poses for a long time. This, together with the wide range of activity of the oil, suggests that garlic oil or its active principle, whether natural or synthetic, could be used as pesticides.

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Hypertension of Renal Origin: Evidence for Two Different Mechanisms

Abstract. *Antibody to angiotensin II, or a specific peptide competitive inhibitor of angiotensin II, was used to investigate the role of the renin-angiotensin system in two types of renal hypertension in rats. The data indicate that angiotensin II is in fact critically involved in the pathogenesis of the form of renal hypertension in which one renal artery is clamped and the contralateral kidney is left in place, but that it probably plays no significant role in the maintenance of experimental renal hypertension in which the opposite kidney has been removed.*

Goldblatt and co-workers first induced chronic arterial hypertension in dogs by partially occluding one renal artery and removing the opposite kidney (*1*). Subsequently, increased renin secretion by such clipped kidneys was demonstrated (*2*). Renin acts enzymatically to release angiotensin I from a plasma globulin (*3*). Angiotensin I is then converted by other enzymes to the active octapeptide angiotensin II, the most potent pressor substance known. Accordingly, it has seemed reasonable to assume that increased plasma angiotensin II is the cause of experimental renal hypertension and its various naturally occurring counterparts in man. However, numerous studies have failed to establish this assumption as fact, and both plasma renin and angiotensin II levels are often normal in chronic renal hypertension of various species, including man (*4*). Furthermore, in other studies in which antibodies to renin or angiotensin were either administered or induced it very often has not been possible to correct this type of hypertension (*5, 6*). Therefore, the etiologic role of angiotensin II in causing or maintaining renal hypertension is open to question.

In our experiments reported here, angiotensin II antibodies were administered intravenously to rats with chronic renal hypertension, and the effects were compared with the administration of a new highly specific synthetic peptide competitive inhibitor of angiotensin II. This compound, [sarcosine^1–Ala^8]angiotensin II, completely blocks the pressor action of exogenous angiotensin II in rats and dogs when given in approximately equimolar amounts (*7*). The compound itself has no pressor or depressor activity when given intravenously. Its biological half-life is approximately 12 minutes. The use of a similar peptide inhibitor of angiotensin II has been described (*8*).

Two types of renal hypertension were studied. In the first type a silver clip was placed on the left renal artery and the other kidney was left untouched (this is referred to as two-kidney Goldblatt hypertension). In the second, a silver clip was placed on the left renal artery, and the contralateral kidney was removed (one-kidney Goldblatt hypertension). The two groups of hypertensive animals, together with an additional control group, were maintained for 6 weeks on Purina rat chow (0.42 percent sodium content) and allowed free access to water. All animals weighed 350 to 450 g. A mean blood pressure of 121.3 ± 6.6 mm-Hg (mean ± standard error) was found in normal rats. The mean blood pressure of two-kidney hypertensive animals was 195.6 ± 10.8 mm-Hg. The difference in blood pressures between the two hypertensive groups was not statistically significant.

Antibodies to angiotensin II were prepared in rabbits (*9*). The apparent affinity constant of the antibody was calculated to be 3×10^{11} liter/mole.

The animals were anesthetized with intraperitoneal pentobarbital (5 mg/100 g). Both jugular veins were cannulated (PE-10 catheter) for injection or infusion, and the blood pressure was continuously monitored with a strain gauge through a carotid artery catheter (PE-50). During an initial control period standard doses of angiotensin II (50 ng) and of norepinephrine (100 ng) were injected through the cannula; pooled rabbit serum was then injected as a control, and the animals were challenged with angiotensin II and norepinephrine standards. Then, either undiluted serum containing angiotensin II antibody (0.3 or 0.6 ml) was injected as a single dose or, alternatively, the angiotensin II inhibitor was infused at a rate of 4 μg/min for 1 hour. After the antibody injection or during infusion of the inhibitor, the pressor effects of the standard amounts of exogenous angiotensin II and norepinephrine were checked periodically.

The blood pressure response to exogenous angiotensin II in normal rats ($n = 10$) was blocked by as little as 0.3 ml of antibody. The amount of antibody also induced an immediate fall in blood pressure of 47.5 ± 2.5 mm-Hg, which was sustained for about 5 minutes, before it gradually returned to baseline levels (in the next 10 minutes). However, the pressor effects of exogenous angiotensin II remained completely blocked for up to 3 hours. Blood pressure response to exogenous norepinephrine was never affected by the administration of the antibody. Administration of the pooled rabbit serum had no effect on the blood pressure or the pressor activity of angiotensin II or norepinephrine.

Twice as much antibody (0.6 ml) was required to abolish the pressor effect of exogenous angiotensin II in the two-kidney hypertensive rats ($n = 6$) and in the one-kidney rats ($n = 6$). However, in the two-kidney animals antibody administration induced a fall in mean blood pressure of 41.0 ± 4.0 mm-Hg ($P < .001$), whereas in the one-kidney rats the blood pressure fell by only 10.0 ± 6.4 mm-Hg, and this change was not significant ($P > .1$) (Fig. 1). The reduction in blood pressure lasted for an average of 16 min-

utes in the two-kidney rat as compared to only 6 minutes in the one-kidney rat. However, in both groups the blood pressure response to exogenous angiotensin II remained blocked for 1 to 3 hours.

Infusion of the peptide inhibitor of angiotensin II into normal rats ($n = 6$) blocked completely the blood pressure response to exogenous angiotensin II. However, no significant change in blood pressure was induced. The blood pressure response to exogenous norepinephrine was not affected by the angiotensin inhibitor.

Infusion of the peptide inhibitor of angiotensin II into the two-kidney animals $n = 6$ induced an immediate progressive fall in blood pressure which reached 33.0 ± 8.3 mm-Hg after 15 minutes, 41.0 ± 3.3 mm-Hg after 30 minutes, and 40.8 ± 4.2 mm-Hg ($P < .001$) after 60 minutes. When the infusion was discontinued, the blood pressure gradually returned to baseline within 30 minutes. In contrast, the reduction in blood pressure induced by the angiotensin inhibitor in the one-kidney hypertensive rats ($n = 6$) was only 6.0 ± 9.9 mm-Hg after 15 minutes, 9.0 ± 11.1 mm-Hg after 30 minutes, and 17.5 ± 10.2 mm-Hg ($P > 0.1$) after 60 minutes (Fig. 2). In both groups, standard test doses of exogenous angiotensin II given during the infusion had no pressor effect, whereas the blood pressure response to exogenous norepinephrine remained unchanged.

Thus, injection of angiotensin II antibody into normal rats can induce a fall in blood pressure of as much as 48 mm-Hg. This fall seems to be quite specific, since pooled rabbit serum did not affect blood pressure, and the pressor activity of norepinephrine was unaffected at a time when the action of angiotensin II was completely blocked. In addition, in studies with nephrectomized normal rats which had no circulating angiotensin II the injection of a blocking dose of antibody failed to produce any change in blood pressure (*9a*). In contrast, infusion of the peptide inhibitor of angiotensin II into normal rats did not affect their blood pressure. This observation confirms the findings of Marshall and coworkers (*8*) who used a similar angiotensin II inhibitor.

Either injection of angiotensin antibody or infusion of angiotensin inhibitor into two-kidney rats caused similar reductions of blood pressure. However, in one-kidney hypertensive animals different effects were observed with either the administration of angiotensin antibody or the inhibitor. The induced change in blood pressure was not significant, and the lowest blood pressure recorded remained in the hypertensive range. That the failure of blood pressure reduction in these animals is not due to administration of insufficient amounts of blocking agent was suggested by the complete abolition of the pressor effects of exogenous angiotensin II. Our data therefore suggest that the mechanisms of these two types of renal hypertension are not the same. If angiotensin II antibody or angiotensin inhibitor can only lower the blood pressure when angiotensin II is actively involved in the maintenance of the hypertension, then angiotensin II may actively participate in the pathogenesis of two-kidney renal hypertension and probably plays no significant role in the one-kidney type.

Several investigators have actively or passively immunized "Goldblatt hypertensive" rabbits (*5*) or rats (*6, 10, 11*) against angiotensin II. In some of these previous studies only one-kidney models were used (*5*); in another experiment (*11*) rapid injection of antibody into two-kidney hypertensive animals produced a transient blood pressure fall similar to that which we describe. This transient fall induced by antibody injection is not likely to occur during a gradual increase in antibody titer, such as produced by an active immunization (*5, 10*) or even a slow infusion of antibody (*6*). These observations are therefore largely in agreement with our findings.

That there are two different mechanisms involved in the development of these two types of renal hypertension has been suggested by other studies (*12*); normal values for renin in plasma and kidney have been described in one-kidney hypertensive animals, whereas the renin is increased in plasma and in the clipped kidney of the two-kidney hypertensive animals. In another study, it was suggested that one-kidney type of hypertension was associated with an increase in total body sodium (*13*). Thus, even normal levels of renin in the plasma in these one-kidney animals might be inappropriately high for the state of sodium balance. In this context, through an enhancement of its pressor effect by sodium retention (*14*), even an apparently normal plasma angiotensin level might induce hypertension. However, our results do not support this thesis because, if circulating angiotensin II had an enhanced pressor effect in one-kidney Goldblatt hypertension, then angiotensin II antibodies or angiotensin inhibitor should become very efficient depressor agents in these animals. It is pertinent that a normal renin level did not prevent angiotensin II antibody from being depressor, as illustrated by the blood pressure reductions found in the normal control group. Furthermore, in other studies in which antibody was injected into sodium-loaded normal rats ($n = 6$), the blood pressure was reduced by 25.2 ± 3.9 mm-Hg. This occurred despite concurrent volume expansion and suppression of plasma renin activity. Therefore, taken altogether these results point to a causal role for the renin-angiotensin system in

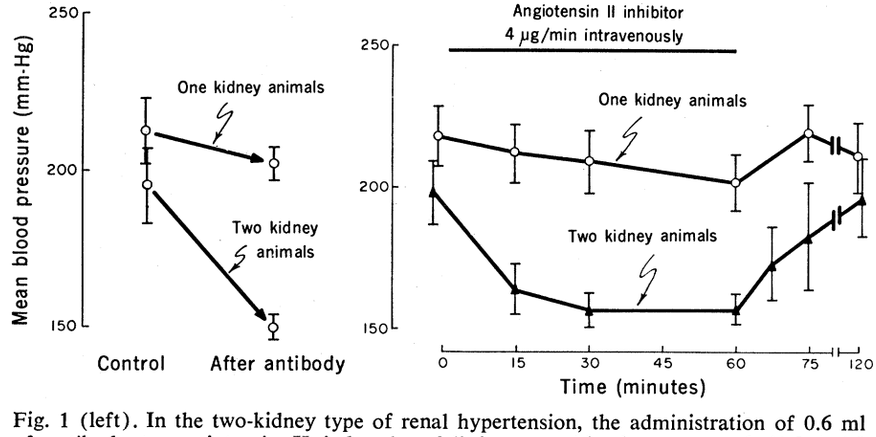

Fig. 1 (left). In the two-kidney type of renal hypertension, the administration of 0.6 ml of antibody to angiotensin II induced a fall in mean blood pressure of 41.0 ± 4.0 mm-Hg ($P < .001$), whereas in the one-kidney type of renal hypertension, the blood pressure fell by only 10.0 ± 6.4 mm-Hg. This latter change was not significant ($P > .1$). Fig. 2. (right). In the two-kidney type of renal hypertension infusion of the angiotensin inhibitor induced an immediate and progressive fall in blood pressure which reached 40.8 ± 4.2 mm-Hg after 60 minutes ($P < .001$). In contrast, the change in blood pressure induced in the one-kidney type of renal hypertension was not significant ($P > .1$).

the two-kidney form of renal hypertension. However, the results also strongly suggest that one-kidney renal hypertension is maintained by pressor mechanisms different from the renin-angiotensin system.

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Dopamine-Sensitive Adenyl Cyclase: Possible Role in Synaptic Transmission

Abstract. *An adenyl cyclase activated by low concentrations of dopamine has been found in the mammalian superior cervical sympathetic ganglion. The existence of this enzyme may account for the increased amount of adenosine 3′,5′-monophosphate associated with synaptic activity in the ganglion. The results suggest that the physiological effects of dopamine in the ganglion, and possibly elsewhere in the nervous system, may be mediated by stimulating the synthesis of adenosine 3′,5′-monophosphate.*

Experiments in our laboratory (*1*) have demonstrated that preganglionic stimulation of the superior cervical sympathetic ganglion of the rabbit, under relatively physiological conditions, produced a severalfold increase in the content of adenosine 3′,5′-monophosphate (cyclic AMP) in the ganglion. In contrast, postganglionic stimulation produced no increase in the cyclic AMP content of the ganglion. From these and other observations, it was concluded that the increased amount of cyclic AMP was associated with the process of synaptic transmission within the ganglion, and that the increase occurred primarily in postsynaptic cells. This and a variety of other evidence, summarized elsewhere (*2, 3*), suggests that the cyclic AMP system may be intimately associated with the physiology of synaptic transmission. It therefore seemed of considerable importance to clarify the mechanism responsible for the increase in cyclic AMP associated with synaptic transmission in the ganglion.

Catecholamines have been shown to increase the cyclic AMP content of many tissues, including brain. There is evidence that suggests adrenergic regulation of synaptic transmission in the superior cervical ganglion (*4–6*). Superior cervical ganglia from several species, including bovine and rabbit, contain two catecholamines, dopamine and norepinephrine, in comparable amounts (*7*); and we have, therefore, studied the ability of these two catecholamines to stimulate the formation of cyclic AMP. Our experiments, with blocks of tissue prepared from bovine ganglia, indicate that dopamine, in low concentrations, and norepinephrine, in higher concentrations, increase the amount of cyclic AMP in intact cells. In addition, in experiments with homogenates of bovine ganglia, it was found that dopamine stimulates adenyl cyclase activity, but does not significantly alter phosphodiesterase activity. These observations support the hypothesis that small chromaffin-like interneurons release dopamine in response to preganglionic stimulation, and that this catecholamine activates adenyl cyclase in postganglionic neurons, thereby mediating the increased amount of cyclic AMP that follows preganglionic stimulation (*1*). Our experiments provide experimental evidence for a new type of adenyl cyclase, one with apparent specificity for dopamine, and suggest a mechanism, at the cellular and molecular levels, for the action of dopamine in the ganglion and possibly in other regions of the nervous system.

The amount of cyclic AMP accumulation in blocks of ganglion tissue was determined by means of the prelabeling technique (*8*) in which the amount of radioactive cyclic AMP formed from prelabeled adenosine triphosphate is determined (*9*). The relative potencies of dopamine and norepinephrine in causing the accumulation of cyclic AMP in blocks of ganglion tissue were compared (Fig. 1). At low concentrations dopamine was more effective than norepinephrine in causing the accumulation of cyclic AMP. The maximum responses to the two catecholamines were approximately equal, although in some experiments the maximum response to norepinephrine was somewhat greater than to dopamine. In each of several experiments similar to that shown in Fig. 1, a half-maximum increase in cyclic AMP accumulation occurred in the presence of 6 to 10 μM dopamine. Moreover, in those experiments, 42 μM norepinephrine produced a response approximately equal to that seen with 7 μM dopamine.

In some experiments, the absolute amount of cyclic AMP was also determined by means of the protein kinase assay method (*11*). The results obtained with the two methods were similar. For instance, in one experiment the absolute amount of cyclic AMP in prelabeled but nonincubated tissue was 14.5 pmole per milligram of protein. Incubation in the presence of 10 mM theophylline alone caused a 2.6-fold increase in the absolute amount of cyclic AMP and a 3.2-fold increase in the amount of radioactive cyclic AMP. Incubation in the presence of 30 μM dopamine plus 10 mM theophylline caused a 9.7-fold increase in the absolute amount of cyclic AMP and a 9.1-fold increase in the amount of radioactive cyclic AMP.

We have studied the effect of agents that antagonize the actions of catecholamines in other tissues on the accumulation of cyclic AMP in the superior cervical ganglion. Phentolamine, an α-adrenergic antagonist, prevented the increase in cyclic AMP produced by