Arterial Hypertension Elicited by Subpressor Amounts of Angiotensin

Abstract. *Long-term infusion of amounts of angiotensin insufficient at the beginning to raise arterial pressure results, after several days in sustained arterial hypertension in unanesthetized dogs. This hypertension is to a large degree dependent on environmental stimuli, and results chiefly from increase in peripheral resistance. As in dogs with renal hypertension, there is increased pressor responsiveness to tyramine. This indirect action of angiotensin to increase total peripheral resistance and arterial pressure by an action on the sympathetic nervous system, along with an upward resetting of the carotid sinus buffering mechanism, might logically account for the neural component of chronic renal hypertension. Such a proposal integrates the humoral and neural elements of the mosaic describing the mechanisms of tissue perfusion.*

It has been shown by McCubbin and Page (*1*) that infusion of angiotensin intensifies the pressor action of ephedrine, tyramine, and a ganglion-stimulating agent; since the action of these drugs is dependent on release of norepinephrine at nerve endings, the conclusion was drawn that angiotensin has an indirect effect on the sympathetic nervous system. Similar increases in responses to tyramine were observed in dogs with chronic renal hypertension. It was desirable to test whether angiotensin, infused at a rate insufficient to raise blood pressure in a short time, could cause hypertension if infused at the same rate over a long period.

We used unanesthetized dogs with implanted arterial and venous catheters. In some of these dogs, we also implanted aortic flow probes to monitor cardiac output. Angiotensin in saline with 1 percent Bacitracin was infused at a rate of 10 μg/kg per day in a total volume of about 10 ml by an electrolytically driven pump strapped to the animal. Arterial pressure and cardiac output were recorded daily for periods of about 20 minutes. Responses to tyramine, norepinephrine, and angiotensin were measured regularly.

With the very small dosage of angiotensin used, there was no increase in mean arterial pressure during the first 1 to 2 hours, but after 24 hours it had risen an average of 33 mm-Hg in 5 of 12 animals. A significant increase occurred after 3 to 9 days in the remainder. Following these periods, there was further progressive rise. The average increase was 30/23 mm-Hg after 1 week and 45/33 mm-Hg after 2 weeks. These levels were maintained for the durations of the infusions which lasted as long as 30 days.

Most characteristic of the pressure rise was its great lability. Before the angiotensin infusion, mean arterial pressure was quite stable at levels of about 85 mm-Hg, even during the normal movements of the animals. But during the infusion it became astonishingly variable. For example, the pressure rose to 230/170 mm-Hg in one experiment while the dog scratched itself. When the infusions were carried out in our usual laboratories without attempts to shield the dogs from environmental disturbance, blood pressures were continuously high except during sleep. Repeating the infusion in a laboratory with minimal environmental disturbance showed basal arterial pressure to be either at control levels or only some 5 to 15 mm-Hg higher, even after long infusion (Fig. 1). But if the animals moved or were handled, pressure rose strikingly, contrasting sharply with the pressure response before the angiotensin. The longer the infusion was continued, the more difficult it was to obtain normal blood pressure.

After a few days of infusion, pressure returned to normal within a few hours after the infusion was stopped, but after a 25-day infusion, it reached normal levels only after 48 hours. As hypertension developed during the first several days, peripheral resistance increased parallel with the rise in pressure. There was a tendency for cardiac output and heart rate to decrease, but this was not clear-cut. The large fluctuations associated with environmental stimulation were caused by change in peripheral resistance rather than by cardiac output. Treatment of the dogs with subcutaneous guanethidene abolished the hypertension.

The responses to tyramine increased with occurrence of hypertension and slowly returned to control values at a rate about parallel with arterial pressure. Response to single injections of angiotensin showed no consistent change. The responses to carotid artery occlusion (Van Leersum loop) often doubled in height.

We propose that angiotensin causes hypertension by an indirect action

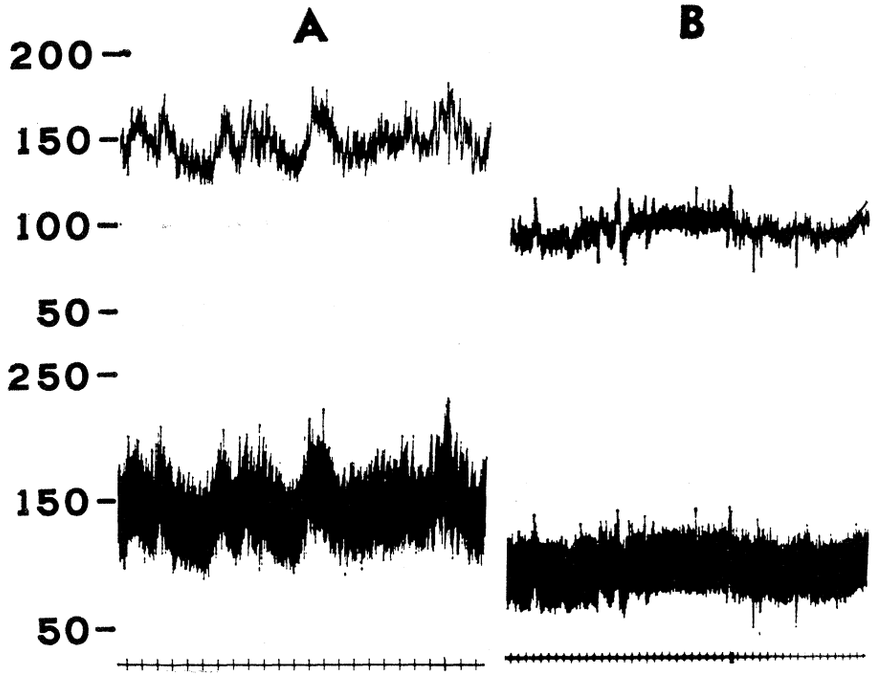

Fig. 1. Arterial pressure of dog after 1 week of infusion of initially subpressor amount of angiotensin. (Top) Mean arterial pressure; (bottom) systolic and diastolic pressures. (*A*) Dog lying quietly on its side but surrounded by normal laboratory activity. (*B*) Same position but with laboratory completely quiet. Time marks: 1 minute.

mediated by the sympathetic nervous system, an action independent of its direct vasoconstrictor action. The parallelism of the increase in response to tyramine and the rise in arterial pressure suggests that a common mechanism is concerned.

These observations bear on the nature of renal and essential hypertension in man. The dogs behaved very much like labile essential hypertensives. The amount of angiotensin need not be large to elicit relatively severe hypertension by its action on the sympathetic nervous system.

Dickinson and Lawrence (*2*) found a delayed rise of pressure in rabbits infused with small amounts of angiotensin. They suggested that the hypertension was due to cerebral vasoconstriction; it seems more likely to us that the peripheral sympathetic nervous system is affected.

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References and Notes

1. J. W. McCubbin and I. H. Page, *Science* **139**, 210 (1963).
2. C. J. Dickinson and J. R. Lawrence, *Lancet* **1963-I**, 1354 (1963).
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Hemolysin Production in the Development of Staphylococcal Lesions

Abstract. *The presence of hemolysin in staphylococcal lesions of the rabbit kidney was detected by overlaying sections of kidney with blood agar. Hemolytic activity against rabbit, human, sheep, calf, and guinea pig erythrocytes was present and was not abolished by heating the sections to 60°C for 30 minutes. In developing lesions, hemolytic activity appeared before necrosis or exudation.*

Although culture filtrates of pathogenic staphylococci contain a large number of substances capable of damaging cells and tissues, none of these toxins have been shown directly to play a role in the pathogenesis of staphylococcal infections. Neither has it been clearly demonstrated that staphylococci produce toxins while growing in a host's tissue, although production of hemolysin in peritoneal exudates has been observed (*1*). Since the production in vitro of many of the toxins is influenced by the conditions of culture, staphylococci could fail to elaborate any or all toxins under the conditions of growth in a particular tissue. If this were true the toxin could have no role as a pathogenic agent in that tissue. On the other hand, if a toxin is produced in a tissue, careful attention must be paid to the temporal relation between the appearance of the toxin and the development of tissue injury in assessing the pathogenic role of the toxin.

Table 1. Hemolytic activity and histologic changes in rabbit kidneys infected with staphylococci. Each animal received 10^8 cells of *Staphylococcus aureus* (Wood 46 strain) per kilogram of body weight by intravenous injection at 0 hour. RBC, red blood cells.

Time after inoculation (hr)	Staphylococci (avg. No. per gram of kidney)	Hemolysis of:*		Histologic findings*			
		Rabbit RBC	Human RBC†	Degeneration	Bacteria	Necrosis	Leukocytes
6 to 8	3.9×10^2	3/6	2/6	4/6	0/6	0/6	0/6
9 to 12	4.3×10^5	7/9	2/9	5/9	2/9	1/9	0/9
18	6.8×10^6	5/5	5/5	5/5	5/5	5/5	2/5

* Number of animals with positive findings in kidney tissue over number infected animals examined. † In each instance, kidney sections showing hemolysis of human erythrocytes also showed hemolysis of rabbit erythrocytes.

Two probably pathogenic staphylococcal products are α-hemolysin and δ-hemolysin, both of which are produced in vitro by most pathogenic strains (*2*, pp. 239, 343, 247). Alpha-hemolysin causes local necrosis when injected directly into the skin and is lethal to rabbits when injected intravenously (*2*, p. 260); δ-hemolysin has much less pronounced toxic properties (*3*). Alpha hemolysin lyses rabbit erythrocytes readily. Sheep, cow, and goat cells are much less sensitive to this hemolysin; human, guinea pig, and horse cells are completely resistant (*2*, p. 231; *3*). Delta hemolysin is active against rabbit, human, guinea pig, monkey, horse, rat, and sheep erythrocytes (*2*, p. 242; *3*). The α- and δ-hemolysins can be further distinguished from each other by tests of their heat stability. In crude preparations, the activity of α-hemolysin is completely abolished by heating at 60°C for 30 minutes (*2*, p. 231). According to some reports, δ-hemolysin is partially inactivated by heating at 65°C for 30 minutes (*3*). Others state that purified δ-hemolysin is stable at 65°C or 100°C for 2 hours (*2*, p. 243).

The production of hemolysin in staphylococcal lesions of the rabbit kidney is reported here. Renal infections were induced by injecting approximately 10^8 staphylococci per kilogram of body weight into the ear veins of white New Zealand rabbits (2 kg). The animals were killed at intervals after inoculation by intravenous injection of sodium pentobarbital. Blocks of renal tissue were frozen with dry ice within 10 minutes of the animal's death. To detect the presence of hemolysin, frozen sections of kidney (prepared in a cryostat), approximately 8μ thick, were covered with a thin layer of agar containing 5 percent of triply washed erythrocytes as well as 5 μg/ml of sodium methicillin to prevent bacterial growth. The diluent was either 0.85 percent NaCl or 5.5 percent glucose in 0.01*M* phosphate buffer, *p*H 6.8 to 7.0. The necessary thinness of the blood-agar layer was achieved by placing a drop of the liquid material on the tissue and rapidly covering it with a glass cover slip (22 × 22 mm). Preparations were incubated for 1 to 3 hours at 37°C in a moist chamber. Hemolysis could be recognized in transmitted light with the naked eye.

In the initial studies, animals were killed 2 to 4 days after intravenous inoculation of a locally isolated, human-pathogenic strain of *Staphylococcus* (Kyser). Grossly visible, small abscesses were present in the kidneys. Hemolytic activities against rabbit, human, sheep, calf, and guinea pig erythrocytes were demonstrated in the lesions. Hemolysis was evident after 30 minutes of incubation in some instances, and, usually, after 2 hours it was fully developed. The hemolytic activity could be reduced, but not abolished, by maintaining the sections at 60°C for 30 minutes before applying the blood agar. Heating the sections for 10 minutes at 100°C abolished all hemolytic activity.

The possibility that the hemolysis was due to the presence of products of damaged tissue in the lesions was considered. Necrotic, 2-day-old lesions of similar histologic appearance to the