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Differentiation of Nephrotensin from the Renin Angiotensin System[1] (38668)

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(Introduced by Bismarck B. Lozzio)

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Several years ago it was suggested (1, 2) that the development of renovascular hypertension involves a vasopressor substance which is independent of the renin–angiotensin system (RAS). Recently, Grollman, *et al.* (3, 4) concentrated a pressor material termed nephrotensin from the plasma of dogs with renal hypertension, and they claimed to have differentiated it from renin, angiotensin I (AI), angiotensin II (AII), and various vasoactive monoamines and kinins. On the other hand, Schweikert (5) has stated that nephrotensin is related to AI.

Since preliminary work from this laboratory (6) indicated that nephrotensin might be involved in the development of renal-clip hypertension in rats, groups of rats were placed under this and other experimental conditions, and an attempt was made to concentrate nephrotensin from their plasmas. When found, nephrotensin was further tested for pressor activity in rats immunized with AII. Since it was reported (7) that AII immunization would not block the pressor activity of AI completely, nephrotensin was also tested in animals treated with AI converting enzyme inhibitor. Thus, the purpose of the present study was to elucidate the relationship between nephrotensin and the RAS.

Materials and Methods. Experiments were performed in male rats of the Memorial Research Center inbred strain (8) weighing from 200 to 300 g. All rats were fed a standard diet (Purina Lab Chow) and given chlorinated (tap) water *ad libitum*.

The animals were divided into five groups of 15 rats each. The first group (control) did not receive any treatment. The second group of animals was treated with a diuretic (Spironolactone) for two weeks. Spironolactone (1.4 mg/kg) was administered daily as an aqueous suspension through a gastric tube. The third group was selected from 26 rats in which renal clip hypertension was induced by placing a silver clip (0.2 mm) on the artery of left kidney, leaving the contralateral kidney untouched. Two weeks later, indirect determinations of systolic blood pressure were made using a tail-cuff (8), and animals for the third group were chosen from the rats with a systolic pressure over 160 mmHg. The fourth group of rats was given a subcutaneous injection of $HgCl_2$ (4.7 mg/kg) and killed 48 hr later. Animals in group 5 were bilaterally nephrectomized, immediately injected intraperitoneally with 3.5 ml/kg of CCl_4 in an equal amount of mineral oil, and killed 24 hr later. Damage to the kidneys or to the liver produced by $HgCl_2$ or CCl_4 respectively was verified by light microscopy. At the end of the treatments, systolic blood pressure was measured in all animals. Six animals from each group were killed by blunt trauma, a laparotomy was made, the renal pedicles were clamped, and specimens for the determination of plasma renin activity were taken from the aorta into prechilled tubes containing 1 mg Na_2EDTA/ml. Plasma renin activity (PRA) was measured by radioimmunoassay as described previously (9).

For the isolation of nephrotensin, the animals were anesthetized with ether, a laparotomy was performed, and blood samples were taken from the aorta into chilled, heparinized centrifuge tubes. After centrifugation at 4°, plasma from all animals in each group was pooled and nephrotensin was concentrated by fractional precipitation with ammonium sulfate as described by Grollman *et al.* (3). The plasma fraction precipitating between 40 and 70% ammonium sulfate saturation was dissolved in a small amount of water and dialyzed against 0.1 *M* sodium acetate buffer (pH

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3.8) at 4° until free of ammonia. The dialyzate was then lyophilized. Approximately 16 mg of material, presumably protein, was obtained from 1 ml of plasma. The angiotensin formed during the concentration procedure was removed by ion exchange chromatography using Dowex 50W-X2 resin (3).

All samples of concentrated plasma were tested for pressor activity in anesthetized, ganglion-blocked rats (Bioassay I). When pressor activity was found, the substance was further tested in rats immunized with AII (Bioassay II) and in animals treated with AI converting enzyme inhibitor (SQ-20,881)[2] (Bioassay III).

Test animals for Bioassay II were immunized against AII by 4 ip injections (1 mg per rat) at 3-wk intervals of (asp^1-val^5) angiotensin II (Hypertensin, Ciba, Summit, NJ) coupled to rabbit serum albumin by the carbodiimide conjugation procedure (10) and homogenized in Freund's complete adjuvant. The degree of the immunization was evaluated by iv injections of AII (25 ng), AI (25 ng), and hog renin (0.05 units) into both the immunized and control rats. Animals for Bioassay III were prepared by iv injection of SQ-20,881 (0.4 mg) followed immediately by injections of the substances to be tested. For all bioassays, animals were anesthetized with pentobarbital (40 mg/kg) and treated with pentolinium tartrate (20 mg/kg) and atropine sulfate (1.5 mg/kg). The right jugular vein and common carotid artery were cannulated and a tracheotomy was performed. The arterial cannula was connected to a pressure transducer, and the blood pressure was continuously recorded on a direct writing recorder (Narco Bio-Systems, Inc.). Samples to be tested were injected in a volume of 0.2 ml into the jugular vein. Blood pressure responses to unknown samples were compared to responses to standard doses of AII (5 ng) and Norepinephrine (50 ng).

Results. As shown in Table I, nephrotensin was found in the plasma of rats with renal-clip hypertension and of animals with chemically induced kidney damage. Its pressor activity was estimated to be equivalent to 6 ng AII per ml of the original plasma volume in renal hypertensive rats and about 3 ng AII/ml in rats treated with $HgCl_2$. No correlation between PRA and nephrotensin was observed. Thus nephrotensin was not found in the plasma of rats treated with diuretics in which the PRA was significantly ($P < 0.01$) elevated, but it was found in the plasma of rats treated with $HgCl_2$ in which very low values for PRA were detected.

TABLE I. SYSTOLIC BLOOD PRESSURE (BP), PLASMA RENIN ACTIVITY (PRA) AND NEPHROTENSIN IN RATS UNDER VARIOUS EXPERIMENTAL CONDITIONS.

Groups	BP (mmHg)	PRA (ng AI/ml/hr)	Nephrotensin
I Controls	119 ± 2	2.2 ± 0.4	—
II Treated with diuretic	117 ± 2	7.7 ± 0.8[a]	—
III Renal-hypertensive	171 ± 4[a]	16.6 ± 1.3[a]	+
IV Treated with $HgCl_2$	144 ± 6[a]	1.3 ± 0.6	+
V Treated with CCl_4	112 ± 5	0.3 ± 0.1[a]	—

Values are means ± one SE.

[a] Significantly different when compared to controls ($P < 0.01$).

Figure 1 illustrates the ability of the pressor bioassays to differentiate nephrotensin from angiotensins I and II. Although the nephrotensin used in this illustration was concentrated from the plasma of renal hypertensive rats, the plasma concentrates of rats treated with mercuric chloride exhibit similar pressor activity. In anesthetized, ganglion-blocked rats 12 mg of protein concentrated from the plasma of renal hypertensive animals evoked a blood pressure response similar to that of 5 ng AII. The shape of the nephrotensin response curve resembled that of the AII although the effect lasted longer. On repeated injections of nephrotensin no tachyphylaxis was observed. The pressor action of nephrotensin was not altered in rats immunized with AII although the effect of AI and AII was abolished. Pretreatment of the test animals with SQ-20,881 did not influence the pressor action of nephrotensin but blocked that of AI.

[2] SQ-20,881 was generously supplied by Squibb Institute for Medical Research.

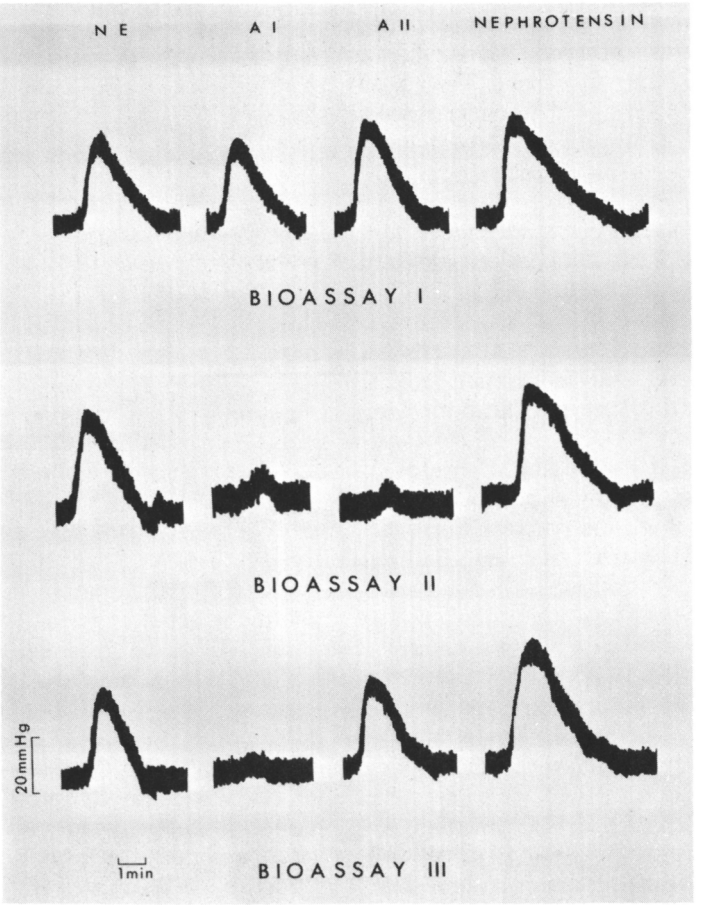

FIG. 1. Effect of norepinephrine (NE), angiotensin I and II (AI and AII), and nephrotensin on the blood pressure of anesthetized ganglion-blocked rats (Bioassay I), rats immunized with AII (Bioassay II), and rats treated with SQ-20,881 (Bioassay III). Doses: 50 ng of NE; 5 ng of AI; 5 ng of AII; and 5 ng AII equivalents of nephrotensin. In Bioassay II, 25 ng of both AI and AII were given.

Discussion. There is general agreement that nephrotensin differs from renin, AII, catecholamines and various vasoactive agents of small molecular weight in its vasoconstrictor action as well as in its physicochemical properties (3–5). However, the relationship between nephrotensin and AI is still a matter of controversy. It was reported (5) that nephrotensin and AI are similar immunologically and that AI bound to plasma proteins exhibits physicochemical properties similar to those of nephrotensin. Neither substance is dialyzable nor are they adsorbed by Dowex 50W-X2 resin or Fuller's earth, which differentiate them from various vasoactive agents of low molecular weight. On the contrary, Grollman *et al.* (3, 4) have reported that although nephrotensin might be immunologically related to AI, it is neither inactivated by AI antibody nor does it exhibit the same action as AI on various smooth muscle preparations.

The results of the present study further support the findings of Grollman and coworkers (3, 4). The failure to demonstrate nephrotensin in the plasma of rats with elevated PRA due to treatment with diuretics, and the demonstration of pressor activity in the plasma of rats with $HgCl_2$ induced kidney damage and low values for PRA make unlikely the hypothesis of Schweikert *et al.* (5) that nephrotensin is preformed AI bound to plasma proteins. Furthermore, immunization of the test

animals with AII did not influence the pressor action of nephrotensin, although the blood pressure response to renin, AI and AII were abolished. The failure of SQ-20,881 to block the pressor activity of nephrotensin further differentiates nephrotensin from AI.

One possible explanation for the presence of nephrotensin in the plasma of animals with renal-clip hypertension has been offered in previous reports (11–13) which indicate that vasoactive peptides different from AI and AII may be formed by the action of tissue peptidases. Also, Regoli (14) and Hofbauer (13) have reported that the vasoconstriction induced in isolated, perfused rabbit or rat kidney by the addition of renin substrate to the perfusate could not be blocked by the simultaneous administration of competitive antagonists of AII nor by AI converting enzyme inhibitor. Since the kidney is rich in tissue peptidases, a possible explanation for their results is that these enzymes act on either renin substrate or intrarenally formed AI to release vasoconstrictor peptides other than AII (13). This peptidase activity is a likely explanation for the presence of nephrotensin in the plasma of animals with renal-clip hypertension and of rats with chemically induced kidney damage. In this context, the failure to demonstrate pressor activity in the plasma of nephrectomized rats in which liver damage had been induced by CCl_4 might indicate that nephrotensin forming enzymes are peculiar to kidney tissue. It is also of interest to note that Powell, *et al.* (15) have reported that a vasopressor material of renal origin, different from the RAS, is involved in the control of vascular capacity during hemorrhage.

Summary. An investigation of the relationship between nephrotensin and the renin angiotensin system was carried out. Nephrotensin was found in the plasma of rats with renal clip hypertension and with chemically induced kidney damage. There was no demonstrable correlation between presence of nephrotensin and plasma renin activity, and the pressor activity of nephrotensin was not altered by previous immunization of test animals with angiotensin II nor by pretreatment with angiotensin I converting enzyme inhibitor. These results indicate that nephrotensin is different from the components of the renin–angiotensin system.

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