRA (Fig. 1). Initially, PRA was markedly elevated as a result of caval constriction, and it more than doubled during infusion of the angiotensin II analog. Both aldosterone and cortisol secretion fell, and some of the values for aldosterone output were very low (Fig. 2).

As a control experiment, arterial pressure and PRA were studied similarly in six normal anesthetized dogs with the angiotensin II antagonist; arterial pressure increased from 5 to 10 mm-Hg during the first 5 minutes, but returned to the control level and remained normal thereafter; PRA was unaltered. Also, three conscious dogs with thoracic caval constriction received the same dose of the angiotensin II analog, and the drop in arterial pressure was essentially the same (Table 1) as that observed for five anesthetized dogs with caval constriction (Fig. 1). It appears, therefore, that the response in arterial pressure was not influenced appreciably by anesthesia.

Pals *et al.* (*3*) have described the action of [Sar^1, Ala^8]-A II as a competitive antagonist to angiotensin II during studies on vascular smooth muscle in the rabbit and the rat. Brunner *et al.* (*8*) have found that the antagonist decreased arterial pressure in rats with two-kidney but not one-kidney hypertension. To examine the response to this angiotensin II antagonist in the dog, synthetic angiotensin II (Hypertensin, Ciba) was infused intravenously into three normal animals before, during, and after the infusion of the angiotensin II antagonist. A typical response is shown in Fig. 3; the angiotensin II antagonist blocked both the vasoconstrictor and steroidogenic effects of angiotensin II. In other studies in our laboratory, the angiotensin II antagonist used here also completely blocked the decrease in renal blood flow produced by angiotensin II in the dog, but was without effect in blocking the action of norepinephrine. Collectively, these data and the data of Pals *et al.* (*3*) and Brunner *et al.* (*8*) indicate that the angiotensin II analog acts as a competitive antagonist to angiotensin II.

Table 1. Effect of the angiotensin II antagonist [Sar^1, Ala^8]-A II on femoral arterial pressure in dogs with thoracic caval constriction. Pressures are given in millimeters of Hg. The antagonist was infused intravenously at a rate of 6 μg kg^{-1} min^{-1}; S.E.M., standard error of the mean; C_1 and C_2, control periods 1 and 2.

Dog	Pressure at control period		Pressure during infusion after:		
	C_1	C_2	15 min	30 min	45 min
1	135	135	120	110	120
2	115	115	90	70	70
3	140	140	125	120	120
Mean ± S.E.M.	130 ± 8	130 ± 8	112 ± 11	100 ± 15	103 ± 17

In the dog with thoracic caval constriction, the cardiac output is frequently decreased and arterial pressure is maintained at the normal level by an increase in peripheral resistance (*4*). The data reported here support the concept that arterial pressure is maintained in this situation by an action of angiotensin II on arteriolar smooth muscle. Indeed, it seems likely that the observed fall in arterial pressure resulted from the competitive displacement of angiotensin II from the receptor sites at the arteriolar level. The important functional role of this mechanism for maintenance of arterial pressure is indicated by an average drop in arterial pressure from 140 to 107 mm-Hg for the five anesthetized dogs (Fig. 1) and from 130 to 100 mm-Hg for the three conscious dogs (Table 1). Since the pathophysiology in dogs with caval constriction is almost identical to that in experimental low-output heart failure (*9*), it seems likely that angiotensin II helps to support arterial pressure in patients with congestive heart failure when PRA is elevated. And the decreased pressor response to exogenous angiotensin II observed in 1962 (*1*) now seems explicable on the basis of "saturation" of available receptor sites in arteriolar smooth muscle with endogenous angiotensin II as a consequence of the hyperangiotensinemia.

A reasonable interpretation of the decrease in aldosterone secretion is that the antagonist replaced angiotensin II at the receptor sites in the adrenal cortex. This suggestion is supported by the associated fall in cortisol secretion since angiotensin II acts early in steroidogenesis at the cholesterol and pregnenolone step to increase cortisol as well as aldosterone secretion (*10*). The finding of an increase rather than a decrease in PRA in response to the antagonist is also consistent with this interpretation. The data reported here for studies with the antagonist agree with the early finding that bilateral nephrectomy of hypophysectomized dogs with caval constriction reduced aldosterone secretion almost to zero (*11*). These results, therefore, support the concept of a renin-angiotensin-aldosterone system in the regulation of blood volume and, indirectly, in the control of blood pressure. These relationships have been described in detail in a control system analysis of the renin-angiotensin-aldosterone system (*12*).

More recently, this angiotensin II analog has been studied (*13*) in sodium-depleted dogs with essentially the same results as described here. These new observations point to the homeostatic role of angiotensin II in its action on vascular smooth muscle and control of blood pressure since the situation of sodium depletion is only one step removed from the day-to-day normal regulation of sodium balance.

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References and Notes

1. J. O. Davis, P. M. Hartroft, E. O. Titus, C. C. J. Carpenter, C. R. Ayers, H. E. Spiegel, *J. Clin. Invest.* **41**, 378 (1962).
2. J. H. Laragh, *Circulation* **25**, 203 (1962).
3. D. T. Pals, F. D. Masucci, G. S. Denning, Jr., F. Sipos, D. C. Fessler, *Circ. Res.* **29**, 673 (1971).
4. J. O. Davis and D. S. Howell, *ibid.* **1**, 171 (1953).
5. J. O. Davis, M. M. Pechet, W. C. Ball, Jr., M. J. Goodkind, *J. Clin. Invest.* **36**, 689 (1957).
6. B. Kliman and R. E. Peterson, *J. Biol. Chem.* **235**, 1639 (1960).
7. E. G. Schneider, J. O. Davis, C. A. Robb, J. S. Baumber, *Circ. Res.* **24**, 213 (1969).
8. H. R. Brunner, J. D. Kirshman, J. E. Sealey, J. H. Laragh, *Science* **174**, 1344 (1971).
9. J. O. Davis, in *Handbook of Physiology*, W. F. Hamilton, Ed. (American Physiological Society, Bethesda, Md., 1965), sect. 2, vol. 3, p. 2071.
10. ———, in *The Adrenal Cortex*, A. B. Eisenstein, Ed. (Little, Brown, Boston, 1967), pp. 203–247.
11. ———, C. R. Ayers, C. C. J. Carpenter, *J. Clin. Invest.* **40**, 1466 (1961).
12. E. H. Blaine, J. O. Davis, P. D. Harris, *Circ. Res.* **30**, 713 (1972).
13. J. A. Johnson and J. O. Davis, *ibid.*, in press.
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