min A deficiency; a smaller proportion of RNA species with low electrophoretic mobility is found in vitamin A–deficient animals than is found in normal controls. This alteration is reversed after vitamin A treatment, with 2 weeks necessary for complete restoration of the normal electrophoretic pattern. Although migration of RNA molecules in gel electrophoresis generally bears an inverse reaction to molecular weight (*10*), variations in secondary structure can alter mobilities (*11*, *12*). The change observed in the vitamin A–deficient animals is a relative lack of RNA molecules with low electrophoretic mobility. It is not known at present whether this represents a deficiency of RNA molecules with specific nucleotide sequences or whether these sequences are present, but with mobilities somehow changed. Alterations in rates of synthesis, processing, or degradation of RNA molecules might account for the observed changes in electrophoretic pattern. Parallel morphologic studies indicate that these changes persist despite the disappearance of squamous metaplasia and restoration of normal cellular populations within 1 week after vitamin A treatment (*13*). Thus, the changes in electrophoretic pattern cannot be completely explained as the result of an altered cellular population. Analogous to this observed effect of vitamin A on the electrophoretic pattern of RNA is the fact that (*14*) there is a relative deficiency of RNA molecules of low electrophoretic mobility in mammary gland organ cultures maintained on medium without hydrocortisone, and an increase in such molecules upon addition of hydrocortisone to the cultures.

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References and Notes

1. S. B. Wolbach and P. R. Howe, *J. Exp. Med.* **42**, 753 (1925); J. J. Salley and W. F. Bryson, *J. Dent. Res.* **36**, 935 (1957); Y. Wong and R. Buck, *Lab. Invest.* **24**, 55 (1971).
2. C. C. Harris, M. B. Sporn, D. G. Kaufman, J. M. Smith, F. E. Jackson, U. Saffiotti, *J. Nat. Cancer Inst.* **48**, 743 (1972).
3. L. De Luca, H. K. Kleinman, E. P. Little, G. Wolf, *Arch. Biochem. Biophys.* **145**, 332 (1971).
4. R. D. Zachman, *Life Sci.* **6**, 2207 (1967).
5. M. Zile and H. F. De Luca, *Arch. Biochem. Biophys.* **140**, 210 (1970).
6. B. C. Johnson, M. Kennedy, N. Chiba, *Amer. J. Clin. Nutr.* **22**, 1048 (1969).
7. A. Leibovitz, *Amer. J. Hyg.* **78**, 173 (1963).
8. D. G. Kaufman, M. S. Baker, M. B. Sporn, U. Saffiotti, *Proc. Amer. Ass. Cancer Res.* **12**, 12 (1971); D. G. Kaufman, M. S. Baker, C. C. Harris, J. M. Smith, H. G. Boren, M. B. Sporn, U. Saffiotti, *J. Nat. Cancer Inst.*, in press.
9. S. Raymond, *Clin. Chem.* **8**, 455 (1962).
10. D. H. L. Bishop, J. R. Claybrook, S. Spiegelman, *J. Mol. Biol.* **26**, 373 (1967); A. C. Peacock and C. W. Dingman, *Biochemistry* **7**, 688 (1968).
11. M. P. Fisher and C. W. Dingman, *Biochemistry* **10**, 1895 (1971).
12. U. E. Loening, *Biochem. J.* **113**, 131 (1969).
13. H. G. Boren, C. C. Harris, D. G. Kaufman, unpublished observations.
14. M. R. Green, S. L. Bunting, A. C. Peacock, *Biochemistry* **10**, 2366 (1971).
15. We thank Dr. W. Dingman for suggestions with respect to acrylamide gel electrophoresis, and Dr. R. Brown and G. Stoner for their assistance with the computer program used to analyze the electrophoretic data.

31 January 1972; revised 3 April 1972

Inhibition of Angiotensin Conversion in Experimental Renovascular Hypertension

Abstract. *Constriction of the renal artery and controlled reduction of renal perfusion pressure is followed by a prompt increase in systemic renin activity and a concomitant rise in blood pressure in trained, unanesthetized dogs. The elevated blood pressure induced by the renal artery stenosis can be prevented by prior treatment with the nonapeptide Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro, which blocks conversion of angiotensin I to angiotensin II. Further, the nonapeptide can restore systemic pressure to normal in the early phase of renovascular hypertension. These results offer strong evidence that the renin-angiotensin system is responsible for the initiation of hypertension in the unilaterally nephrectomized dog with renal artery constriction.*

Although some evidence suggests that the renin-angiotensin system may become relatively unimportant in the maintenance of blood pressure in chronic renovascular hypertension in animals with one kidney removed (*1*), data are not available to define the role of this system in the initiation of the elevated blood pressure. Gutmann and co-workers (*2*) demonstrated in unanesthetized dogs previously subjected to unilateral nephrectomy that both systemic renin activity and blood pressure rise within minutes after renal artery constriction and controlled reduction of renal perfusion pressure. To determine whether the rise in renin activity and blood pressure are causally related, similar experiments were performed in which animals were given the nonapeptide Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro (*3*), a potent inhibitor of the angiotensin-converting enzyme (angiotensin I to angiotensin II).

Under sterile conditions, polyvinyl catheters were inserted into the aorta, renal artery, and inferior vena cava of each of ten anesthetized male mongrel dogs (26 to 34 kg), and an externally inflatable cuff was placed about

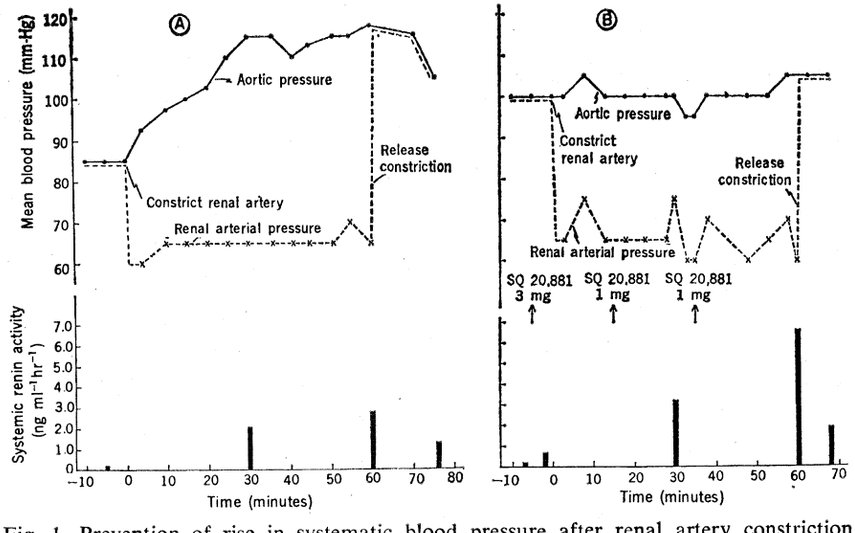

Fig. 1. Prevention of rise in systematic blood pressure after renal artery constriction by blockade of conversion of angiotensin I to angiotensin II by the nonapeptide. Data are for (A) an untreated dog and for (B) the same dog after intravenous administration of the drug.

the origin of the renal artery (*2*, *4*). The proximal portions of the catheters were brought through the skin, and the contralateral kidney was excised. The catheters were filled with heparin (1000 units per milliliter) daily and stoppered with stainless steel obturators. The dogs were maintained on a constant diet and given free access to water.

Experiments were begun after a 2-week recovery period, and were in all instances performed on conscious animals trained to lie quietly on a padded table. Aortic and renal arterial pressures were measured with matched Statham P23-H pressure transducers and recorded on a Grass polygraph. All drug injections and blood withdrawals were made through the venous catheter. Blood was collected in iced sterile tubes containing the disodium salt of ethylenediaminetetraacetic acid and centrifuged in the cold. The plasma renin activity was measured by the radioimmunoassay technique of Haber *et al.* (*5*) and expressed as angiotensin I generated (nanograms per milliliter per hour). The packed cells were returned to the animal after each experiment. In ten dogs systemic renin activity and aortic pressure rose rapidly when renal arterial pressure was reduced to 50 to 70 mm-Hg. At 60 minutes pressure was elevated by an average of 20 mm-Hg (range, 5 to 35 mm-Hg). A representative experiment is illustrated in Fig. 1A. There was no consistent change in either heart rate or behavior following constriction. After release of the constriction, both renin activity and pressure fell.

To determine whether the rise in angiotensin II was responsible for the elevation of blood pressure, inhibition of the angiotensin-converting enzyme was produced by administration of the nonapeptide Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro, a bradykinin-potentiating peptide isolated from the venom of the Brazilian snake *Bothrops jararaca* and synthesized by Ondetti *et al.* (*6*). Bakhle (*7*) first demonstrated that extracts of the venom also markedly inhibited angiotensin-converting enzyme, and at a dose lower than that required for potentiation of bradykinin.

In the present study we first demonstrated in five experiments in three normal dogs that intravenous injection of the nonapeptide caused either no change or a transient fall in blood pressure of 5 mm-Hg. Systemic renin activity was not altered by the administration of the peptide in the normal dog. Plasma bradykinin was undetectable before and after the injection of 5 to 10 mg of the drug (*8*). Intravenous administration of 3 to 5 mg of the nonapeptide was found to block the usual pressor response (25 to 30 mm-Hg) induced by intravenous injection of 1.0 μg of angiotensin I. The pressor response to angiotensin II was unchanged.

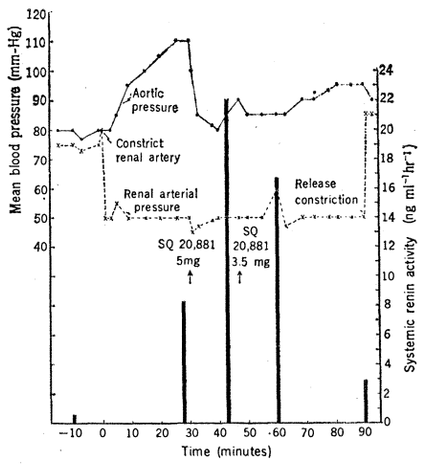

Fig. 2. Restoration of normal blood pressure by blockade of conversion of angiotensin I to angiotensin II 30 minutes after renal artery constriction.

In six experiments in four dogs, the peptide (3 to 5 mg), administered before renal artery constriction, blocked the usual rise of aortic pressure (Fig. 1B). The mean change in pressure was +2.5 mm-Hg, with a range of −5 to +10 mm-Hg; the difference from control experiments was highly significant ($P < .001$). Renin activity was more markedly elevated following renal artery constriction in the animals treated with peptide than in the untreated dogs. These observations provide strong evidence that the renin-angiotensin system initiates the rise in blood pressure that follows renal artery constriction. No rise in pressure is seen after blockade of conversion of angiotensin I to angiotensin II.

To provide further evidence that angiotensin II is responsible for the elevation of systemic pressure, the renal artery was constricted to lower pressure to 50 to 60 mm-Hg, and systemic pressure was allowed to rise. After 30 to 60 minutes, when pressure was elevated 20 to 30 mm-Hg, 3 to 5 mg of the peptide was administered. In seven experiments performed in four dogs, systemic pressure fell to baseline or near baseline levels (Fig. 2); further elevation in renin activity was measured after drug administration.

The rise in systemic pressure which occurs following renal artery constriction in the unilaterally nephrectomized dog is accompanied by an increase in plasma renin activity and (by inference) angiotensin I and II. The development of hypertension in this animal can be prevented by inhibiting conversion of angiotensin I to angiotensin II by a specific peptide inhibitor. Further, the pressure, which is elevated following renal artery constriction, can be restored to normal by administration of a peptide dose that does not raise plasma bradykinin. These results provide strong evidence that angiotensin II is responsible for the initiation of renovascular hypertension. It will be of interest to determine whether hypertension can be abolished in the dog with long-term constriction of the renal artery, and whether renovascular hypertension can be induced during long-term administration of the nonapeptide.

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References and Notes

1. E. M. Krieger, H. C. Salgado, C. J. Assan, L. L. J. Greene, S. H. Ferreira, *Lancet* **1971-I**, 269 (1971); H. R. Brunner, J. D. Kirshman, J. E. Sealey, J. H. Laragh, *Science* **174**, 1344 (1971); D. T. Pals, F. D. Masucci, G. S. Denning, Jr., F. Sipos, D. C. Fessler, *Circ. Res.* **29**, 673 (1971); H. R. Brunner, P. Chang, R. Wallach, J. E. Sealey, J. H. Laragh, *J. Clin. Invest.* **51**, 58 (1972).
2. F. D. Gutmann, H. Tagawa, E. Haber, A. C. Barger, *Fed. Proc.* **31**, 317 (1972).
3. The nonapeptide was a gift of Squibb Corp., designated SQ 20,881. The abbreviations are Pyr, pyroglutamic acid; Trp, tryptophan; Pro, proline; Arg, arginine; Gln, glutamine; and Ile, isoleucine.
4. J. A. Herd and A. C. Barger, *J. Appl. Physiol.* **19**, 791 (1964).
5. E. Haber, T. Koerner, L. B. Page, B. Kliman, A. Purnode, *J. Clin. Endocrinol.* **29**, 1349 (1969).
6. M. A. Ondetti, N. J. Williams, E. F. Sabo, J. Pluscec, E. R. Weaver, O. Kocy, *Biochemistry* **19**, 4033 (1971).
7. Y. S. Bakhle, *Nature* **220**, 919 (1968).
8. We are indebted to R. C. Talamo for assaying the plasma samples by the method of R. C. Talamo, E. Haber, and K. F. Austen [*J. Lab. Clin. Med.* **74**, 816 (1969)].
9. Supported by PHS grant 1 P17 HE14150 for a specialized center of research.
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24 April 1972; revised 26 June 1972