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Research Article

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ABSTRACT During intravenous administration of varying doses of angiotensin II antibody to anesthetized rats, apparently specific vascular receptors were characterized. These receptors compete with administered antibody to bind circulating angiotensin. This competitive phenomenon was used to evaluate the affinity of these receptors for angiotensin. Apparent vascular receptor affinity was defined by the amount of antibody required to block the blood pressure response to exogenous angiotensin. It was found that this receptor affinity varies directly with sodium intake so that the amount of antibody required to block was eightfold greater in normal animals on a high sodium intake, as compared with those on a low sodium intake. Sodium dependence of receptors was also demonstrated in nephrectomized animals, in desoxycorticosterone (DOC)-treated rats, and in chronic renal hypertension. Thus the observed changes in receptor affinity were usually inversely related to measured endogenous angiotensin II levels. Ganglionic blockade increased antibody requirement eightfold. All of these changes were consistent, with no overlap observed in response of individual animals from different groups. These results may explain the variation in pressor activity of angiotensin associated with changes in salt balance and ganglionic blockade.

In general, when sufficient antibody was injected to block the effect of exogenous angiotensin a blood pressure lowering effect was also observed. Two exceptions were the nephrectomized and the one-kidney renal hypertensive animals, in both of which antibody administration had no effect on blood pressure.

Additional results suggest that changes in receptor affinity are involved in the pathogenesis of various types of experimental hypertensions because the amount of antibody required to block angiotensin was enhanced in renal (twofold), DOC (fourfold), and genetic (fourfold) hypertension. Accordingly, changes in the affinity of these receptors could be critically involved in normal blood pressure control and in various forms of experimental and clinical hypertension, even when circulating angiotensin II levels are normal.

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INTRODUCTION

The renin-angiotensin system plays an important role in sodium homeostasis. In states of sodium depletion plasma renin levels are elevated (1). Furthermore, angiotensin II, formed when renin reacts with a plasma alpha-2-globulin (renin substrate), has been shown to stimulate aldosterone secretion (2). Angiotensin is by weight the most potent pressor substance known (3). Notwithstanding, the role of the renin-angiotensin system in the maintenance of normal blood pressure and in the pathogenesis of various forms of experimental and naturally occurring hypertensive disorders remains to be defined.

Sodium depletion, which is associated with high levels of circulating angiotensin II is not associated with any increase in blood pressure, and this lack of hypertension has been attributed to volume depletion and reduction of pressor responsiveness to angiotensin (4). However, in other studies, elevated plasma renin levels have been observed and implicated in experimental and clinical forms of renal or malignant hypertension (5, 6), and it has been assumed that increased circulating angiotensin II plays a causal role in these hypertensions. However, results obtained in a number of other clinical and experimental studies have shed serious doubt on the role of the renin-angiotensin system in the pathogenesis of hypertensive disorders, because the measured plasma

renin and angiotensin levels have often been found to be normal (7, 8). Furthermore, in other studies utilizing the induction or administration of antibody to angiotensin II it has not been possible to correct the hypertension (9–11).

Despite the demonstration of normal circulating levels of renin and angiotensin II in experimental or naturally occurring hypertension of renal origin it is possible that angiotensin may still play a causal role in the maintenance of the elevated blood pressure. For example, there could be an increase in the sensitivity of critical vascular receptors to circulating angiotensin II. If such is the case, then renin and angiotensin might be critically involved in the maintenance of various other forms of experimental or clinical hypertension in which plasma levels are known to be normal or even subnormal.

To study this question of altered vascular receptor affinity or increased pressor responsiveness, we have administered varying amounts of specific angiotensin antibody to rates under different physiological conditions. The data obtained suggest the existence of apparently specific vascular receptors for angiotensin II which have the capacity to compete with the administered antibody to bind endogenous angiotensin II. This competitive phenomenon has then been used to reveal changes in the apparent affinity of these receptors. We have found that vascular receptor affinity varies directly with sodium intake. Furthermore, this receptor affinity is enhanced during ganglionic blockade and by nephrectomy. Receptor affinity to angiotensin II was also consistently increased in the several forms of experimental hypertension tested to date.

The whole experience describes a role for the renin angiotensin system in both normal blood pressure control and in the maintenance of various types of experimental hypertension. The data suggest that because of changes in affinity of angiotensin receptors, angiotensin II could be critically involved in blood pressure regulation even when measured circulating levels of renin and angiotensin are normal.

METHODS

Preparation of the experimental animal models. Male Sprague-Dawley rats weighing from 150 to 250 g were divided into 15 groups of 15 animals each and were maintained for 6 wk on one of three different diets. (*a*) normal sodium intake: Purina rat chow (Ralston Purina Co., St. Louis, Mo.) containing 0.42% sodium. (*b*) sodium-free intake: sodium-free rat chow from General Biochemicals, Chagrin Falls, Ohio. (*c*) high sodium intake: Purina rat chow containing 0.42% sodium and 0.9% saline provided as drinking water. The rats were allowed free access to food and fluid. After 5 wk of the constant diet, jugular vein blood was collected for determination of circulating angiotensin II. This sample was collected at least 1 wk before the standard antibody injection procedure to minimize the effect of the venosection on the response to antibody injection. The animals were weighed each week and their blood pressure was measured using the microphonic tail method (12). At the end of the balance period the animals weighed from 350 to 500 g.

TABLE I
Characteristics of Experimental Groups

Preparation	Na intake (n)	Final weights*	BP‡
		g	*mm Hg*
Normals	0 (6)	344.2 ±4.2	129.2 ±2.4
	Normal (6)	350–450 (r)§	120.0 ±2.9
	Normal (4)	421.3 ±41.1	121.3 ±6.6
	High (6)	460.8 ±13.6	118.7 ±2.9
Goldblatt two kidneys	0 (6)	400–490 (r)	175.8 ±3.3
	Normal (7)	451.5 ±9.2	182.5 ±4.2
	Normal (6)	384.7 ±20.8	195.8 ±12.0
Goldblatt one kidney	Normal (8)	391.6 ±15.4	212.6 ±10.8
DOCA-treated	0 (6)	337.5 ±6.7	125.8 ±1.5
	High (6)	340.0 ±10.6	151.0 ±2.0
Spontaneously hypertensive	0 (6)	426.7 ±13.3	198.3 ±7.6
	Normal (9)	422.3 ±18.3	195.0 ±2.4
Adrenalectomy	High (6)	394.2 ±18.2	101.7 ±1.7
Nephrectomy	0 (6)	320.0 ±15.5	100.0 ±2.6
	Normal (6)	436.7 ±7.6	105.0 ±1.8

* Values given as mean ± standard error.
§ (r), Range for entire group.
‡ Blood pressure, measured directly in carotid artery.

In addition to the groups of normal animals, renal hypertensive rats were prepared by placing a silver clip on the left renal artery. The opposite kidney was left untouched. Other rats were prepared in the same manner but the contralateral kidney was removed (one-kidney renal hypertension). DOC-hypertension was induced by placing two 25 mg pellets of desoxycorticosterone acetate (DOCA[1]) subcutaneously every 4 wk and feeding a high sodium intake. Spontaneously hypertensive rats (Japanese strain) (13) were bred in our laboratory. Adrenalectomized animals were maintained on the high sodium diet. Two groups of normal rats maintained on either the normal or sodium-free diet for 6 wk were nephrectomized 18 hr before the antibody administration. Following nephrectomy these animals had no access to food or water. The diets employed in the various experimental groups are tabulated in Table I.

Preparation of the specific antibody to angiotensin II. The immunization procedure has been described previously (14). Undiluted sera obtained from rabbits immunized against angiotensin II were employed. In early pilot studies serum from one particular bleeding of one rabbit (AB-A193) was used. However, later studies were carried out with a pool of sera from several bleedings of a number of different rabbits (Pool-AB). Sera were stored at −20°C in 2 ml portions. The antibody titers were determined at the

[1] *Abbreviations used in this paper:* DOC, desoxycorticosterone; DOCA, desoxycorticosterone acetate.

beginning of the study and were defined by the dilution at which the sera bound 50% of 4 pg of ^{125}I-angiotensin II. AB-A193 had a titer of 1:250,000 and Pool-AB 1:80,000. Despite the difference in titer, the two sera have proven to be equally potent when compared in our standard antibody injection procedure (see Tables II and III, normal rats and two-kidney Goldblatt rats on normal sodium intake). The apparent dissociation constant (K) for Pool-AB was quite high (3×10^{11} liters/mole). Utilizing this dose at a dilution of 1/80,000, the lower limit of sensitivity for assay of angiotensin II was 2 pg.

Standard antibody injection procedure. The animals were weighed and the blood pressure measured by the microphonic tail method on the day of assay (Table I). Then each rat was anesthetized with pentobarbital (5 mg/100 g) and prepared surgically according to procedures utilized for the bioassay of plasma renin activity with the exception that pentolinium tartrate (5 mg subcutaneously) was omitted in all but one group of rats. Both jugular veins were cannulated (PE-10 catheter) for injection of samples and the blood pressure was monitored through a carotid artery catheter (PE-50). After an initial control period of about 30 min during which the blood pressure stabilized, a standard dose of norepinephrine (100 ng) and then of angiotensin II (50 ng) was injected. A control injection of pooled rabbit serum was tested and then the bioassay rat was rechallenged with the same doses of norepinephrine and angiotensin II. Subsequently, undiluted rabbit serum containing specific antibodies to angiotensin II (AB-A193 or Pool-AB) was injected slowly. When the blood pressure had returned to the original base line (approximately 15 min) norepinephrine and angiotensin II were again assayed. These intravenous injections of norepinephrine and angiotensin II were repeated until responsiveness to exogenous angiotensin II had recovered.

The approximate amount of antibody required to block the blood pressure response to exogenous angiotensin II was established for each experimental group by injecting 0.15 ml of serum containing antibody into one animal and then increasing the dose by two-, four-, and eightfold to a maximum of 1.2 ml. Subsequently, this minimum antibody dose required to block ("antibody requirement") was determined in each of at least six additional animals of the same group, and then half of this blocking dose was injected in order to establish that the minimum blocking dose was the same in each animal of the group. A dose of antibody half that of the minimum blocking dose had either a slight or no effect on the blood pressure response to exogenous angiotensin II. At least 4 hr elapsed before the second dose of antibody was administered. The sequence of these two antibody injections was alternated among individual animals of the same group in order to exclude any possible cumulative effect of antibody remaining in the test animal.

Radioimmunoassay of circulating rat angiotensin II. To measure plasma angiotensin levels we used the method previously described (14) with the following modifications: The concentration of lysozyme was doubled to prevent adsorption of angiotensin II to glass and plastic tubes. ^{125}I-angiotensin was diluted to approximately 8,000 cpm/ml. Pooled plasma, obtained from rats which were nephrectomized 24 hr before bleeding was added to the standard curve in a concentration of 10% in order to diminish the nonspecific effect of plasma on the antibody-angiotensin reaction.

RESULTS

Effect of antibody administration. The blood pressure response to exogenous angiotensin II could always be blocked if sufficient amounts of antibody were given. Furthermore, whenever a dosage sufficient to block the blood pressure response to exogenous angiotensin II was given, it also caused an immediate fall in arterial blood pressure of 20–50 mm Hg. The blood pressure invariably returned to base line levels in from 5 to 15 min. However, the pressor action of exogenous angiotensin II remained blocked for up to 2 or 3 hr. Continuous infusion of relatively large doses of angiotensin II (10 μg) during this period did not diminish the duration of the blocking effect of the antibody. At the same time, the blood pressure response to exogenous norepinephrine was not affected by the antibody injection. Administration of pooled rabbit serum did not interfere with the pressor effect of either angiotensin II or norepinephrine. A representative study illustrating these

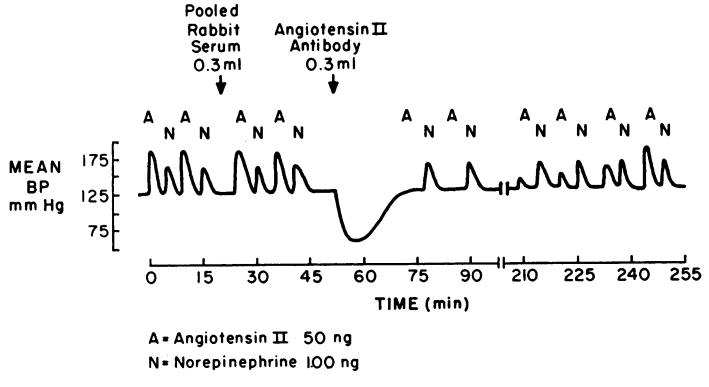

FIGURE 1 Representative study of a normal rat on normal sodium intake. 0.3 ml of antibody induced an immediate fall in blood pressure and blocked the blood pressure response to exogenous angiotensin II. The latter returned towards control levels after 2–3 hr. Response to exogenous norepinephrine was not affected.

TABLE II

*Blood Pressure Response to Exogenous Angiotensin II**

Preparation	Na intake (n)	Before antibody	After blocking dose of antibody	After half blocking dose of antibody
		mm Hg	*mm Hg*	*mm Hg*
Normals	0 (6)	36.8 ±4.8	2.0 ±1.5	—
	Normal (6)‡	32.7 ±2.4	12.2 ±4.5	24.6 ±2.7
	Normal (4)	25.0 ±2.1	4.5 ±2.6	20.0 ±2.0
	High (6)	45.2 ±4.8	6.8 ±4.2	20.0 ±0.4
Goldblatt two kidneys	0 (6)‡	25.0 ±2.5	4.7 ±2.2	18.0 ±2.0
	Normal (7)‡	29.0 ±1.7	0.9 ±0.6	14.6 ±4.9
	Normal (6)	41.7 ±4.5	1.2 ±1.2	15.8 ±1.4
Goldblatt one kidney	Normal (8)	34.6 ±4.9	6.6 ±4.6	31.8 ±8.6
DOCA	0 (6)	31.5 ±4.1	1.2 ±1.2	28.6 ±5.0
	High (6)	44.0 ±3.5	(28.7§) ±5.9	—
Spontaneously hypertensive	0 (6)	36.5 ±4.8	(19.3§) ±3.4	—
	Normal (9)	38.6 ±3.7	(17.0§) ±2.5	—
Adrenalectomy	High (6)	48.8 ±5.0	0	20.7 ±5.1
Nephrectomy	0 (6)	43.0 ±3.9	3.7 ±1.8	31.0 ±11.5
	Normal (6)	55.3 ±6.1	2.0 ±2.0	24.4 ±3.8
Normals Pentolinium tartrate	0 (6)	28.7 ±4.9	(29.5§) ±4.7	—

* Values as mean ± standard error.

‡ Antibody AB-A193 used.

§ Blood pressure response to exogenous angiotensin II not blocked with 1.2 ml of antibody serum.

features in a normal animal fed a normal sodium diet is depicted in Fig. 1.

Physiological definition of the "blocking dose" of antibody. The blocking dose was defined as the minimal dose of antibody required to prevent completely the pressor effect of 50 ng of angiotensin II. This minimal dose was confirmed in each case by demonstrating that when half of the blocking dose was injected the blood pressure response to angiotensin II was either unchanged from the preinjection response or was only partially blocked (Table II). The antibody requirement in each rat of any one experimental group was identical so that there was no overlap in results of individual animals from different groups.

Influence of dietary sodium intake on the amount of antibody required to block the blood pressure response to exogenous angiotensin II. Fig. 2 illustrates the influence of sodium intake on the antibody requirement of the normal rat. While 0.15 ml was sufficient to block the pressor effect of exogenous angiotensin II in the sodium-depleted animals, 0.3 and 1.2 ml of antibody respectively were required to achieve the same blocking effect in rats fed a normal or high sodium intake. This direct relationship between the antibody requirement and the sodium intake could also be demonstrated in DOC-treated, in nephrectomized, and in renal hypertensive animals (Table III). The amount of antibody required

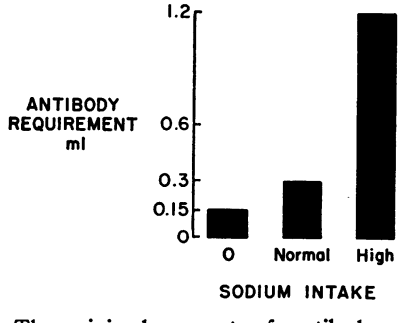

FIGURE 2 The minimal amount of antibody required to block the blood pressure response to exogenous angiotensin II in normal rats was directly related to the state of sodium balance.

TABLE III

Antibody Requirement and Induced Fall in Blood Pressure

Preparation	Na intake	(n)	Antibody requirement	Mean blood pressure fall*	Plasma angiotensin II*
			ml	*mm Hg*	*pg/ml*
Normals	0	(6)	0.15	34.3 ±5.2	150.2 ±19.8
	Normal	(6)	0.3‡	42.6 ±7.3	66.7 ±6.8
	Normal	(4)	0.3	47.5 ±2.5	—
	High	(6)	1.2	25.2 ±3.9	<20
Goldblatt two kidneys	0	(6)	0.3‡	42.7 ±9.8	—
	Normal	(7)	0.6‡	51.3 ±4.7	—
	Normal	(6)	0.6	41.0 ±4.0	—
Goldblatt one kidney	Normal	(8)	0.6	10.0 ±6.4	—
DOCA	0	(6)	0.6	20.7 ±9.7	—
	High	(6)	>1.2§	30.2 ±7.7	<20
Spontaneously hypertensive	0	(6)	>1.2§	0 ±10.8	<20
	Normal	(9)	>1.2§	11.2 ±13.4	<20
Adrenalectomy	High	(6)	0.3	20.4 ±3.4	—
Nephrectomy	0	(6)	0.3	5.7 ±3.4	<20
	Normal	(6)	1.2	0	<20
Normals Pentolinium tartrate	0	(6)	>1.2§	8.0 ±4.1	—

* Values as mean ± standard error.

‡ Antibody AB-A193 used.

§ Blood pressure response to exogenous angiotensin II not blocked with 1.2 ml of antibody serum.

as affected by changes in salt intake increased by greater than 0.6 to more than 1.2 ml in the DOC-treated groups, by 0.9 to 1.2 ml in the nephrectomized groups, and by 0.3 to 0.6 ml in renal hypertension.

Influence of ganglionic blockade on the antibody requirements. Subcutaneous injection of 5 mg of pentolinium tartrate into a group of normal rats maintained on a sodium-free diet increased the antibody requirement from 0.15 ml to more than 1.2 ml (Fig. 3 and Table III). The effect of ganglionic blockade was evaluated in animals from other experimental groups; antibody requirement was always greater than 1.2 ml.

Antibody requirement in adrenalectomized animals. Adrenalectomy decreased the antibody requirement to 0.3 ml as compared to the 1.2 ml required to block the blood pressure response in normal rats fed a similar high sodium intake (Table III).

Antibody requirement in nephrectomized animals. Nephrectomy enhanced the amount of antibody required to block the blood pressure response to exogenous angiotensin II (Table III). This effect could be demonstrated at the two different levels of sodium intake which was fed before total nephrectomy. Since the overall sodium balance was unchanged during the 18 hr after nephrectomy, the enhancement of the antibody requirements could not be explained by an increased total body sodium.

Antibody requirement in different forms of hypertension. All groups of hypertensive animals required more antibody to block the blood pressure response to exogenous angiotensin II than did normal animals. Fig. 4 illustrates the antibody requirement of normal rats, one- and two-kidney Goldblatt animals, and spontaneously hypertensive rats, all maintained on a normal sodium intake. In addition, the results of DOC-hypertensive animals fed a high sodium intake are depicted. While, in normal animals, 0.3 ml of antibody were sufficient to block angiotensin II, 0.6 ml were required in one- and two-kidney Goldblatt rats, and 1.2 ml of antibody was not sufficient to block the pressor action of exogenous angiotensin II in either DOC-treated or spontaneously hypertensive animals. Furthermore, while the antibody requirement fluctuated with sodium balance in hypertensive models in the same way as in normal animals, the requirements at all levels of sodium intake were always higher in the hypertensive animals than they were in normals.

Blood pressure response to antibody injection. In all groups except the nephrectomized and the one-kidney renal hypertensive animals, whenever the blood pressure response to exogenous angiotensin II could be blocked by the antibody injection, the blood pressure fell by 20–50 mm Hg (Table III). The difference between the blood pressure fall in one-kidney renal hypertensive as compared to two-kidney Goldblatt rats is illustrated in Fig. 5. In the two-kidney animals, antibody administration induced a mean blood pressure fall of 41.0 ±4.0 mm Hg ($P < 0.01$), whereas in the one-kidney rats the blood pressure fell by only 10.0 ±6.4 mm Hg and this change was not significant ($P > 0.1$). No significant correlation was observed between the antibody-induced blood pressure fall and the base line blood pressure or the state of sodium balance. The blood pressure always returned to base line during the 5–15 min after the antibody injection.

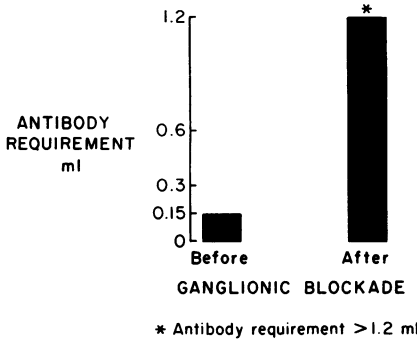

FIGURE 3 Ganglionic blockade of normal rats maintained on a low sodium intake increased the minimal antibody requirement by more than eightfold.

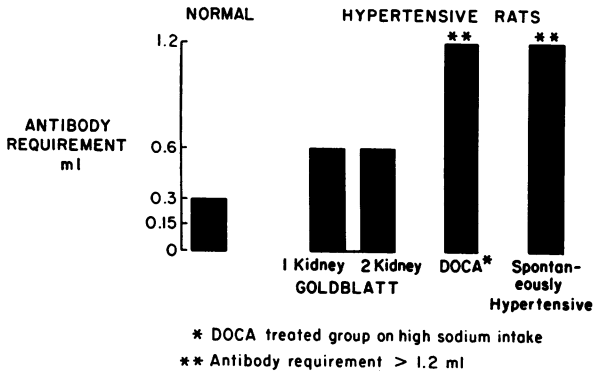

FIGURE 4 The amount of antibody required to block exogenous angiotensin was increased in two types of renal hypertension and was greatly increased in DOCA-treated animals and in spontaneously hypertensive rats. All hypertensive animals were fed a normal sodium intake except the DOCA-treated animals, which were maintained on a high sodium intake.

Relationship of antibody requirements to circulating angiotensin II levels. In general, an inverse relationship was noted between antibody requirements and the concentration of circulating endogenous angiotensin II. (Table III). Two notable exceptions were the nephrectomized group and the one-kidney renal hypertensive animals.

DISCUSSION

In the present series of experiments, the amount of angiotensin antibody required to block the pressor effect of a standard dose of exogenous angiotensin II was determined. It was then shown, under various experimental conditions, that the amount of antibody required to block exogenous angiotensin II varied in a consistent way. Sodium-depleted animals required the least amounts of antibody and as much as eightfold the amount was required to block the action of angiotensin II in sodium-loaded animals. In various forms of experimental hypertension and in totally nephrectomized animals the same relationship to salt intake was demonstrated. However at all levels of dietary sodium the hypertensive or nephrectomized animals required significantly more antibody than did the normotensive controls. Ganglionic blockade also increased the antibody requirement in all animals tested. This increase was more than eightfold in sodium-depleted normal rats.

Because of the marked difference in the response obtained by doubling the antibody dose it seems that the antibody concentration in the plasma must reach a certain level in order to completely inhibit the pressor effect of exogenous angiotensin II. Accordingly, if enough antibody is injected, all of the exogenous angiotensin II is apparently bound by the antibody and none is available to act at the vascular receptor to increase the blood pressure.

One might expect this antibody requirement to vary directly with the preexisting endogenous plasma level of angiotensin II. In fact the data indicate that in general there exists an inverse relationship between the antibody requirements and the endogenous plasma angiotensin II level. However, this inverse relationship was by no means consistent. For instance, the antibody requirement of nephrectomized animals varied according to sodium intake and yet circulating angiotensin II was undetectable in these rats. Furthermore, the two different types of Goldblatt hypertension exhibited similar antibody requirements even though circulating angiotensin II levels are presumably different. Normal plasma renin levels in one-kidney Goldblatt hypertension have been described (15). We have demonstrated in a separate experiment that there is no difference in the binding capacity of the antibody when tested in plasma from rats on low, normal, or high sodium intake. Since it is unlikely that the affinity of the injected antibodies varies, we therefore postulate that antibody competes with receptors and that the different antibody requirements found in our experimental models are mediated by differences in vascular receptor affinity to angiotensin II.

Our findings seem to suggest that changes in vascular receptor affinity are not primarily affected by changes in endogenous angiotensin II levels. However, it is apparent that under most conditions an inverse relationship does exist between antibody requirement and plasma angiotensin II levels. This might indicate that receptor sensitivity changes because of previous exposure to circulating angiotensin so that if there has been a high

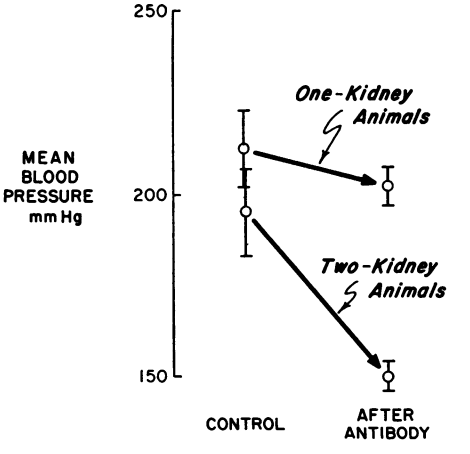

FIGURE 5 In the two-kidney type of renal hypertension the administration of 0.6 ml of angiotensin II antibody induced a mean blood pressure fall of 41.0 ±4.0 mm Hg ($P < 0.01$), whereas in the one-kidney type of renal hypertension the blood pressure fell by only 10.0 ±6.4 mm Hg. This latter change was not significant.

level of circulating angiotensin, the receptor sensitivity might be greatly depressed, while if there has been a low level of angiotensin, receptor sensitivity might be highly increased. Similar adaptation processes have been observed in response to other hormones and drugs. Yet, still another sequence of events might cause the observed inverse relationship in the hypertensive animals. It may be that hypertension is primarily a disorder of the vascular angiotensin receptors so that increased receptor affinity in the face of a normal angiotensin level induces hypertension which in turn, through a feedback mechanism, tends to reduce renin release and thereby lower plasma angiotensin. If the degree of renin suppression is limited, normalization of the blood pressure could not be achieved. The low plasma renin levels seen in a large subgroup of essential hypertensives may be induced by such a mechanism. Obviously, our data do not allow us to choose which of the possible mechanisms is prevailing. It is conceivable that both mechanisms can function simultaneously.

Many investigators have reported that the responsiveness to exogenous angiotensin II is dependent on the state of sodium balance. Thus, sodium depletion of normal rats decreases and sodium loading increases the blood pressure response to a standard dose of exogenous angiotensin II (16). Reduced pressor responsiveness to angiotensin II has also been demonstrated in adrenalectomized rats (17), in patients with Addison's disease (18), in nephrosis (19), and in cirrhosis (19, 20). Furthermore, patients with primary aldosteronism exhibit an increased pressor responsiveness to angiotensin II (21). In more prolonged studies, Ames, Borkowski, Sicinski, and Laragh found that in order to maintain a constant blood pressure rise during angiotensin infusions it was necessary to progresively reduce the dose of angiotensin II (22). The decreased angiotensin requirement appeared to be directly related to progressive sodium retention caused by the angiotensin-induced stimulation of aldosterone secretion. All these reported changes in vasoactivity of angiotensin II are consistent with the sodium-dependent changes in antibody requirement observed herein. Because changes in apparent vascular receptor affinity fluctuated in a manner entirely parallel to these previously described circumstances in which pressor responsiveness to angiotensin II is known to change, it seems likely that the observed changes in angiotensin receptor affinity are meaningful physiological indicators of pressor responsiveness.

Physiological properties of receptors to angiotensin I and II have been studied extensively in vitro by Goodfriend and Lin (23). The experiments described herein could involve these same or other angiotensin receptors. Whatever the case, the present study provides new information about the physiological behavior of angiotensin II receptors.

Sodium depletion increases angiotensin II production by stimulating renin release. However, there is no increase in blood pressure associated with the hyperreninemia and the hyperangiotensinemia of sodium depletion. This lack of increase may now be explained by the concomitant decrease in vascular receptor affinity to angiotensin II. The observations suggest a balanced control mechanism such that low sodium intake stimulates angiotensin production but at the same time lowers vascular receptor affinity to angiotensin II. Thus the renin-angiotensin system can respond freely to the needs of sodium conservation without affecting blood pressure homeostasis. Since sodium depletion leads to increased angiotensin II production and since in man this has been shown to stimulate aldosterone secretion, it would appear that the adrenal cortical receptors for angiotensin II are not sodium-dependent in the same way as the vascular receptors. Indeed there is evidence, that sodium excess may actually blunt these receptors (24).

When adrenalectomized animals were maintained on a high sodium intake, vascular receptor affinity to angiotensin II was reduced so that 0.3 ml of antibody was required as compared to the blocking dose of 1.2 ml in a normal animal on a high sodium intake. Since adrenalectomized animals are "salt losing," it is reasonable to suspect that the decrease in vascular receptor affinity is due to a reduction in total body sodium. However, the present data do not permit the exclusion of a direct influence of the adrenal glands on the receptors.

In contrast, nephrectomy enhanced vascular receptor affinity to angiotensin II and this effect could be demonstrated at the two different levels of sodium intake fed before nephrectomy. However, a change in sodium balance did not seem to be responsible for the increase in receptor affinity since overall sodium balance was unchanged during the 18 hr between nephrectomy and the injection of antibody. This observation suggests that the kidney, like the autonomic nervous system, exerts some moderating influence on receptor affinity to angiotensin II by a mechanism that apparently does not involve renin release. Thus, the kidney appears to play a key role in blood pressure regulation, since it can control simultaneously both the liberation of angiotensin II via renin release, and also the pressor activity of this liberated angiotensin II via changes in vascular receptor affinity. Such renally induced changes in receptor affinity may explain instances of renal hypertension associated with normal plasma renin levels.

Ganglionic blockade with pentolinium tartrate induced a striking increase in vascular receptor affinity. This observation is in keeping with the enhanced sensitivity to angiotensin II and also to norepinephrine which

has been observed during ganglionic blockade. Many laboratories have used ganglionic blockade to increase the sensitivity of their bioassay animal to injected angiotensin II. Since ganglionic blockade increases vascular receptor affinity to angiotensin II it is probable that the converse is true and that an increase in the activity of the autonomic nervous system results in a reduction in receptor affinity. The sympathetic nervous system has been shown to stimulate renin release (25). Thus the stimulatory effect of sympathetic discharge on renin release may be counterbalanced by a concomitant decrease in vascular receptor affinity.

Vascular receptor affinity was found to be moderately increased in two different forms of experimental renal hypertension, which are thought to exhibit different, i.e. normal or slightly elevated plasma renin levels (15). Vascular receptor affinity was also found to be strikingly increased, in fact unmeasurable by our present technique, in two forms of hypertension which exhibit low levels of circulating plasma renin, i.e. genetic and DOC-hypertension. Such normal or low plasma renin levels have been thought to exclude a role for renin in the pathogenesis of these hypertensions. However, the marked increase in vascular receptor affinity provides some evidence for the idea that even abnormally low plasma renin or angiotensin II levels may be sufficient to cause high blood pressure. Therefore, the present results suggest that renin and angiotensin II may play a role, even in genetic and DOC-hypertension, in maintaining an enhanced blood pressure. Plasma levels of renin or angiotensin II per se may not be sufficient indices of their role in blood pressure control and it may be necessary to take into consideration the concurrent influence of vascular receptor affinity.

Tobian and Binnion have reported that the sodium content of arterial walls of renal and DOC-hypertensive rats is significantly greater than that of normal animals (26). Since increases in sodium intake can enhance vascular receptor affinity to angiotensin II, this finding of increased sodium concentration close to the angiotensin II receptors may be pertinent. Thus increased vascular receptor affinity in hypertension may be mediated by changes in arterial wall sodium concentration.

It is possible that increased sodium in arterial walls leads to increased swelling and rigidity of the arteries. Such an effect might also lead to an increase in the availability of receptors for binding of angiotensin. By our present methods it is not possible to distinguish between a change in the affinity of vascular receptors and an increase in the number of receptors.

Several investigators have actively or passively immunized Goldblatt hypertensive rabbits (9, 10), dogs (27, 28), or rats (29–31) against angiotensin II or renin. The interpretation of all these results is somewhat difficult because various techniques and hypertensive models have been used, and very often important information such as antibody titers achieved in vitro or in vivo is incomplete. Nevertheless, it should be noted that, particularly in the more recent reports, one-kidney Goldblatt hypertension is not consistently corrected by the inhibition of the renin-angiotensin system.

In most of the situations studied whenever the respone to exogenous angiotensin II was blocked, antibody injection also induced a considerable transient fall in blood pressure (Table III). Two exceptions were the nephrectomized animals and the one-kidney renal hypertensive rats. It seems reasonable to assume that injected antibody lowers the blood pressure only when angiotensin II is actively involved in the maintenance of blood pressure. Consistent with this reasoning is the failure of depression of blood pressure in nephrectomized animals even when exogenous angiotensin II was completely blocked, and the normalization of blood pressure after the administration of antibody to dogs with acute renovascular constriction (32). Therefore, our results suggest that normal blood pressure is maintained at least in part by circulating angiotensin II. They further seem to indicate that maintenance of blood pressure in the two types of renal hypertension involve two different mechanisms. It appears that angiotensin II participates in the maintenance of two-kidney renal hypertension and probably plays no significant role in one-kidney renal hypertension.

After injection of angiotensin antibody, the reduced blood pressure returned to control levels at a time when antibody could still be demonstrated to be present in amounts sufficient to block the pressor effect of exogenous angiotensin II. These data confirm the observations of Meyer and Worcel (33). However, this does not rule out angiotensin II as an important factor in the pathogenesis of hypertension or in the maintenance of normal blood pressure. Various other compensatory mechanisms may well be responsible for this secondary restoration of blood pressure. The sympathetic nervous system, catecholamines, or other pressor substances such as the one isolated by Ebihara and Grollman from the kidney (34) could act to replace the circulating angiotensin II that has been blocked by the antibody. This interpretation would automatically imply that though angiotensin II may be actively involved in the maintenance of hypertension, in its absence other mechanisms come into play to keep the blood pressure elevated and to maintain tissue perfusion.

The secondary blood pressure recovery after antibody administration could be interpreted in still another way. Though circulating angiotensin II is completely bound by antibody and therefore inactive, renin secretion might increase in compensation for the loss of active angio-

tensin II. It is conceivable that this increased renal renin, by liberating angiotensin I and II locally at an intrarenal but extravascular site not reached by the relatively large antibody molecules, could induce systemic hypertension by increasing renal resistance. It is of note that the intrarenal generation of angiotensin II, a prerequisite for this hypothesis, has been demonstrated recently (35).

Taken altogether the data suggest the following conclusions: (*a*) Vascular receptor affinity to angiotensin II can fluctuate. (*b*) Increased sodium intake stimulates the affinity of these receptors and a decreased intake reduces their activity. The affinity is also increased by ganglionic blockade and by nephrectomy. (*c*) Apparent vascular receptor affinity is greatly enhanced in experimental genetic and DOC-hypertension, and is also increased in two types of renovascular hypertension. (*d*) Vascular receptor affinity may change independently of the plasma angiotensin II levels. Therefore, the role of angiotensin II in blood pressure control cannot be evaluated without taking into account possible changes in vascular receptor activity. (*e*) The data suggest that angiotensin II by interacting with its vascular receptors is actively involved in normal blood pressure control and in the maintenance of genetic, DOC-, and two-kidney Goldblatt hypertension. (*f*) Angiotensin II does not appear to play a role in the maintenance of one-kidney Goldblatt hypertension.

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REFERENCES

1. Vander, A. J. 1967. Control of renin release. *Physiol. Rev.* **47**: 359.
2. Laragh, J. H., M. Angers, W. G. Kelly, and S. Lieberman. 1960. Hypotensive agents and pressor substances: the effect of epinephrine, norepinephrine, angiotensin II and others on the secretory rate of aldosterone in man. *J. Amer. Med. Ass.* **174**: 234.
3. Khairallah, P. A., I. H. Page, and R. K. Turker. 1966. Potentiation of vascular myotropic responses by metanephrine and other noncatecholamines. *Circ. Res.* **19**: 538.
4. Laragh, J. H. 1966. Dependence of the vasoactivity of angiotensin on the state of sodium balance. *In* L'Hypertension Arterielle. International Club on Arterial Hypertension: First Meeting, 5–7 July 1965, Paris. P. Milliez and P. Tcherdakoff, editors. L'Expansion Scientifique Française, Paris. 92.
5. Helmer, O. M., and W. E. Judson. 1960. The presence of vasoconstrictor and vasopressor activity in renal vein plasma of patients with arterial hypertension. *Hypertension, Proc. Counc. High Blood Pressure Res.* **8**: 38.
6. Creditor, M. C., and U. K. Loschky. 1967. Plasma renin activity in hypertension. *Amer. J. Med.* **43**: 371.
7. Brown, J. J., D. L. Davies, A. F. Lever, and J. I. S. Robertson. 1964. Variations in plasma renin concentration in several physiological and pathological states. *Can. Med. Ass. J.* **90**: 201.
8. Pickens, P. I., H. P. Dustan, F. M. Bumpus, and I. H. Page. 1965. Measurement of plasma renin activity in hypertension. *Hypertension, Proc. Counc. High Blood Pressure Res.* **13**: 90.
9. Eide, I., and H. Aars. 1970. Renal hypertension in rabbits immunized with angiotensin II. *Scand. J. Clin. Lab. Invest.* **25**: 119.
10. Macdonald, G. J., W. J. Louis, V. Renzini, G. W. Boyd, and W. S. Peart. 1970. Renal-clip hypertension in rabbits immunized against angiotensin II. *Circ. Res.* **27**: 197.
11. Johnston, C. I., J. S. Hutchinson, and F. A. Mendelsohn. 1970. Biological significance of renin angiotensin immunization. *Circ. Res.* **27** (Suppl. II): 215.
12. Friedman, M., and S. C. Freed. 1949. Microphonic manometer for the indirect determination of systolic blood pressure in the rat. *Proc. Soc. Exp. Biol. Med.* **70**: 670.
13. Okamoto, K. 1969. Spontaneous hypertension in rats. *Int. Rev. Exp. Pathol.* **7**: 227.
14. Gocke, D. T., T. Gerten, L. M. Sherwood, and T. H. Laragh. 1969. Physiological and pathological variations of plasma angiotensin II in man. Correlation with renin activity and sodium balance. *Circ. Res.* **24** and **25** (Suppl. I): 131.
15. Miksche, L. W., U. Miksche, and F. Gross. 1970. Effect of sodium restriction on renal hypertension and on renin activity in the rat. *Circ. Res.* **27**: 973.
16. Reid, W. D., and J. H. Laragh. 1965. Sodium and potassium intake, blood pressure, and pressor response to angiotensin. *Proc. Soc. Exp. Biol. Med.* **120**: 26.
17. Ostrovsky, D., and A. G. Gornall. 1964. Effects of aldosterone and other adrenal hormones on the blood pressure responses to renin and angiotensin. *Can. Med. Ass. J.* **90**: 180.
18. Kuchel, O., K. Horky, M. Pazourek, and I. Gregorova. 1964. Pressor hyporeactivity to angiotensin in Addison's disease. *Lancet* **2**: 1316.
19. Laragh, J. H. 1962. Hormones and the pathogenesis of congestive heart failure: vasopressin, aldosterone and angiotensin II. Further evidence for renal-adrenal interaction from studies in hypertension and in cirrhosis. *Circulation.* **25**: 1015.
20. Johnston, C. J., and A. D. Jose. 1963. Reduced vascular response to angiotensin II in secondary hyper aldosteronism. *J. Clin. Invest.* **42**: 1411.
21. Kaplan, N. M., and J. G. Silah. 1964. The effect of angiotensin II on the blood pressure in humans with hypertensive disease. *J. Clin. Invest.* **43**: 659.
22. Ames, R. P., A. J. Borkowski, A. M. Sicinski, and J. H. Laragh. 1965. Prolonged infusions of angiotensin II and norepinephrine and blood pressure, electrolyte balance, aldosterone and cortisol secretion in normal man and in cirrhosis with ascites. *J. Clin. Invest.* **44**: 1171.
23. Lin, S-Y., and T. L. Goodfriend. 1970. Angiotensin receptors. *Amer. J. Physiol.* **218**: 1319.
24. Davis, W. W., L. R. Burwell, and F. C. Bartter. 1969. Inhibition of the effects of angiotensin II on adrenal steroid production by dietary sodium. *Proc. Nat. Acad. Sci. U. S. A.* **63**: 718.
25. Gordon, R. D., O. Kuchel, G. W. Liddle, and D. P. Island. 1967. Role of sympathetic nervous system in

regulating renin and aldosterone production in man. *J. Clin. Invest.* **46**: 599.
26. Tobian L., and J. T. Binnion. 1954. Artery wall electrolytes in renal and DCA hypertension. *J. Clin. Invest.* **33**: 1407.
27. Deodhar, S., E. Haas, and H. Goldblatt. 1963. Production of antirenin to homologous and to human renin and its effect on experimental renal hypertension. *Fed. Proc.* **22**: 486.
28. Wakerlin, G. E. 1958. Antibodies to renin as proof of the pathogenesis of sustained renal hypertension. *Circulation.* **17**: 653.
29. Weiser, R. A., A. G. Johnson, and S. W. Hoobler. 1969. The effect of antirenin on the blood pressure of the rat with experimental renal hypertension. *Lab. Invest.* **20**: 326.
30. Hedwall, P. R. 1968. Effect of rabbit antibodies against angiotensin II on the pressor response to angiotensin II and renal hypertension in the rat. *Brit. J. Pharmacol. Chemother.* **34**: 623.
31. Christlieb, A. R., T. U. L. Biber, and R. B. Hickler. 1969. Studies on the role of angiotensin in experimental renovascular hypertension: an immunological approach. *J. Clin. Invest.* **48**: 1506.
32. Cowley, A. W., J. P. Miller, and A. C. Guyton. 1971. Open-loop analysis of the renin-angiotensin system in the dog. *Circ. Res.* **28**: 568.
33. Meyer, P., and M. Worcel. 1970. Role of angiotensin in the salt-hypertension of rats. *Pflugers Arch. Gesamte Physiol. Menschen Tiere.* **317**: 327.
34. Ebihara, A., and A. Grollman. 1968. Pressor activity of renal venous effluent following constriction of renal artery in dogs. *Amer. J. Physiol.* **214**: 1.
35. Bailie, M. D., R. C. Rector, Jr., and D. W. Seldin. 1971. Angiotensin II in arterial and renal venous plasma and renal lymph in the dog. *J. Clin. Invest.* **50**: 119.