Differentiation of Nephrotensin from Angiotensin I and II[1] (37258)

ARTHUR GROLLMAN AND V. S. R. KRISHNAMURTY[2]

Department of Pathology, University of Texas, Southwestern Medical School, Dallas, Texas 75235

It is well established that experimental hypertension induced acutely by drastic restriction of the renal artery and rare instances of hypertensive disease in the human differ in several fundamental respects from chronic sustained hypertensive disease (1–5). Only in the former is the elevation in blood pressure alleviated by nephrectomy and is the presence of a pressor agent demonstrable in the renal venous effluent from the affected kidney (3, 5). Prepossession with renin has led to the assumption that this pressor agent is angiotensin II. In previous reports from this laboratory (4, 6), it has been shown, however, that a hitherto unrecognized pressor agent is formed in ischemic renal tissue and that it is this agent which is responsible for the elevation in blood pressure in so-called "renovascular" (more accurately designated as surgically remediable) and in acute experimental hypertension. Nephrotensin, as this pressor agent has been designated has been claimed to be identical or immunologically related to angiotensin I (7) but incubation with angiotensin I antibody makes it possible to differentiate them. The results of this study and the demonstration of other differences between the angiotensins and nephrotensin add further support to the view that the latter is a unique pressor agent which is responsible for the elevation of blood pressure observed in acute experimental and other forms of surgically remediable hypertension.

Materials and Methods. Nephrotensin was obtained by drastically restricting the renal arteries of 6 mongrel dogs of either sex, 20–25 kg in weight, as previously described (6). This procedure concentrates the nephrotensin present in the peripheral blood 10–20-fold and yields a product containing 25 ng pressor equivalents of synthetic angiotensin II per ml which is free of angiotensin I and II, catecholamines, histamine and 5-hydroxytyramine (6).

The vasoactivity of the pressor agents used in the present study was determined by their effect on the blood pressure of the anesthetized, ganglion-blocked rat and on the perfusion pressure of the isolated rabbit's ear by the previously described methods (6). In the latter procedure, the rabbit was pretreated with 2.5 mg/kg of reserpine on the day prior to use in order to avoid any indirect effects due to the release of catecholamines. Each experiment was performed on at least 5 animals.

The pressor agents, angiotensin I and nephrotensin, were incubated with commercially available 5 asp[1]-ileu[5]-angiotensin I antibody at 4° for 24 hr as suggested by Haber *et al.* (8). In preliminary experiments the amount of angiotensin I antibody required to abolish the pressor action and increased perfusion pressure of the isolated rabbit's ear of 5 ng of asp[1]-ileu[5]-angiotensin I was determined. Twice this amount of antibody was dissolved in 0.2 ml normal saline and incubated with 5 ng of angiotensin I dissolved in 0.2 ml normal saline. An equal amount of the antibody in 0.2 ml normal saline was incubated with 5 ng angiotensin II equivalents of nephrotensin prepared as described above and dissolved in 0.4 ml of normal saline. The pressor activity of the angiotensin I and nephrotensin and their action on the perfusion pressure of the isolated rabbit's ear were

[1] Aided by U.S. Public Health Service Institutional Grant 5 SO1 RR 05426-10 and the Lilien K. Christy Bequest.

[2] Present address: Department of Pharmacology, University of Baroda, Baroda, India. Mr. Krishnamurty was a pre-doctoral fellow during the period of this study.

determined prior to the incubation and that of the pressor agent–antibody solution following their incubation.

Drugs. The following drugs were used: Synthetic val^5-angiotensin II-asp^1-β-amide (Hypertensin, Ciba); reserpine (Serpasil, Ciba); guanethidine (Ismelin monosulfate, Ciba); asp^1-ileu^{5}angiotensin I and asp^1-ileu^5-angiotensin I antibody (Schwarz–Mann); and α-norepinephrine bitartrate monohydrate (Sigma). Stock solutions were prepared every 5 days in normal saline, kept at 4°, and diluted just before use. The angiotensin I antibody was stored at —20°. Volumes of solution injected never exceeded 0.4 ml into rats and 0.2 ml into the isolated rabbit's ear.

Results. Nephrotensin, as shown in Figs. 1–3, differs in its action from angiotensin I and II in many ways. The pressor action of norepinephrine is facilitated by the prior injection of nephrotensin (Fig. 1 A and H). Unlike angiotensin I, the pressor activity of which is abolished by incubation with angiotensin I antibody (Fig. 1 B, C, and F) the pressor action of nephrotensin is not altered by this procedure (Fig. 1 E and G).

Angiotensin II is a vasoconstrictor of the isolated rabbit's ear (Fig. 2 A) but its action is abolished by the development of tachyphylaxis (Fig. 2B). Norepinephrine (Fig. 2 C and D) and nephrotensin (Fig. 2 E and F), on the other hand, manifest no tachyphylaxis and hence, unlike angiotensin may be envisaged as potential mediators of a sustained rise in blood pressure. The vasoconstrictor action of norepinephrine is facilitated by the prior injection of nephrotensin (Fig. 2G) but is not affected by prior treatment with guanethidine (Fig. 2 H) or reserpine (Fig. 2 I).

Incubation with angiotensin I antibody abolishes the vasoconstrictor effect of angiotensin I on the isolated rabbit's ear (Fig. 3 A). The effect of nephrotensin (Fig. 3 D and E) on the other hand, is not altered by prior incubation with angiotensin I antibody (Fig. 3 F and G).

Discussion. That the acute elevation in blood pressure observed following drastic restriction of the renal artery and in surgically remediable hypertension of the human differ pathogenetically from the elevation observed in the chronic stage of hypertensive disease is apparent from the fact that only the former are relieved by nephrectomy and are accompanied by the presence in the renal venous effluent of a demonstrable pressor agent (1–5). The results of the present study

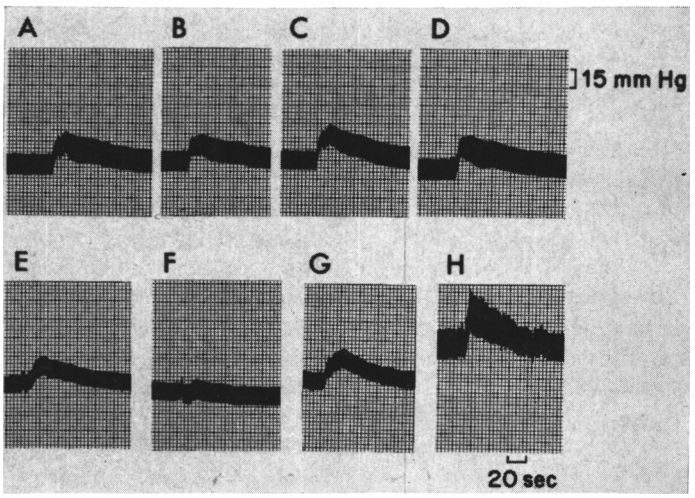

FIG. 1. Effect of norepinephrine (NE), angiotensin I and II (Angio I and II), and nephrotensin on the blood pressure of the anesthetized ganglion-blocked rat. Pressor response to: A, 50 ng of NE; B and C, 5 and 10 ng of Angio I, respectively; D, 50 ng NE; E, 5 ng Angio II equivalents of nephrotensin; F, 5 ng of Angio I after incubation with Angio I antibody; G, 5 ng Angio II equivalents of nephrotensin after incubation with Angio I antibody; H, 50 ng NE following injection of 5 ng Angio II equivalents of nephrotensin.

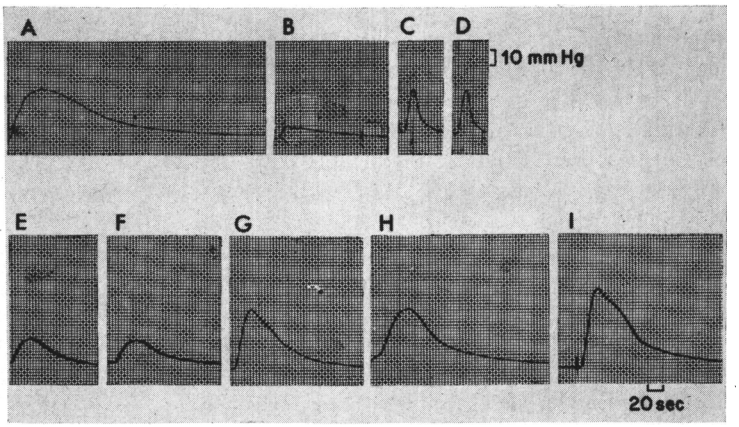

FIG. 2. The effect of nephrotensin and its facilitation of the vasoconstrictor response to NE of the isolated ear of a rabbit pretreated with 2.5 ng reserpine per kg of body weight. A, the effect of 5 μg of Angio II; B, as in A, 10 min later, showing tachyphylaxis; C and D, equipotent responses to 10 ng of NE; E and F, equipotent response to 1.25 ng Angio II equivalents of nephrotensin; G, potentiation of response to 10 ng of NE by prior injection of 1.25 ng Angio II equivalents of nephrotensin; H, as in G, but after pretreatment with 100 μg guanethidine; I, as in G, but after pretreatment with reserpine (2.5 mg per kg body weight).

support the claim that nephrotensin, a newly described pressor agent, and not angiotensins I or II resulting from an excessive production of renin by the ischemic kidney is responsible for the observed rise in blood pressure in acute and surgically remediable hypertension.

Nephrotensin differs in its pressor and vasoconstrictor action as well as in its physicochemical properties (5, 6) from angiotensin I and II and other vasoactive monamines and kinins. Unlike the latter, it is not dialyzable at acid pH, is not adsorbed by the cationic exchange resin Dowex-50X2, exerts a potent vasopressor action without tachyphylaxis, and sensitizes vascular tissue to NE after prior treatment with reserpine and guanethidine. This sensitization cannot be attributed to the release or interference with the storage mechanisms of catecholamines since reserpine and guanethidine which interrupt the storage and reuptake of catecholamines at neuronal endings do not affect the pressor response to nephrotensin. The pressor action of angiotensin I on the anesthetized ganglion-blocked rat and on the perfusion pressure of the isolated rabbit's ear is abolished by incubation with angiotensin I antibody; the action of nephrotensin is not altered by the same procedure.

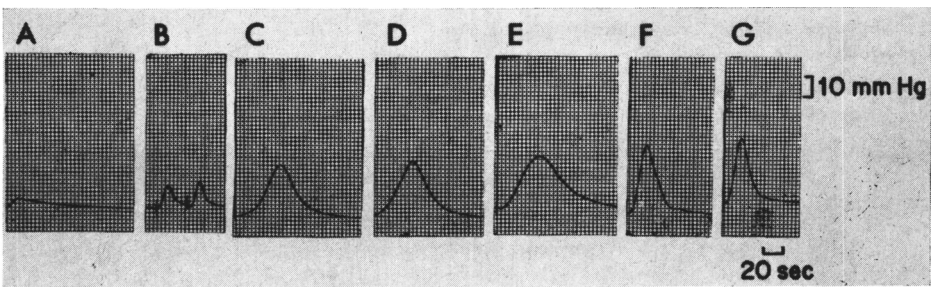

FIG. 3. Effect of Angio II, NE, and nephrotensin, before and after incubation with Angio I antibody on the perfusion pressure of the isolated ear of the rabbit pretreated with 100 μg guanethidine. A, insignificant effect of 5 μg angiotensin I; B and C, of 5 and 10 ng NE, respectively; D and E, of 1.25 ng angiotensin II pressor equivalents of nephrotensin; F and G, as in D and E but after incubation with angiotensin I antibody.

Although nephrotensin may be immunologically related to angiotensin I, as suggested by Schweikert *et al.* (7), the fact that unlike the latter it is not inactivated by angiotensin I antibody proves that the two compounds are not identical. The fact that prophylactic preimmunization against angiotensin II (9) has no effect on the acute hypertension produced by clipping the renal artery is further evidence against the assumption that liberation of its precursor, angiotensin I, mediates the rise in blood pressure in acute hypertension.

The liberation from the kidney of a humoral agent that enhances adrenergic responsiveness during acute renal ischemia has also been demonstrated by Sweet *et al.* (10). Their suggestion that this agent may be prostaglandin E_2 appears unlikely since this compound, unlike nephrotensin, is lipid soluble and stimulates contraction of the rat's rectum (6).

That the secretion of excessive quantities of renin are not responsible for the rise in blood pressure in acute experimental and surgically remediable hypertension in the human is supported by the lack of correlation between the renin activity of the blood and the response to nephrectomy and the high levels of renin observed in normotensive patients suffering from cirrhosis of the liver and Bartter's syndrome (6, 11). The occasional occurrence of a renal tumor in association with hypertension and an increased secretion of renin (12) may be attributed to the ischemia induced by compression of the adjacent tissue by the tumor. Surgically remediable hypertension has also been noted in patients with cysts or other nonrenin-producing masses.

Summary. Nephrotensin, a newly described pressor agent which appears in the renal venous effluent of the ischemic kidney is not inactivated by angiotensin I antibody. It can also be differentiated from angiotensin I and II by the response to its injection of the rat's blood pressure and the perfusion pressure of the isolated rabbit's ear. These findings add further evidence that nephrotensin is a unique pressor agent responsible for the elevation in blood pressure observed in renovascular (surgically remediable) hypertension as observed in man and in the acute stage of experimental hypertension induced by drastic restriction of the renal artery or infarction of the kidney.

1. Ogden, E., Collings, W. D., and Saperstein, L. A., Special Publications N. Y. Acad. Sci. **3**, 153 (1946).

2. Scornik, O., and Paladini, A., Amer. J. Physiol. **201**, 526 (1961).

3. Koletsky, S., and Pritchard, W. H., Circ. Res. **13**, 552 (1963).

4. McPhaul, J. J., McIntosh, D. A., Williams, L. F., Gritti, E. J., and Grollman, A., Circulation **33**, 781 (1966).

5. Grollman, A., Clin. Pharmacol. Ther. **10**, 755 (1969).

6. Grollman,, A., and Krishnamurty, V. S. R., Amer. J. Physiol. **221**, 1499 (1971).

7. Schweikert, J. R., Cary, R. M., and Liddle, G. W., Clin. Res. **19**, 32 (1971).

8. Haber, E., Koerner, T., Page, L. B., Kliman, B., and Purnode, A., J. Clin. Endocrinol. Metab. **29**, 1349 (1969).

9. Johnston, C. I., Hutchinson, J. S., and Mendelsohn, F. A., Circ. Res. **27**, 215 (1970).

10. Sweet, C. S., Kadowitz, P. J., and Brody, M. J., Nature (London) **231**, 263 (1971).

11. Grollman, A., and Ebihara, A., Texas Rep. Biol. Med. **26**, 313 (1968).

12. Robertson, P. W., Klidjian, A., Harding, L. K., Walters, G., Lee, M. R., and Robb-Smith, A. H. T., Amer. J. Med. **43**, 963 (1967).

Received Nov. 6, 1972. P.S.E.B.M., 1973, Vol. 143.