STUDIES ON THE MECHANISM OF RENOVASCULAR HYPERTENSION

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RAUL CARDONA, M. D.

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ABSTRACT

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By

Raul Cardona, M.D.

The etiology of renovascular hypertension (RVHPT) has been studied for many years. After the renin-angiotensin system was discovered, several studies showed that this system may be the main cause of this type of hypertension. However, in the last decade, several investigators have reported some evidences which support the hypothesis that another pressor substance or system may play role in the etiology of RVHPT.

The objectives of this thesis work were: (1) To test the hypothesis that during the chronic stage of RVHPT unknown humoral factor(s), different from, but not necessarily independent of the renin-angiotensin-aldosterone system, is (are) involved; and (2) To determine certain physicochemical and/or biological characteristics of that factor.

Three groups of experiments were attempted. First, studies were performed to find out if chronic (four or more

weeks after hypertension) one-kidney Goldblatt type hypertension in rats (1-KHR) is related to plasma renin activity (PRA) levels. There was no significant correlation between blood pressure (BP) and PRA (correlation coefficient: $r = 0.002$, $n = 27$, $p > 0.05$). It was also found that in one-kidney perinephritis hypertension in dogs (1-KHD) there was a significant suppression of both PRA and aldosterone concentration ($p < 0.05$). After three weeks of hypertension, aldosterone concentration became normal but PRA was still low and it remained so for three months after the development of hypertension.

Secondly, it was attempted to determine the effect of the plasma obtained from normotensive and hypertensive rats and dogs, on the pressor activity of angiotensin II (AII) and norepinephrine (NE). Blood pressure (BP) response of bilaterally nephrectomized-pentolinium treated rats (Bioassay I) was used as model to study the effect of the plasma on AII vasopressor activity. There was an increased response to AII after injection of plasma from hypertensive, but not after the injection of plasma from normotensive rats. The maximum effect was usually seen between 20 to 40 minutes after plasma administration. Additionally, isolated mesentery arteriole (Bioassay II) was used to study the effect of the plasma, obtained from hypertensive dogs, on the vasopressor activity of NE. The dose-response curve of

NE was significantly increased ($p < 0.05$) during perfusion with the plasma from hypertensive dogs.

Thirdly, two types of fractionation were done to determine whether any specific fraction may increase the vasopressor activity of AII in Bioassay I. Plasma was separated into four main fractions by using Amicon Diaflo Ultramembranes: (1) UM-10 retainer, substances with molecular weight more than 10,000. This fraction was not tested because of the very high concentration of large molecules; (2) UM-2 retainer, substances with molecular weight between 1,000 and 10,000; (3) UM-05 retainer, substances with molecular weight between 500 and 1,000; and (4) UM-05 filtrate, substances with molecular weight less than 500. In initial experiments, fraction 4 failed to affect the pressor activity of AII in Bioassay I and, for this reason, this fraction was not used in any further study. Fractions 2 and 3 enhanced the action of AII in Bioassay I; the effect of fraction 3 was significantly higher than that observed with fraction 2 ($p < 0.05$).

UM-05 retainer was further fractionated on Bio-Gel P-2 column (1.5 x 90 cm) and 6 ml eluate was collected in each tube. Fraction No. 20 (115 to 120 ml of eluted volume) from 1-KHD produced a significant increase of AII vasopressor activity, but no effect was observed with fraction No. 20 from normotensive dogs. These tractions contain substances with molecular weight close to 1,000.

It is concluded that a humoral factor of small molecular weight may exist during one-kidney renovascular hypertension and it may play an important role in maintaining the high blood pressure by increasing the pressor activity of angiotensin II and norepinephrine.

STUDIES ON THE MECHANISM
OF
RENOVASCULAR HYPERTENSION

by

Raul Cardona, M.D.

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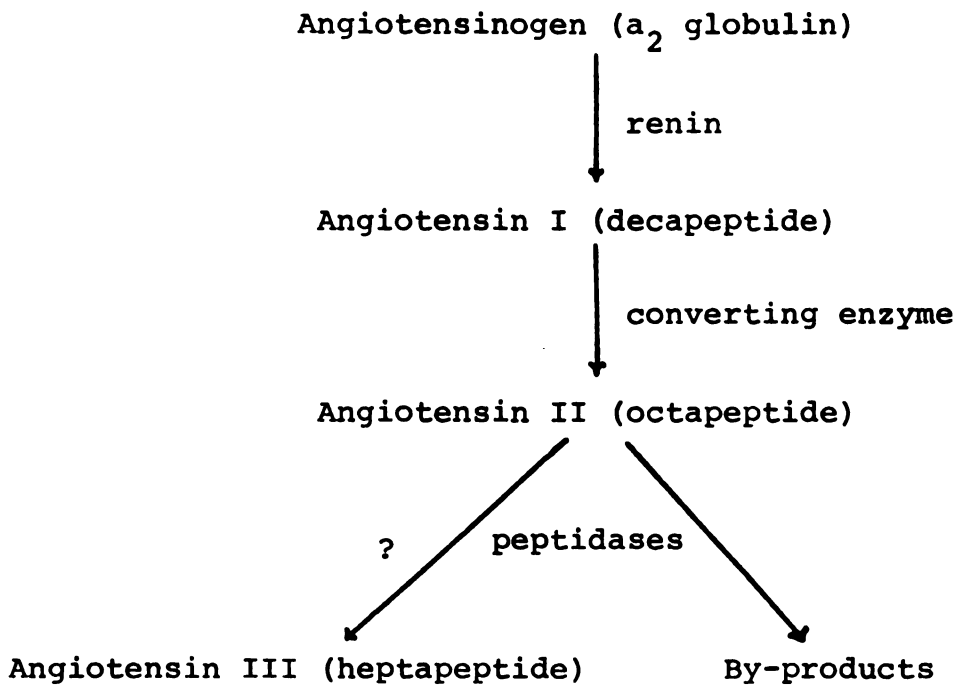
INTRODUCTION

1. General background

After Goldblatt's studies (1934, 1937) on experimental reno-vascular hypertension, and the description of angiotensin by Page et al., (1940) and Braun-Menendez et al., (1940), the study on the pathogenesis of RVHPT has been centered on the renin-angiotensin-aldosterone system (R-A system). The following is a simplified scheme on the main points of this system:



Angiotensin II (AII), on one hand produces a direct smooth muscle contraction, probably by acting on Ca^{++} firmly bound to the membrane and increasing the free Ca^{++} inside the cell (Somlyo and Somlyo, 1970). On the other hand, AII increases the release of aldosterone (Gross, 1968), and catecholamines (Douglas et al., 1967) from adrenal glands. In addition, it has been reported that AII may act on the central sympathetic system (Severs and Daniels-Severs, 1973) and also on sympathetic nerve terminals (Distler et al., 1965). Peripherally, this peptide may increase the catecholamine levels either by releasing norepinephrine from nerve endings (Gascon and Walaszek, 1968), by blocking the reuptake of this neurotransmitter (Khairallah et al., 1971) or by accelerating the synthesis of norepinephrine from precursors (Davila et al., 1971). It appears that the R-A system is essential in the circulatory homeostasis in normotensive animals.

Although the R-A system seems to play an undoubtedly very important role in RVHPT, some experimental data have raised the question of whether or not it is the only contributor in this type of hypertension. Shortly after the Goldbatt studies, it was reported that in dogs with RVHPT in the chronic state, the blood pressure (BP) may remain high even though the plasma renin levels are usually normal (Haynes and Dexter, 1947). This finding has been confirmed by others (Blair-West et al., 1968; Harris and

Ayers, 1972). The same non-relationship between experimental chronic RVHPT and angiotensin levels has been reported by several workers (Brunner et al., 1972; Scormik and Paladin, 1961). Furthermore some investigators have not found a consistent relationship between PRA and BP in clinical RVHPT (Bath et al., 1968). Also, aldosterone secretion is usually normal in patients with unilateral renal hypertension (Laragh et al., 1966).

It is generally accepted that RVHPT can be divided into acute and chronic phases. These two phases may exhibit differences in their relationship with the R-A system depending upon the experimental design. In one-kidney renal hypertension (1-KH), which can be done by clamping one renal artery and contralateral nephrectomy (Goldblatt et al., 1934) or by wrapping one kidney and nephrectomy (Page, 1939), PRA have been found to be either normal (Helmchen et al., 1972b), low (Mogil et al., 1969), or high (Ayers et al., 1969) in the acute phase. However, after two or three weeks (chronic phase) PRA levels are often normal (Brown et al., 1966; Koletsky and Rivera-Velez, 1967; Fujii and Ikeda, 1971; Liards and Peters, 1973). Furthermore, AII inhibitors (Bumpus et al., 1973; Skeggs et al., 1975), converting enzyme inhibitors (Engel et al., 1973), and immunization against AII (Eide and Aars, 1969; Johnston and Mendelson, 1970; Louis et al., 1970), in many instances could not influence the high BP in chronic 1-KH. Also, active

immunization with "renin" (renal extracts) have decreased the BP in some chronic 1-KH (Wakerlin et al., 1958) but not in others (Hill et al., 1970). The fact that impure renin extracts have been used in those studies makes it difficult to interpret these findings. Skeggs et al., (1976) have been able to obtain a kidney cortex preparation with little or no renin; active immunization with that extract lowered the BP in rabbits with chronic 1-KH, although there was no formation of plasma anti-renin. These authors produced high plasma anti-renin titers in another group of 1-K hypertensive rabbits without affecting the BP levels. In two-kidney renal hypertension (2-KH), which can be done by clamping one renal artery or wrapping one kidney and leaving the contralateral organ intact (Skulan et al., 1974; Thurston and Swales, 1974; Liard and Peters, 1973), or by clamping both renal arteries (Wakerlin et al., 1958), PRA is usually increased in both acute and chronic phase (Skulan et al., 1974). Moreover, AII inhibitors (Bumpus et al., 1973) or anti-angiotensin antibodies (Brunner et al., 1971) can decrease the BP in chronic 2-K RVHPT. Several reviews have supported a significant relationship between PRA and BP in 2-K hypertension (Liard and Peters, 1973; Oparil and Heber, 1974; Laragh et al., 1975).

2. Main factors related to increased vascular resistance in Renovascular Hypertension

A. Fixed Factors

1) Increased in the wall to lumen ratio (Folkow et al., 1958; Folkow and Neil, 1971; Sivertson and Olander, 1968). The initial increase in BP, which may be due to a high PRA or an early increase in cardiac output (Ferrario, 1974), causes generalized hypertrophy of the vascular wall of the arterioles and therefore, produces an increase in the wall to lumen ratio. As it has been proposed (Folkow et al., 1958), the higher the wall to lumen ratio, the higher the reduction in luminal diameter (or the higher the vascular resistance) for any degree of smooth muscle shortening.

Even though this mechanism may play a significant role, it fails to explain, for example, the fall in blood pressure after removing the renal artery constriction in animals with low renin chronic RVHPT as it has been reported in sheep (Blair-West et al., 1970), dogs (Tagawa et al., 1974) and rats (Neubig and Hoobler, 1975).

2) Water and electrolytes retention: Some investigators have found an increased amount of water and electrolytes (Na^+) in the wall of both large (aorta) and small (mesenteric arterioles) vessels from hypertensive animals and/or humans (Tobian and Binion, 1952, 1954; Tobian et al., 1961; Tabian et al., 1969). On one hand, the

electrolyte changes could alter the vascular response by acting at some levels of the excitation-contraction coupling of vascular smooth muscle (Jones, 1974), and on the other, the water effect would be indirect, mainly by altering the wall to lumen ratio (Tobian et al., 1969).

3) Enhanced vascular response due to an increase in adrenergic nerve innervation: It has been reported that during hypertension, the adrenergic innervation of blood vessels as well as synthesis of NE could be enhanced (Bevan et al., 1974; Bevan et al., 1975; De Quattro and Alexander, 1974; Samir Amev et al., 1975). However, it is not clear what the actual reson for these changes would be.

B. Humoral Factors

During the last 10 years, some investigators have raised the possibility that an unknown renal substance may be responsible for the maintenance of chronic RVHPT (Genest et al., 1964; Skeggs et al., 1975, 1976). Recently, it has been suggested that one pressor substance different from renin and angiotensin is present in the acute phase of RVHPT (Grollman, 1970; Grollman and Krishnamurty, 1971; Susic and Sparks, 1975); it was called nephrotensin. However, studies brought about by others have related nephrotensin to angiotensin I (Schweikert et al., 1972).

Mizukoshi and Michelakis (1972) have found that peripheral venous plasma from chronic hypertensive patients

(PVP) has a moderate pressor effect and also potentiates the activity of AII and NE on the blood pressure of small (body weight 180 to 200 gm), ganglion-blocked and bilaterally nephrectomized rats. Greenberg et al. (1974) reported that in big rats (body weight 525 to 585), neither pretreated with pentolinium nor nephrectomized, PVP enhanced the pressor response to tyramine, but did not affect the pressor response to AII and NE. The fact that the Greenberg's study was done with old rats may be important in the difference observed with Mizukoshi and Michelakis' report as it has been shown that receptors to catecholamines decrease with increasing age (Fleish et al., 1970; Fleish, 1971). Also, in Greenberg's study animals were neither pretreated with pentolinium nor nephrectomized; therefore, rats could have been able to compensate BP changes after plasma administration and to excrete rapidly the injected substance by the urine.

Recently, it has been reported that plasma fractions from one-kidney hypertensive dogs can potentiate the pressor activity of AII and NE (Michelakis et al., 1975).

SPECIFIC OBJECTIVES

The objectives underlying this thesis work were: (1) To determine if the chronic one-kidney hypertension is related to plasma renin activity and aldosterone concentration; (2) To determine whether an unknown circulating factor is presented in chronic one-kidney hypertension; and (3) To attempt to determine approximated molecular weight, stability and pharmacodynamics of that factor.

MATERIALS AND METHODS

1. Clamping the renal artery in rats

Sprague Dawley male rats weighing between 180 to 200 gm were used. Under sodium pentobarbital anesthesia (35 mg/kg., i.p.), a dorsal incision of about 2 cm was made to expose the left kidney and the ipsilateral renal artery was partially isolated. For clamping the artery, the Schaffenburg's method (1959) was used. Clips (bent strips of fine silver, 0.005 inches thick, 6 x 2 mm) were applied with a calibrated forceps (0.2 mm i.d.). Another incision was made on the other side and the right kidney was removed. Incisions were closed with separate suturing of muscle and skin.

In the sham operated rats, one kidney was removed but the renal artery of the other kidney was left without clamping. All animals in both groups had free access to commercial rat food (Wane-Lab-Blox) and tap water.

2. Wrapping the kidney in dogs

Male, young and healthy dogs weighing about 18 kg were anesthetized with sodium pentobarbital (25 mg/kg., i.v.) and under sterile conditions a flank incision of about 12 cm was

made and the left kidney was wrapped in a silk bag. After one week, another incision was performed on the other side and the right kidney was removed.

After surgery, dogs received penicillin (100,000 units) and streptomycin (0.1 gm) i.m. daily for 5 days. They had a free access to commercial dog food and tap water.

3. Blood pressure measurement and blood collection

a. Rats: Systolic BP was measured in conscious rats. They were warmed at 45°C in a box for a 5 minute period. Afterward, rats were removed from the box and placed in a restraining cage (Narco-Bio Systems, Inc.), where a cuff was put on the proximal part of the tail and the BP was measured under heating (40°C) and after allowing 4 to 5 minutes for stabilization. Pneumatic pulse transducer, Electrosphygmograph ESG 3000 and Physiograph Desk Model DMP 4A (Narco-Bio Systems, Inc.) were used in BP measurement.

Blood collection in rats was done under sodium pentobarbital anesthesia (35 mg/kg., i.p.). About 10 ml of arterial blood samples were withdrawn from the carotid artery in anesthetized rats, and the blood sample was placed in a cold plastic centrifuge tube which contained 5 mg of disodium-EDTA (disodium ethylenediamine tetracetate), and was centrifuged for 20 minutes at 3,000 r.p.m. Plasma was kept at -20°C until assay.

b. Dogs: Male, young and healthy dogs weighing about 18 kg were trained for at least four weeks in order to take BP directly from a femoral artery in conscious and calmed dogs, using a transducer P23DC Statham and Grass Model 7D Polygraph. BP was measured once every week before and after surgery. After blood pressure determination, about 20 ml of venous plasma was withdrawn from the jugular vein, blood was placed in a cold siliconized glass tube which contained 10 mg of disodium-EDTA, and centrifuged for 20 minutes at 3,000 r.p.m. Plasma was kept at -20°C until assay.

4. Plasma fractionation

a. Ultrafiltration: Under an applied pressure (30 lb/sq. in.) of N_2 atmosphere, 50 ml of plasma was filtrated through Amicon Diaflo Ultramembrane 10 (UM-10). The filtrated solution was passed through UM-2, and the filtrated solution from UM-2 was further filtrated on UM-05. The retainers from UM-2 and UM-05 were dissolved in 5 ml of 0.9% NaCl and kept under N_2 atmosphere at -20°C until assay (not more than 3 days).

At the end, four fractions were obtained:

UM-10 retainer	Molecular weight > 10,000
UM-2 retainer	Molecular weight > 1,000, <10,000
UM-05 retainer	Molecular weight > 500, <1,000
UM-05 filtrate	Molecular weight <500.

b. Gel filtration: Solution obtained from UM-05 retainer was further fractionated on a gel filtration column to sieve substances of small molecular weight (Bio-Gel P-2, 1.5 x 90 cm). It was eluted at 4°C with destilled water, and 6 ml fractions were collected. Each fraction was freeze-dried in a Thermocouple Vacuum Gauge (Vitris) and kept at 0°C until assay (not more than 2 days).

5. Radioimmunoassay

a. Plasma Renin Activity: One ml of each plasma sample was transferred to a polystyrene tube in a ice bath and to each 10 µl of 8-OH Quinoline, 10 µl of BAL (dimercaprol), and 2 ml of pH 6.0 maleate buffer was added. Each sample was mixed thoroughly. One set of the 0.5 ml plasma aliquot was put into Dubnoff Metabolic Shaking incubator at 37°C and shaken at low speed for one hour. Another set of 0.5 ml plasma was kept in the ice for the same period. At the end of incubation period both sets of tubes were matched in the ice bath.

A serie of 18 tubes to be used for the standard curve and 4 tubes for each plasma were enumerated.

Tubes 1 and 2 (total count tubes) received 1600 µl of Tris buffer. Tubes 3 and 4 (blank tubes), 500 µl of Tris buffer and 100 µl of 5% bovine albumin (BSA). Tubes 5 and 6 ("0" standard tube), 100 µl of 5% BSA. The rest of the tubes

received 700 µl of Tris buffer. One hundred µl of each standard of angiotensin I (AI) was pipetted into the tubes:

0.10 ng/ml standard into tubes 7 and 8

0.25 ng/ml standard into tubes 9 and 10

0.50 ng/ml standard into tubes 11 and 12

1.00 ng/ml standard into tubes 13 and 14

2.50 ng/ml standard into tubes 15 and 16

5.00 ng/ml standard into tubes 17 and 18.

In tubes with unknown samples, 100 µl of plasma (4°C sample) was pipetted into tubes 19 and 20 and 100 µl of plasma (37°C sample) into tubes 21 and 22. One hundred µl of ^{125}I-labelled angiotensin I was added to all tubes, and 100 µl of antiserum to all tubes except tubes 1 through 4. All of them were mixed very well, and then incubated at 4°C for 24 hours. After this time, 1 ml of charcoal suspension in barbital buffer (pH 7.4) was added to each tube. They were centrifuged at 3,000 r.p.m. for 15 minutes. Supernatant (bound AI) was separated from the precipitate (free AI). The bound/free ratio for the standard and the unknown samples was calculated after counting the radioactivity of both supernatant and precipitate in a Gamma Counter (Searle, Model 1185 Dual Channel).

Plasma renin activity was calculated in terms of nanograms of AI formation per milliliter per hour, by substracting the preformed AI (plasma at 4°C) from the total AI present at the end of incubation period (37°C).

b. Aldosterone: Extraction and separation procedure was done according to the method of Ito et al., (1972). Aliquot of the extract in methanol (0.4 ml) was taken and transferred to a counting vial. Sample was left in the hood and allowed to dry out overnight. In duplicate assay, 0.2 ml of sample was pipetted to a polystyrene tube next day.

Standards of ^{3}H-aldosterone were added in 0.1 ml of methanol as follows:

0 pg standard into tubes 1 and 2

5 pg standard into tubes 3 and 4

10 pg standard into tubes 5 and 6

25 pg standard into tubes 7 and 8

50 pg standard into tubes 9 and 10

100 pg standard into tubes 11 and 12

10,000 pg standard into tubes 13 and 14

Each assay tube received 0.3 ml of the following solution:

a. Purified ^{3}H-aldosterone to give 1600 count per minute per tube.

b. One hundred µl of phosphate buffered saline-0.1% gelatine (PBSG-0.1%).

c. Two hundred µl of antibody with a titer of 1:1,000,000 in PBSG-0.1%.

The total count tubes (tubes 1 and 2), also received 0.4 ml of PBSG-0.1%. All the samples were mixed very well, and then incubated at 4°C for 24 hours. After this time,

0.1 ml of PBSG-0.5% and 0.5 ml of dextran coated charcoal were added to each tube. They were centrifuged at 2,500 r.p.m. for 10 minutes. Ten ml of aquasol and 10 ml of toluene cocktail were added to each vial. After counting in a Beta Counter (Seale, Model 6847 Liquid scintillation system), aldosterone concentration was calculated in nanograms (ng) per 100 ml of plasma.

6. Bioassays

Bioassays were used to test the hypothesis that an unknown humoral factor may be involved in the maintenance of chronic RVHPT. Since individual responses are expected to vary from one animal to another, for example genetical and environmental conditions alone can provide important variation (Harpley et al., 1973), one must use highly standardized criterion in these assays. We used a direct type of bioassay in which we studied the effect of a single injection of a small amount of plasma or plasma fractions on the vasopressor activity of AII, and also the effect of a slow continuous infusion of plasma on the activity of NE in a small resistance vessel was studied.

a. Blood pressure in ganglion-blocked, bilaterally nephrectomized rats: (Bioassay I): This bioassay has been used to study the pressor activity of renin and angiotensin (Grollman, 1964; Gunnells et al., 1967), and to test the

existance of unknown pressor substances in acute (Grollman, 1970) or chronic (Mizukoshi and Michelakis, 1972; Michelakis et al., 1975) RVHPT.

Rats weighing between 140 to 180 gm were bilaterally nephrectomized 18 to 20 hours prior to the experiment. Each kidney was removed through a flank incision. Care was taken to strip the capsule and fatty tissue, avoiding damage to adrenal gland. Under sodium pentobarbital (35 mg/kg., i.p.) and urethane anesthesia (1.1 gm/kg., i.p.), the carotid artery was isolated and carefully separated from the vagus-sympathetic trunk. With a PE-50 tube (Intramedic Polyethylene tubing) the artery was connected to a pressure transducer P-1,000-A and Physiograph Desk Model DMP-4A (Narco Bio System, Inc.). Two PE-10 tubes (Intramedic Polyethylene tubing) were placed in the left jugular vein, one for injections of plasma or plasma fraction samples and the other for AII administration. The dosage of AII was either 0.4 or 0.8 ng per rat.

Once the preparation was set up, a sixty minute period was allowed before the start of the experiments. A dose-response curve for AII (bolus injection of 0.2, 0.4, 0.6, 0.8 and 1.0 ng per rat) was determined after equilibration and, afterwards, a constant dose of AII (0.4 or 0.8 ng per rat) was given every 5 to 10 minutes for at least 30 minutes. Then, injection of 20 or 50 µl of plasma or 50 µl of plasma fraction were given i.v. to study their

effect on the BP response to the constant dose of AII (0.4 or 0.8 ng per rat) for 30 to 60 minutes. A cross-over random design was used to administer plasma or plasma fraction from normotensive and hypertensive rats and dogs to a single bioassay preparation. Recovery was allowed for any previous change in the response to AII.

Special care was taken of some criteria of acceptability in Bioassy I. No animal was allowed to continue in the experiment if:

1. It was not a stable preparation. Comparable BP response to a constant amount of AII for 30 minutes qualified a preparation as "stable".
2. It was not a sensitive preparation; i.e., first, AII must show a clear dose-response relationship with the BP in each animal, and second, 0.4 ng of AII must produce a rise of at least 8 mm/Hg in BP.
3. Bleeding was present.
4. Blood pressure baseline was not stable; and
5. The animal needed a new anesthetic dose, unless a new stabilization period was completed. Experimental responses were matched only with their own controls.

Also, it was attempted to determine whether plasma from normotensive and/or hypertensive rats changes the response of the bioassay to a constant amount of AII in a

dose-response relationship. The amounts (dose) of the plasma given were: 5, 10, 15, 17, 20 and 50 µl. The response was the difference between the rise in BP (mm/Hg) caused by AII before and after the injection of plasma.

b. Isolated mesenteric arteriole (Bioassay II): The direct effect of plasma from both normotensive and one-kidney hypertensive dogs on small resistance vessels was also studied. Uchida et al., (1967), developed a technique which makes possible to perfuse isolated resistance vessels (50 to 250 microns o.d.). The vessel used, mesentery arteriole, is sensitive to a wide range of doses of NE.

Male Sprague-Dawley rats weighing 300 to 350 gm were killed by a blow in the head and quickly exsanguinated. An abdominal incision was made and, after clamping the thoracic aorta with a forcep, Evan's blue was injected below the occlusion in order to visualize clearly the mesenteric vessels. Afterwards, the mesenteric arteriole was isolated from surrounding tissues. One distal artery was chosen, and its lateral branches were tied off. The artery-arteriole segment was cannulated at its proximal end with a glass cannula (Uchida et al., 1967). The vessel connected to the glass cannula was placed in a horizontal glass chamber which was surrounded by a water jacket. We maintained the temperature at 37°C using a thermostat unit. Vessels were perfused at a constant flow rate by a polystaltic pump

(Buchler Instruments). The perfusion pressure was recorded by a pressure transducer (P-1,000-A Narco Bio System Inc.) through a side arm of the cannula, proximal to the vessel. In our design, a flow rate of 2 ml/min produced a perfusion pressure of about 30 mm/Hg. This pressure was chosen because it has been reported that the response of mesenteric arteriole to vasoactive substances is almost independent of perfusion pressure when this pressure is between 25 to 60 mm/Hg (Uchida et al., 1967). Therefore, one may leave out a possible source of variability.

Perfusate, which was aerated with 95% O_2 and 5% CO_2, had the following composition in millimoles per liter:

NaCl	119.0
KCl	4.7
$CaCl_2$	1.6
$MgSO_4(7\ H_2O)$	2.9
KH_2PO_4	1.1
$NaHCO_3$	14.9
Dextrose	5.5
Sucrose	50.0

In addition, cocaine was added to the perfusate in order to block the effect of AII, which may be present in the plasma, on the reuptake of NE by the sympathetic nerve ending. A concentration of 0.05 mM/L of cocaine was found to be sufficient for this purpose: i.e., 1.0 ng of AII injected into the perfusate caused about 34% increase in the

vascular response to 100 ng of NE; however, when other preparations were perfused with Krebs buffer solution containing 0.05 mM/L of cocaine, 1.0 ng of AII did not further potentiate the response to NE.

Sixty minute equilibration period was allowed before the start of the experiments. After equilibration, a dose-response curve for NE was determined. Dose-response curve during perfusion with the Krebs buffer solution was studied at least twice to ascertain the stability of the preparation. After two near identical dose-response curves for NE were obtained, the vessel was then perfused with buffer solution containing 0.5 ml of plasma from normotensive or hypertensive dogs in 100 ml perfusate. A cross-over random design was used to perfuse plasma from normotensive or hypertensive dogs to a single bioassay preparation. Recovery of any previous change in the response to NE was allowed.

Three basic criterions were used for the acceptability of Bioassay II:

1. It must be stable. Comparable does-response curves of NE during buffer perfusion qualified a preparation as stable.
2. It must be a sensitive tissue.
    a) Threshold dose of NE must be less than 50 ng per preparation.
    b) 100 ng of NE must produce a minimum increase of 30 mm/Hg in the perfusion pressure.

3. No preparation was allowed to continue if any leaking was seen or suspected.

7. Definitions

Systemic arterial hypertension or hypertension has been defined in many ways without reaching good agreements between authorities (Pickering, 1968).

We defined:

a. Normal blood pressure: A mean blood pressure (BP) value below 130 mm/Hg in conscious animals.

b. Hypertension or high blood pressure: A BP value at the level of 150 mm/Hg or above in conscious animals.

c. Hypertensive stage: A BP value at 150 mm/Hg or above for at least two consecutive weeks.

d. Acute hypertension: A BP value at 150 mm/Hg or above during the first four weeks of hypertensive stage.

e. Chronic hypertension: A BP value at 150 mm/Hg or above after the fourth week of hypertensive stage.

In addition, the surgical procedure to make hypertensive animals was defined as:

a. Success: BP became high (150 mm/Hg or above) in the first two weeks after surgery.

b. Fail: BP was below 150 mm/Hg two weeks after surgery. Fourteen (23.3%) rats were left out

because of "fails". There was no "fail" in dogs.

8. Statistical analysis

Data were evaluated by student's t-test. Level for significance was a probability less than 5% ($p < 0.05$).

To study the relationship between BP and PRA, a correlation coefficient value was determined by the least squares method.

RESULTS

1. Relationship among plasma renin activity and aldosterone concentration and blood pressure in one-kidney Reno-vascular Hypertension

a. Dogs: Male, young and healthy dogs weighing about 18 kg were anesthetized with sodium pentobarbital (25 mg/Kg, i.v.) and under sterile conditions the left kidney was wrapped in a silk bag. After one week, another incision was performed on the other side and the right kidney was removed. One week after the surgery, BP increased up to significantly higher level (166 ± 9.62 mm/Hg, $p < 0.05$) compared to the pre-operative BP values (105 ± 7.07 mm/Hg) the high BP was maintained for the entire 11 weeks of observation (Fig. 1). In the same animals, PRA was significantly suppressed ($p < 0.05$) after a week and remained at that low level during the entire observation period (Fig. 1). Aldosterone concentration was determined in three dogs during the first five weeks of 1-KHD. Two consecutive values significantly lower than the control were found in the first two weeks, but aldosterone became variable and within control limits after the third week (Fig. 1).

Figure 1 shows that wrapping one kidney and contralateral nephrectomy had significant effect on BP, PRA, and aldosterone concentration. However, the fact that changes in BP, on one hand, and PRA and aldosterone concentration, on the other, followed oppositive directions make it likely that the increase in BP was not directly related to the renin-angiotensin-aldosterone system.

Suppression of aldosterone concentration was transient, and there was a dissociation between PRA and aldosterone concentration after the third week of hypertension, which suggests that in addition to renin-angiotensin, there are other factors affecting the aldosterone secretion.

In order to study the relationship between BP and PRA in chronic one-kidney hypertension, correlation analysis was performed (Fig. 2). There was a significant negative correlation between BP and PRA ($r = 0.785$, $n = 12$, $p < 0.05$). This result gives no supportive role for renin-angiotensin in the chronic maintenance of the 1-KHD.

In additional study, we used the body weight determination as an indirect method to evaluate whether there was water retention in those dogs in which PRA and aldosterone concentration were measured. Dogs weighed 18.6 $\pm$1.3 Kg (SEM) on the day of wrapping the kidney. Then, they were weighed once every week for eleven weeks, showing a variation within 18.8$\pm$1.3 to 20$\pm$1.2 Kg (SEM). Dogs weighed 19.3$\pm$1.5 Kg (SEM) at the end of the observation period (11th

week). None of these values was significantly different compared to pre-operative dogs weight ($p > 0.05$).

b. Rats: Sixty Sprague Dawley rats weighing between 180 to 200 gm were used. Under sodium pentobarbital anesthesia (35 mg/Kg., i.p.), the left renal artery was clamped and, in the same operation time, the right kidney was removed. BP was significantly higher ($p < 0.05$) than the pre-operative values one week after the surgery (Fig. 3A. Close circles). After the second week of hypertension, we randomly chose a group of hypertensive rats every week; the BP values of those groups (Fig. 3A. Close circles) were significantly higher ($p < 0.05$) than those of sham operated rats (Fig. 3A. Open circles) during the entire period of observation. The group of randomly chosen hypertensive rats, and also a group of sham operated rats, were sacrified every week, 24 hours after the BP determination, to obtain the plasma. PRA showed no significant changes during the nine week period either in hypertensive (Fig. 3B. Close circles) or normotensive (Fig. 3B. Open circles) rats.

Additionally, a group of four hypertensive rats was followed over a nine week period to figure out the usual development of BP in one-kidney hypertensive rats. The control BP of this group was 125 ± 5.0 mm/Hg (SEM); two weeks after the operation the BP value was significantly higher than those before surgery ($p < 0.05$) and then BP constantly increased up to the end of the period of study (Fig. 3C).

In order to evaluate the relationship between BP and PRA in the chronic stage of 1-KHR, correlation analysis was performed by the least squares method (Fig. 4). There was no significant correlation between BP and PRA ($r = 0.002$, $n = 27$, $p > 0.05$).

Clamping one renal artery in contralaterally nephrectomized rats had a significant effect on BP level but did not effect PRA, suggesting that renin-angiotensin system does not play a significant role in the maintenance of BP in chronic one-kidney hypertension.

Figure 1. Effect of wrapping the left kidney in uninephrectomized dogs on: Blood pressure (BP, n = 5); Plasma renin activity (PRA, n = 4), and Aldosterone concentration (Aldo, n = 3). Vertical lines represent standard error of the mean (SEM). (*) Significantly different compared to pre-operative values ($p < 0.05$).

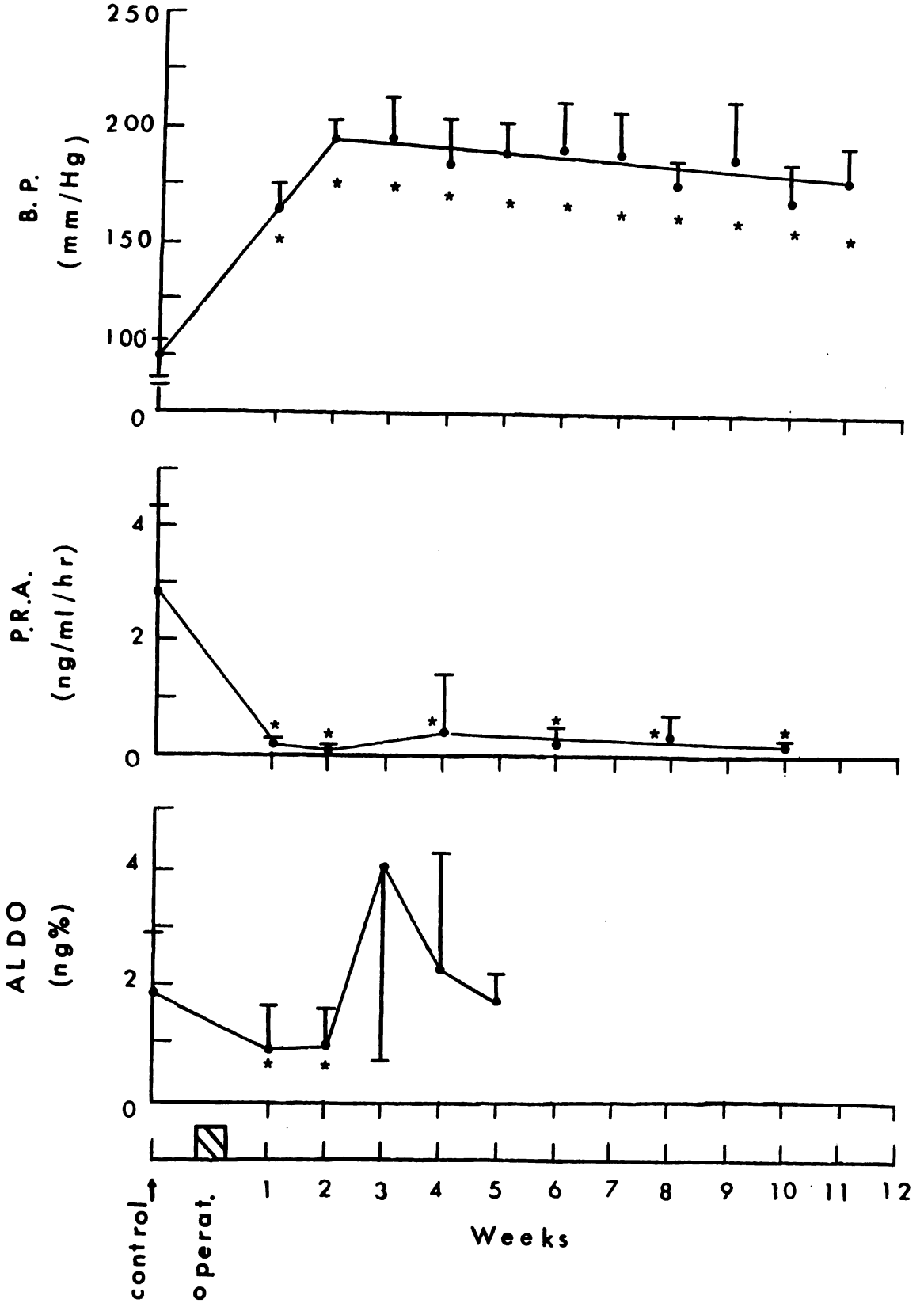

B.P.
(mm/Hg)
250
200
150
100
0
P.R.A.
(ng/ml/hr)
4
2
0
ALDO
(ng%)
4
2
0
control
operat.
1
2
3
4
5
6
7
8
9
10
11
12
Weeks

Figure 1

Figure 2. Relationship between BP and PRA in four dogs during the chronic stage of one-kidney perinephritis hypertension. There was a significant negative correlation between BP and PRA ($r = 0.785$, $n = 12$, $p < 0.05$). Correlation coefficient was calculated by the least squares method.

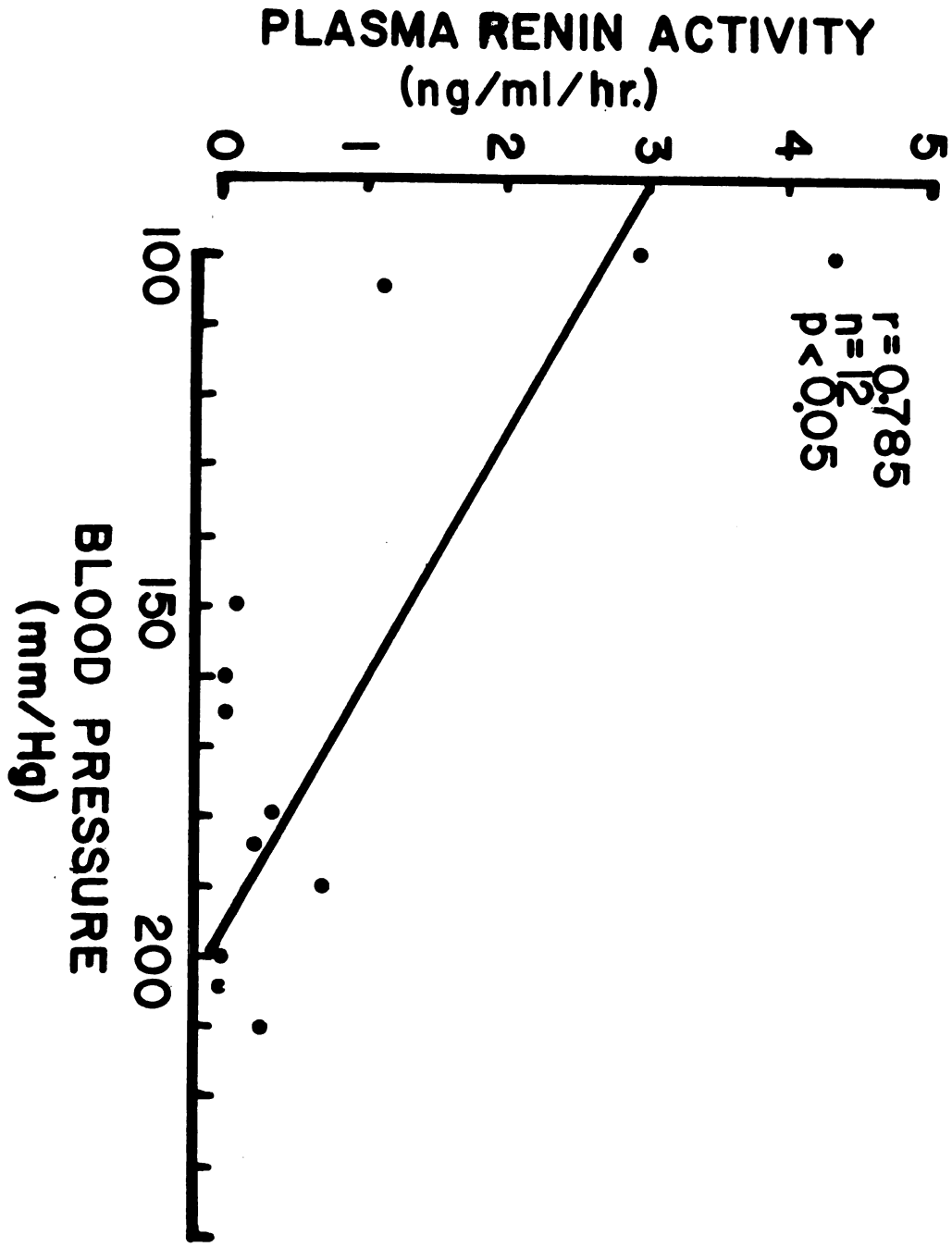

PLASMA RENIN ACTIVITY
(ng/ml/hr.)
0
1
2
3
4
5
BLOOD PRESSURE
(mm/Hg)
100
150
200
r=0.785
n=12
p<0.05

Figure 2

Figure 3. A. Mean value of blood pressure (SEM) in one-kidney hypertensive (close circles, n = 2 to 13) and sham operated (open circles, n = 2 to 10) rats over a nine-week period after operation. Points without SEM are the mean value of 2 rats. (*) Significantly different compared to preoperative values ($p < 0.05$).

B. Mean value of plasma renin activity (SEM) in one-kidney hypertensive (close circles, n = 2 to 13) and sham operated (open circles, n = 1 or 2) rats over a nine-week period after operation. Points without SEM are the value of 1 or the mean value of 2 rats. There were no significant changes during the entire period of observation.

C. Mean value of blood pressure (SEM) in a group of four hypertensive rats over a nine-week period. (*) Significantly different compared to preoperative values (Op. means operation time).

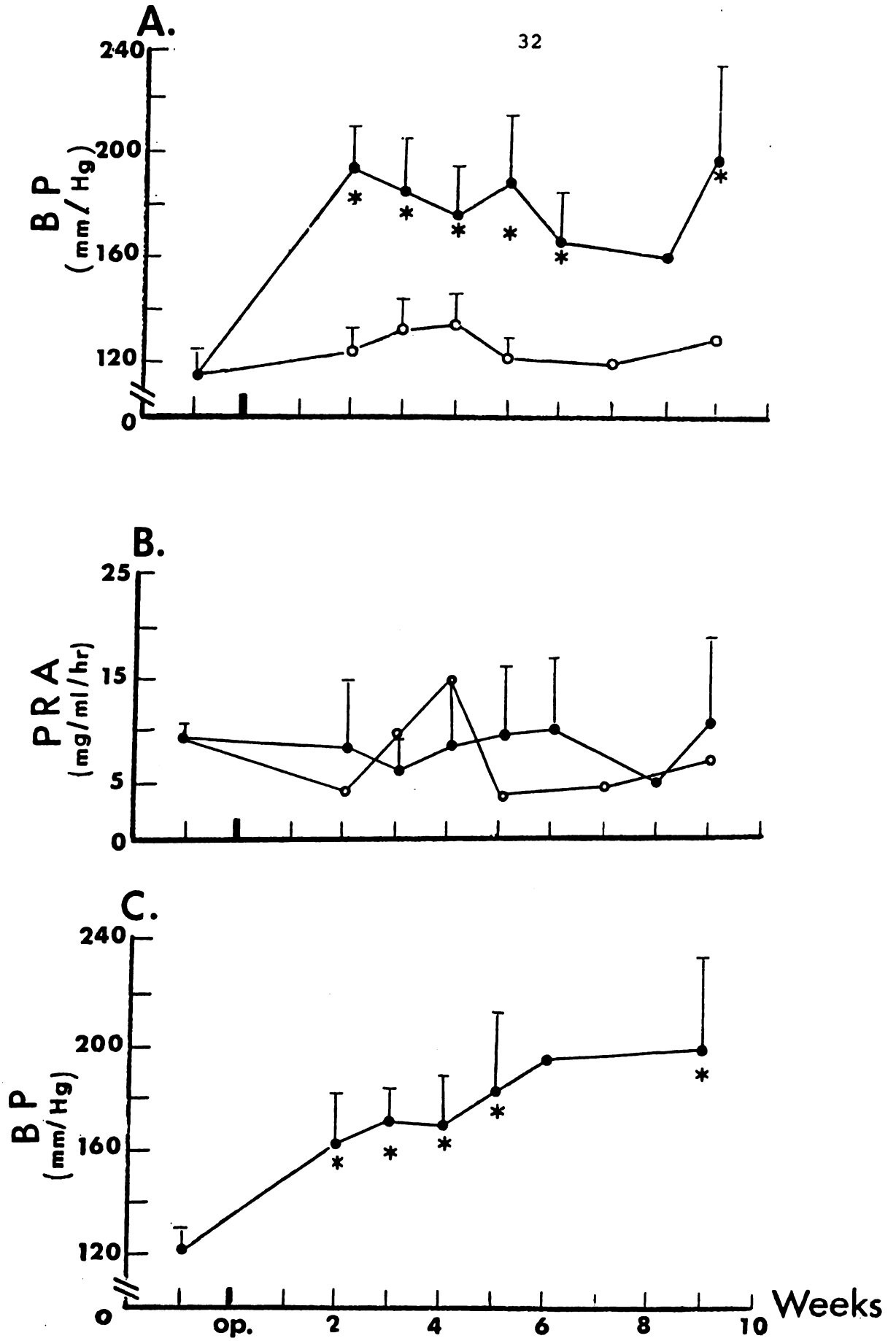

A.
240
200
160
120
0
B P
(mm/Hg)
B.
25
15
5
0
P R A
(mg/ml/hr)
C.
240
200
160
120
0
B P
(mm/Hg)
op.
2
4
6
8
10
Weeks

Figure 3

Figure 4. Relationship between BP and PRA in 27 rats during the chronic one-kidney hypertension. There was no significant correlation ($r = 0.002$, $n = 27$, $p > 0.05$). Correlation coefficient was calculated by the least squares method. Open circles represent values from sham operated rats. Close circles represent values from one-kidney hypertensive rats.

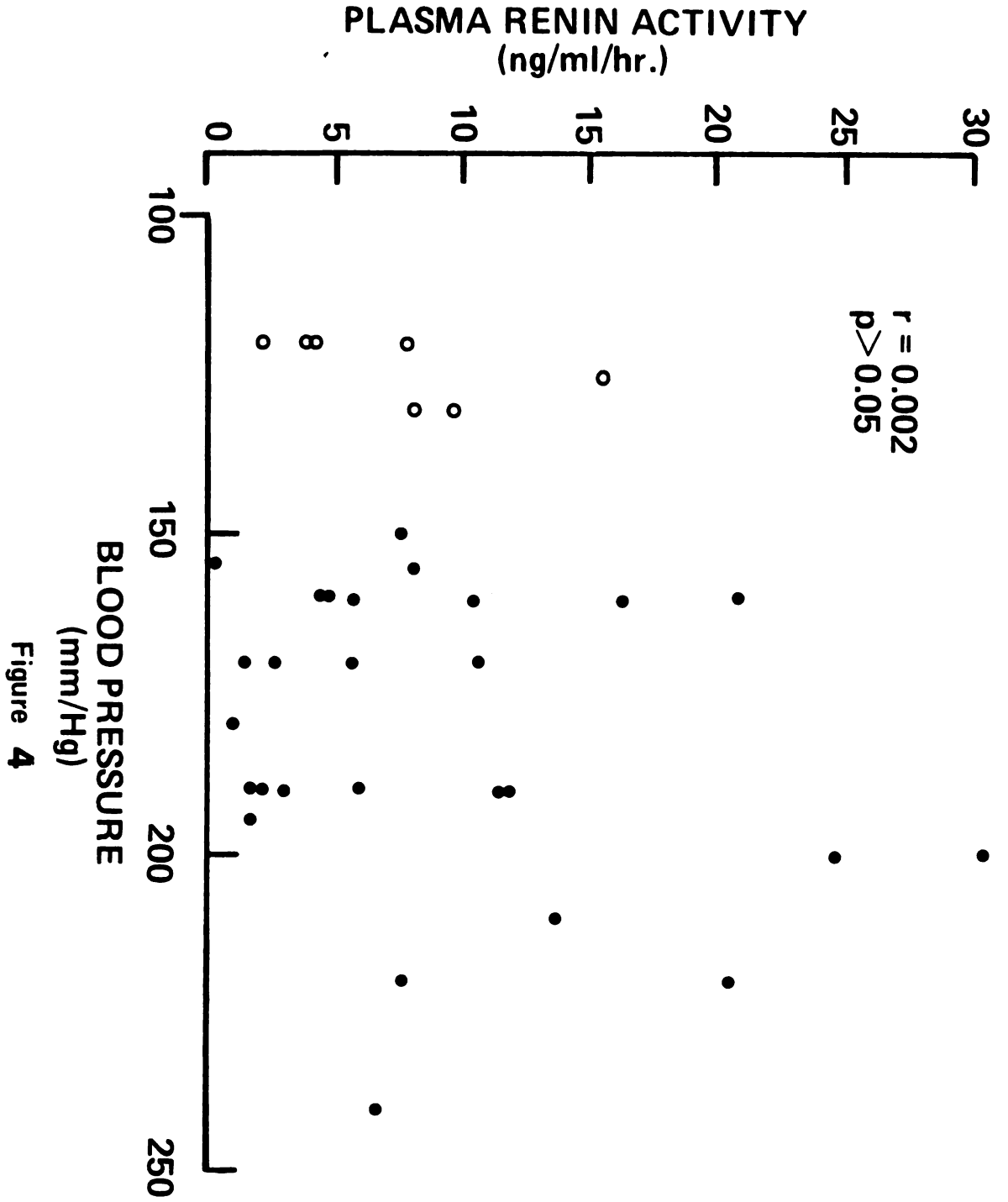

PLASMA RENIN ACTIVITY
(ng/ml/hr.)
0
5
10
15
20
25
30
100
150
200
250
BLOOD PRESSURE
(mm/Hg)
r = 0.002
p < 0.05

Figure 4

2. Effect of plasma on the activity of angiotensin II on blood pressure of ganglion-blocked and bilaterally nephrectomized rats

In the first serie of Bioassay I, we studied the change in the response of the assay rat to a constant amount of AII after the administration of plasma from normotensive and hypertensive rats. There was a rise in mean BP (base line) and vasopressor response to All after intravenous injection of 20 µl or 50 µl of plasma from hypertensive but not from normotensive rats. Figure 5 shows a typical response of the assay. Vascular responsiveness to AII usually increased 10 to 15 minutes after the administration of plasma from 1-KHR. The maximal increase was seen 20 to 25 minutes after the injection of plasma, and the effect continued for 30 to 60 minutes (Fig. 6). The difference between the values obtained with plasma from hypertensive and normotensive rats was statistically significant ($p < 0.05$) 10, 15, 20 and 25 minutes after the injection of plasma.

In the second serie of experiments, we evaluated whether the effect of plasma on the pressor activity of AII in Bioassay I exhibits a dose-response relationship. A constant dose of AII was intravenously injected every 5 to 10 minutes after the administration (i.v.) of 5, 10, 15, 17, 20 and 50 µl of plasma per 130 gm rat. Ten microliters of plasma from 1-KHR was the minimal amount which could enhance the vascular response to AII. Maximal plasma effect was

obtained with 20 µl or 50 µl of plasma from 1-KHR. Plasma from normotensive rats did not produce any significant increase in the response of this bioassay to AII (Fig. 7).

Figure 5. Typical response of the blood pressure of bilaterally nephrectomized rats to the intravenous injection of angiotensin II (AII) before and after the administration of plasma from chronic one-kidney hypertensive (HPT) and normotensive rats (NT). There was a rise in both mean blood pressure (baseline) and response to angiotensin II after injection of plasma from hypertensive but not from normotensive rats.

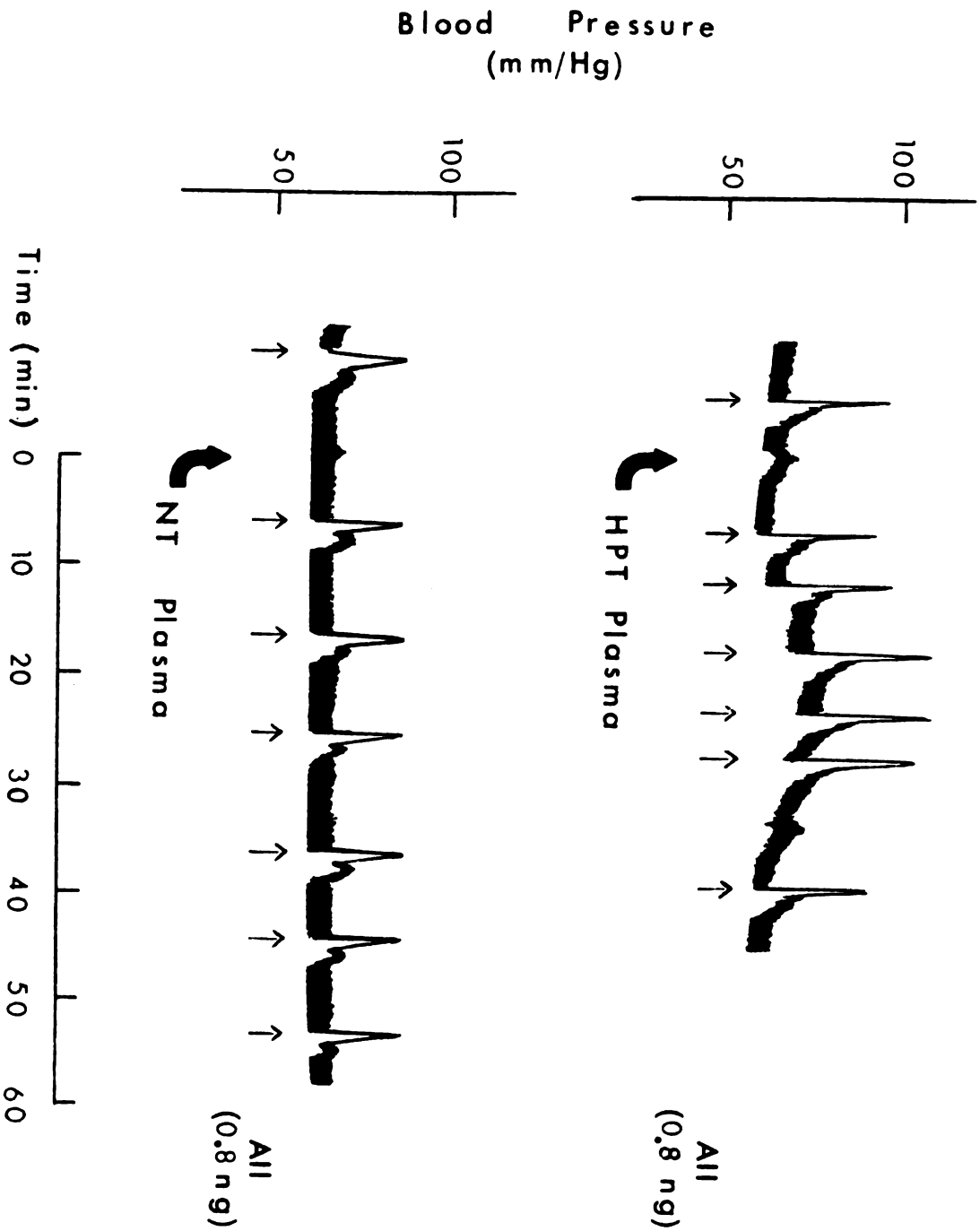

Blood Pressure
(mm/Hg)
50
100
50
100
HPT Plasma
NT Plasma
All
(0.8 ng)
All
(0.8 ng)
Time (min) 0
10
20
30
40
50
60

Figure 5

Figure 6. Effect of plasma from chronic one-kidney hypertensive (●-●) and normotensive (o-o) rats on angiotensin II vasopressor activity in bilaterally nephrectomized rats. Each point is the mean of 13 values. Vertical lines represent the standard error of the mean. (*) Significantly higher ($p < 0.05$) than the effect obtained after the administration of plasma from normotensive rats.

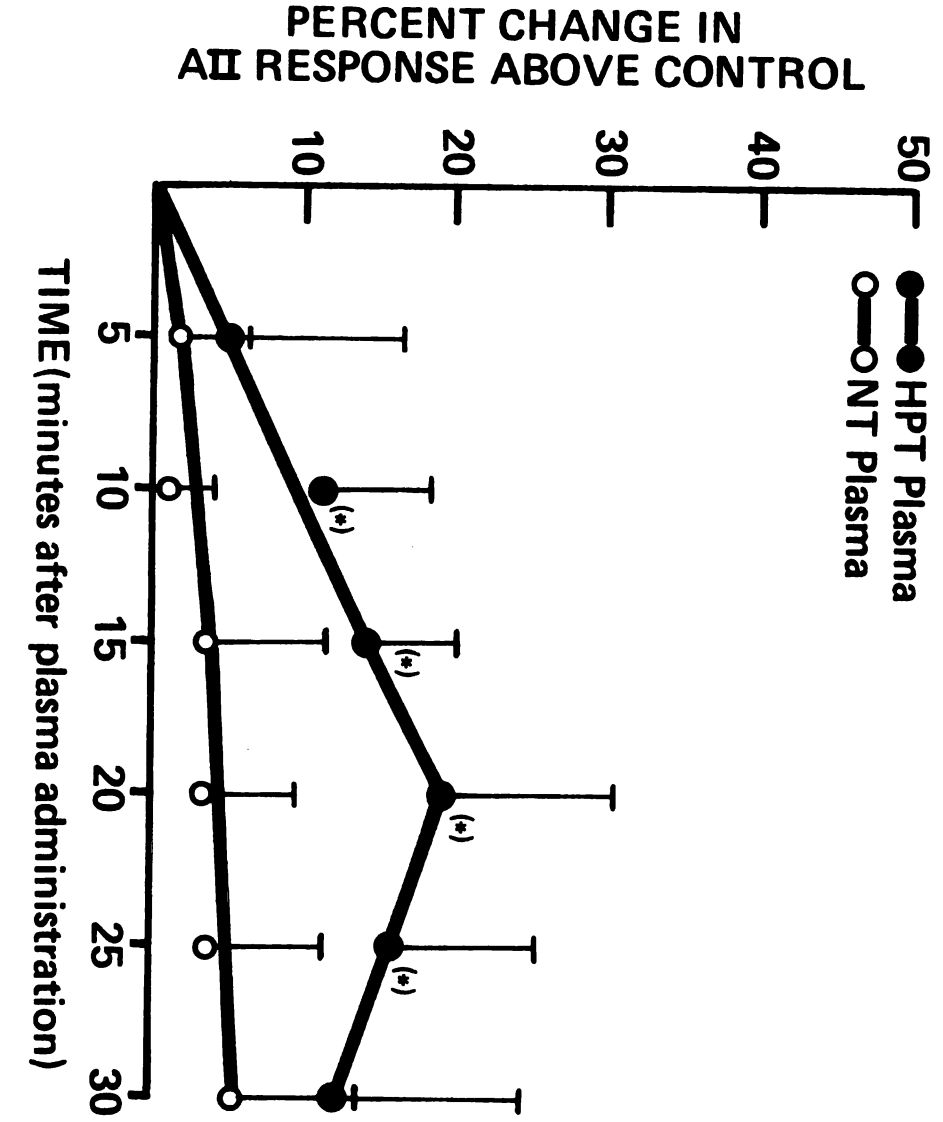

PERCENT CHANGE IN
AII RESPONSE ABOVE CONTROL
10
20
30
40
50
HPT Plasma
NT Plasma
(*)
(*)
(*)
(*)
TIME(minutes after plasma administration)
5
10
15
20
25
30

Figure 6

Figure 7. The dose-response relationship of the percent difference between the rise in BP of bilaterally nephrectomized rats caused by AII before and after the injection of different amounts of plasma from chronic one-kidney hypertensive (●-●) and normotensive (o-o) rats. Each point is the mean of three experiments. Vertical lines represent the standard error of the mean. (*) Significantly higher ($p < 0.05$) than the effect obtained after the administration of plasma from normotensive rats.

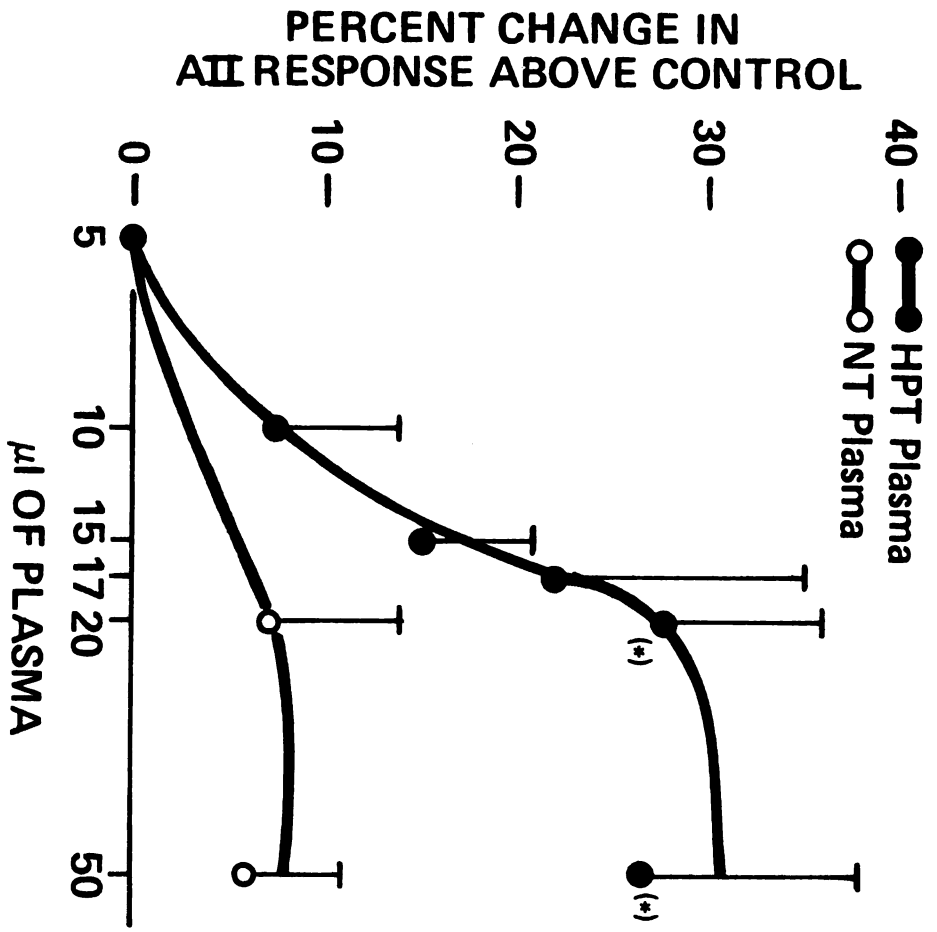

PERCENT CHANGE IN
AII RESPONSE ABOVE CONTROL
0
10
20
30
40
HPT Plasma
NT Plasma
5
10
15
17
20
50
μl OF PLASMA
(*)
(*)

Figure 7

3. Effect of plasma on the pressor response of the isolated mesenteric arteriole to norepinephrine

The dose-response curve for NE during perfusion with Krebs buffer solution was studied. At basal perfusion pressure close to 30 mm/Hg the threshold dose of NE was often 50 ng in bolus injection. We could not determine the maximal response of the assay to NE because this catecholamine usually produced an out of scale increase of perfusion pressure with any dose above 300 ng per preparation. Figure 8 shows a typical response of Bioassay II to NE during the perfusion of Krebs buffer solution, plasma from normotensive and hypertensive dogs.

The dose-response curve for NE was significantly ($p < 0.05$) increased when the mesenteric arteriole was perfused with the buffer solution containing 0.5 ml of plasma from hypertensive dogs per 100 ml of perfusate. There was no significant change with perfusion of plasma from normotensive dogs (0.5 ml of plasma per 100 ml of perfusate). Figure 9 compares dose-response curve for NE obtained during perfusion of plasmas.

Figure 8. Typical response of the pressure of isolated mesentery artery to different doses of NE during perfusion of buffer solution (see method for detailed composition), plasma from chronic one-kidney perinephritis hypertensive (HPT Plasma) and normotensive dogs (NT Plasma). Plasma perfusion was 0.5 ml of plasma per 100 ml of perfusate and flow rate was 2 ml/min.

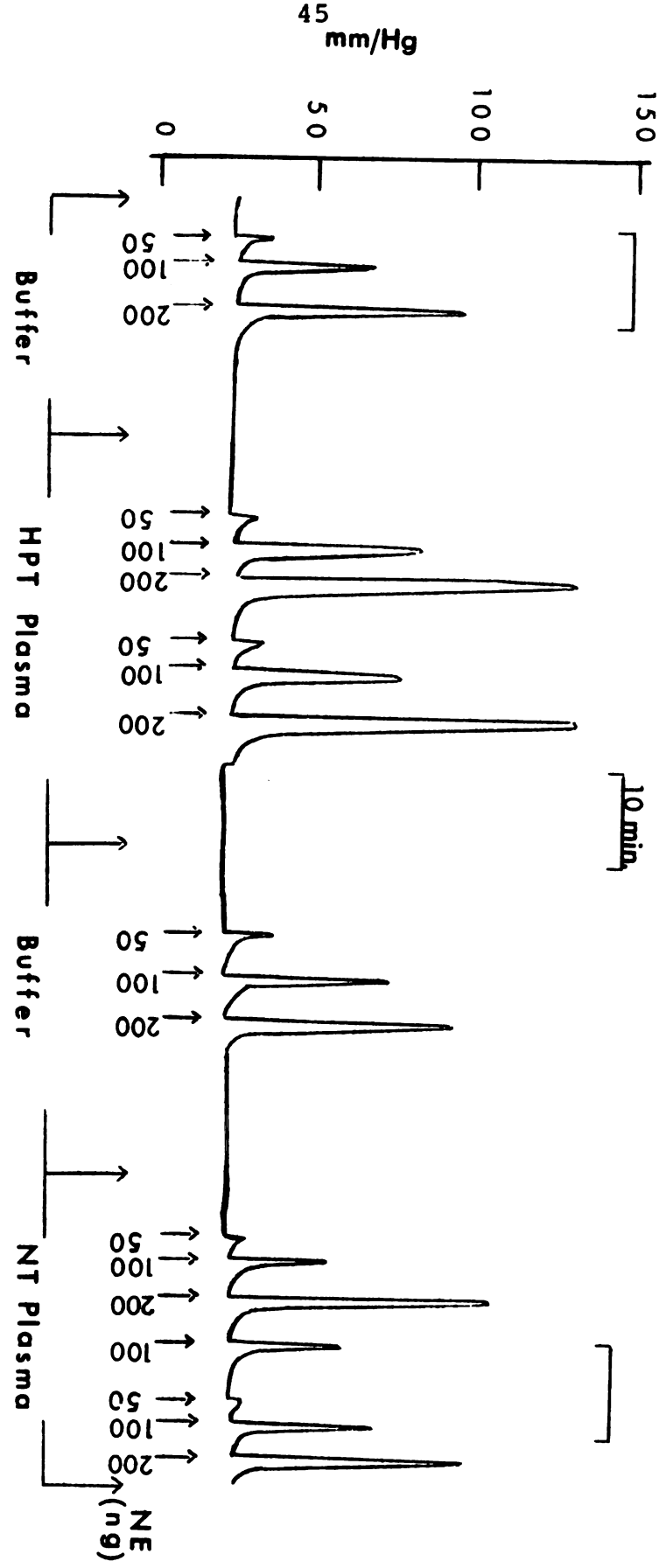

mm/Hg
0
50
100
150
Buffer
50
100
200
HPT Plasma
50
100
200
50
100
200
10 min
Buffer
50
100
200
NT Plasma
50
100
200
100
50
100
200
NE (ng)

Figure 8

Figure 9. Dose-response curve for NE in isolated mesenteric artery during perfusion of plasma from chronic one-kidney hypertensive (●-●) and normotensive (o-o) dogs. Plasma perfusion was 0.5 ml of plasma per 100 ml of perfusate. Percent change in response above control is the percent difference between the rise in perfusion pressure caused by NE before and after the injection of plasma. Each point is the mean of 6 experiments. Vertical lines represent standard error of the mean. (*) Significantly higher ($p < 0.05$) than the effect obtained after the administration of plasma from normotensive dogs.

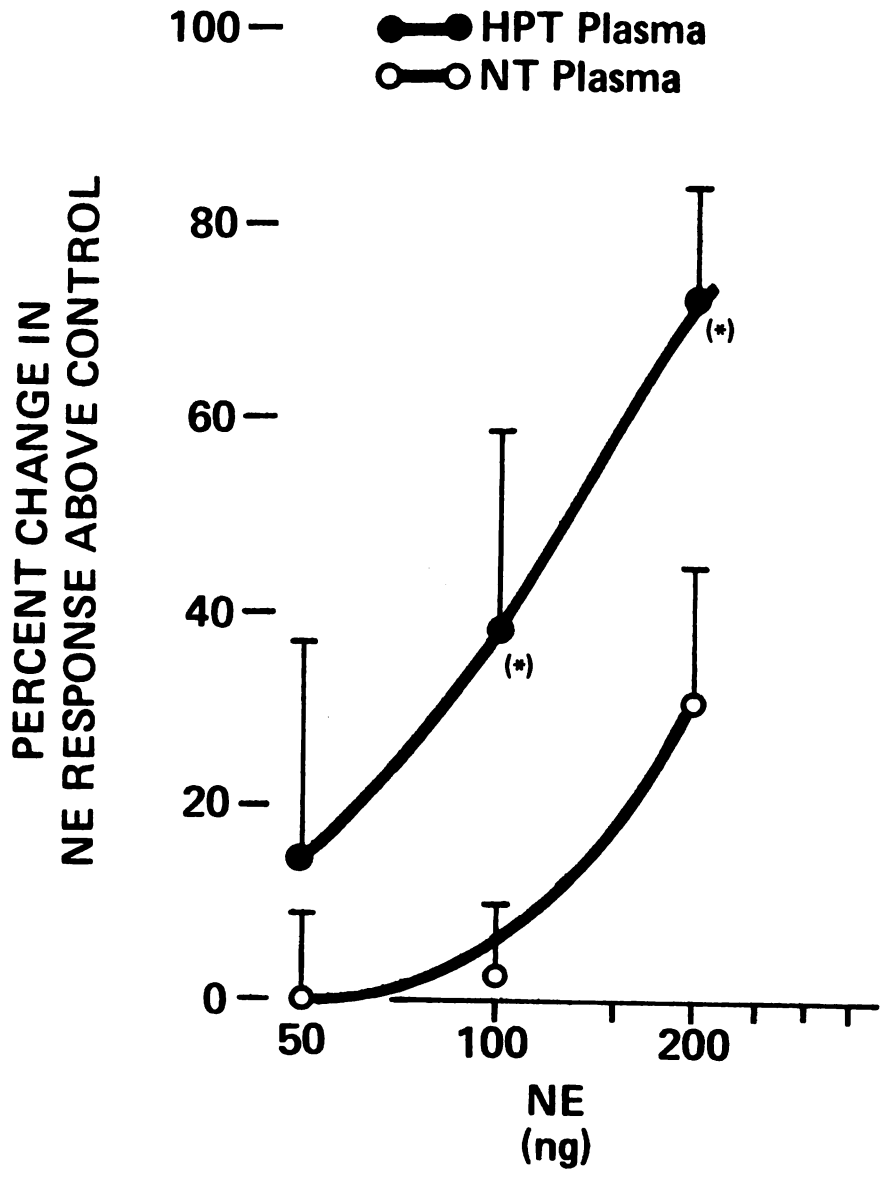

100
HPT Plasma
NT Plasma
80
60
40
20
0
PERCENT CHANGE IN
NE RESPONSE ABOVE CONTROL
(*)
(*)
50
100
200
NE
(ng)

Figure 9

4. Effect of plasma fractions on the activity of angiotensin II on blood pressure of ganglion-blocked and bilaterally nephrectomized rats

We studied the effect of plasma fractions containing substances with small molecular weight on the pressor activity of AII in Bioassay I.

a. Plasma from one-kidney perinephritis hypertensive dogs was passed through Amicon Diaflo Ultramembrane 2 (UM-2 retainer fraction) and 05 (UM-05 retainer fraction). Substances retained by those membranes were tested in 8 rats in Bioassay I.

The pressor activity of AII began to increase 5 to 10 minutes after the intravenous administration of both fractions, but the effect of UM-05 retainer was significantly higher ($p < 0.05$) as compared to the effect of UM-2 retainer (Fig. 10). Also, after the administration of UM-05 retainer fraction the rise in the response to AII was usually longer than after the administration of UM-2 retainer fraction.

b. UM-05 retainer was further fractionated on gel filtration column (Bio-Gel P-2 1.5 x 90 cm) in order to determine the molecular weight of the humoral factor more closely. Figure 11 shows the ultraviolet absorbance at 230 nm of Bio-Gel P-2 fractions (close circles). Two pepetides, angiotensin II (M.W. 1100) and glycyl-glycine (M.W. 132), were used as molecular markers. It can be seen from Figure 11 that there were two main peaks; the substances represented

by the first peak (eluted volume between 102 to 148 ml) have molecular weights close to 1,000; and the substances represented by the second peak (eluted volume between 150 to 212) have molecular weights around 700 or 800. Fractions were freeze-dried and then redissolved in 0.9% NaCl, and bioassay was performed immediately after plasma fraction was passed through Dowex 50W-X2 resin to remove any angiotensin formed during the procedure or that may be in the plasma fraction. Fraction No. 20 (115 to 120 ml of eluted volume) from 1-KHD produced a significant increase of the activity of AII in Bioassay I (Fig. 11. Open circles). Also, there was no significant increase in the bioassay response to AII after the administration of other fractions included in the first peak of eluted volume. These results were consistent with the previous step of the fractionation, which showed that substance(s) with molecular weight(s) close to 1,000 may be responsible for the effect of plasma from one-kidney hypertensive dogs and rats on the pressor response to AII and NE.

Figure 12 shows the ultraviolet absorbance at 230 nm of Bio-Bel P-2 plasma fractions from normotensive dogs (close circles). The elution pattern was essentially the same as that obtained with plasma from hypertensive dogs. Fraction No. 20 from normotensive dogs produced a moderate increase in the response of Bioassay I to AII (Fig. 12. Open circles), but this effect was significantly lower ($p < 0.05$)

than that observed after the administration of fraction No. 20 from 1-KHD.

Fifty µl of fraction No. 20 from 1-KHD raised the vasopressor activity of AII within the first five minutes after the administration of this fraction, and the effect always lasted more than 30 minutes. Fifty µl of fraction No. 20 from normotensive dogs did not produce significant changes in the response of the assay rat to AII (Fig. 13).

These results suggest that in one-kidney Goldblatt type hypertension in rats and in one-kidney perinephritis hypertension in dogs a small molecular weight circulating substance may be responsible for an increased vasopressor response to AII and NE in those types of hypertension.

Figure 10. Effect of plasma fractions from chronic one-kidney perinephritis hypertensive dogs on AII vasopressor activity in bilaterally nephrectomized rats. Both Amicon Diaflo Ultrafiltration membrance UM-05 (●-●) and UM-2 (o-o) retainer fractions were assay in the same animals. Percent change in AII response above control is the percent difference between rise in BP caused by AII before and after the injection of plasma fractions. Each point is the mean of four to eight experiments. Vertical lines represent standard error of the mean. (*) Significantly higher ($p < 0.05$) than the effect obtained after the administration of UM-2 retainer plasma fraction.

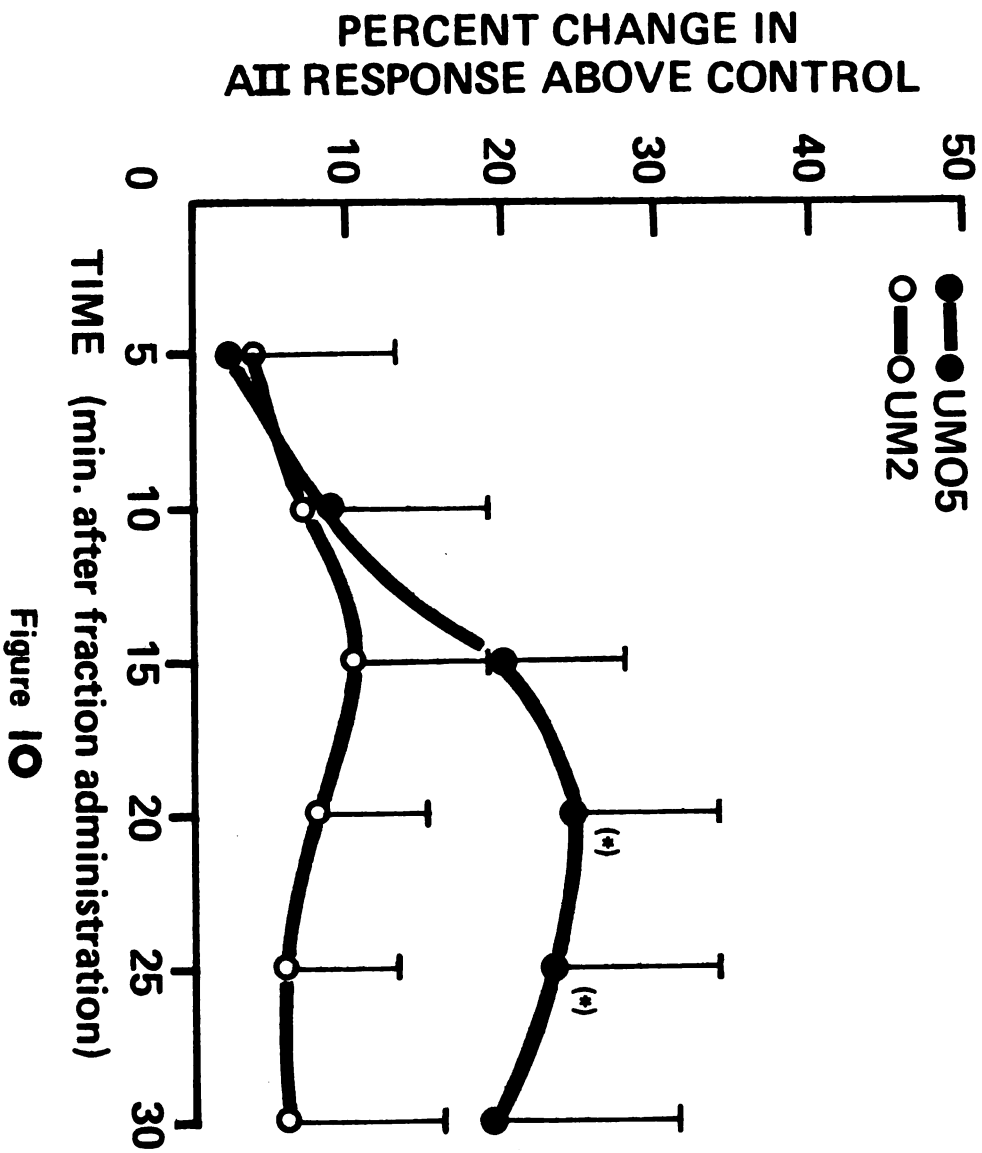

PERCENT CHANGE IN
AII RESPONSE ABOVE CONTROL
0
10
20
30
40
50
TIME (min. after fraction administration)
0
5
10
15
20
25
30
UMO5
UM2
(*)
(*)

Figure 10

Figure 11. Effect of plasma fractions from chronic one-kidney perinephritis hypertensive dogs on AII vasopressor activity in bilaterally nephrectomized rats (o-o). Bioassays were performed immediately after fraction was passed through Dowex 50W-X2 resin column. Percent change in AII response above control is the percent difference between rise in BP caused by AII before and after the injection of plasma fraction. Each point is the mean of 5 experiments. Vertical lines represent the standard error of the mean. (*) Response was significantly higher ($p < 0.05$) than that obtained after the administration of plasma fraction from normotensive dog (see Fig. 11). Close circles (●-●) represent the ultraviolet absorbance of each fraction at 230 nm. Angiotensin II (M.W. 1,100) and glycyl-glycine (M.W. 132) were used as molecular weight markers.

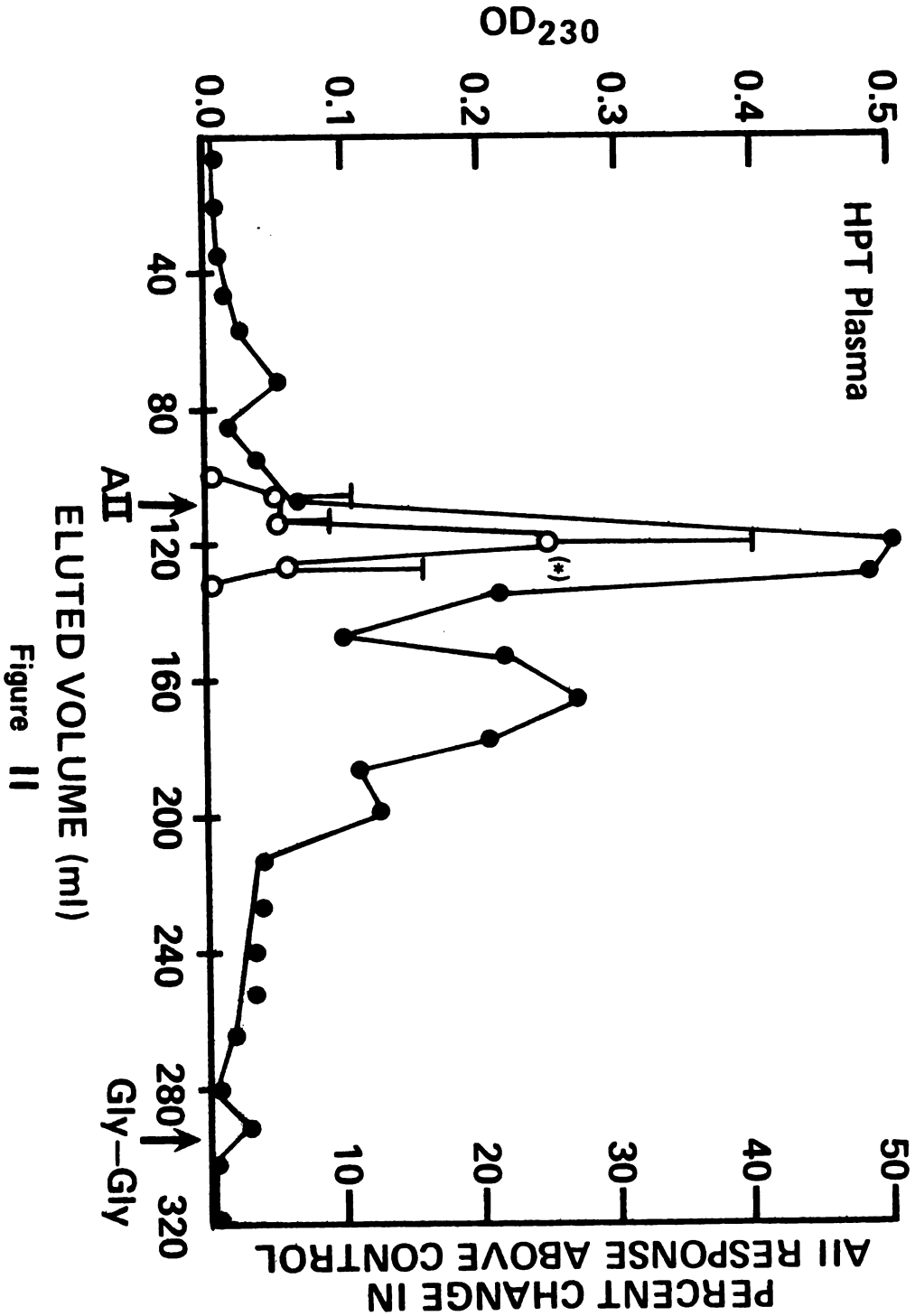

OD230
0.0
0.1
0.2
0.3
0.4
0.5
HPT Plasma
(*)
40
80
AII
120
160
200
240
280
Gly–Gly
320
ELUTED VOLUME (ml)
PERCENT CHANGE IN
AII RESPONSE ABOVE CONTROL
10
20
30
40
50

Figure 11

Figure 12. Effect of plasma fraction from normotensive dogs on the vasopressor activity of AII in bilaterally nephrectomized, ganglion-blocked rats (o-o). Bioassays were performed immediately after fraction was passed through Dowex 50W-X2 column. Percent change in AII response above control is the percent difference between rise in BP caused by AII before and after the injection of plasma fraction. Each point is the mean of 3 experiments. Vertical lines represent standard error of the mean. Close circles (●-●) see legend to Fig. 10.

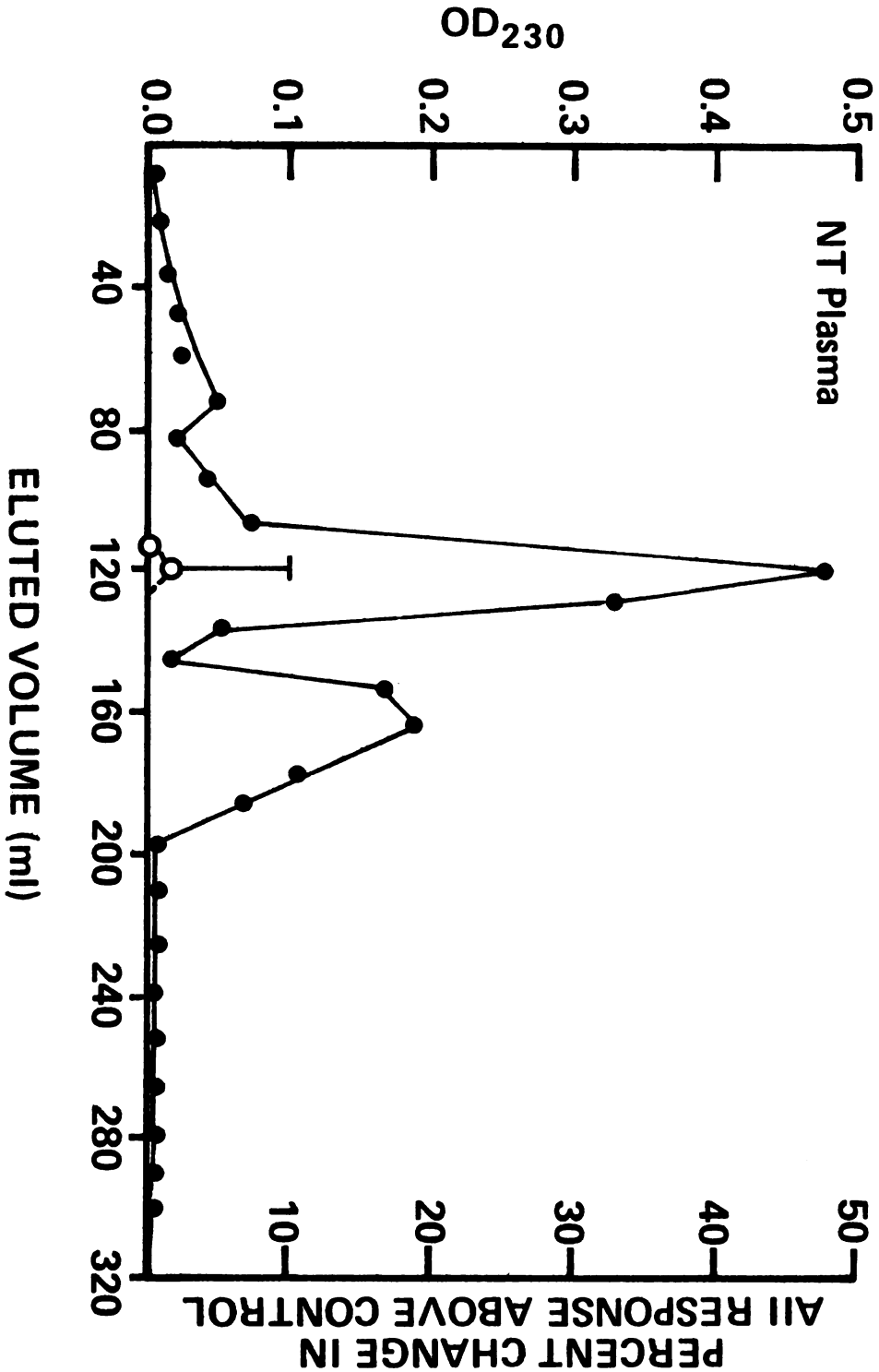

OD230
0.0
0.1
0.2
0.3
0.4
0.5
NT Plasma
40
80
120
160
200
240
280
320
ELUTED VOLUME (ml)
PERCENT CHANGE IN
AII RESPONSE ABOVE CONTROL
10
20
30
40
50

Figure 12

Figure 13. Effect of plasma fraction no. 20 (between 114 to 120 ml of eluted volume on Bio-Gel P-2, 1.5 x 90 cm column) from chronic one-kidney perinephritis hypertensive (●-●) and normotensive (o-o) dogs on AII vasopressor activity in ganglion-blocked and bilaterally nephrectomized rats. Amicon Diaflo Ultrafiltration membrane UM-05 retainer was further fractionated on Bio-Gel P-2, 1.5 x 90 cm, and 6 ml were collected in each tube. Fractions were lyophilized and bioassays were performed immediately after fractions were redissolved in 2 ml of 0.9% NaCl, and passed through Dowex 50W-X2 resin. Percent change in AII response above control is the percent difference between rise in BP caused by AII before and after the injection of plasma fraction. Each point is the mean of 5 (plasma fraction from hypertensive dogs) or 3 (plasma fraction from normotensive dogs) experiments. Vertical lines represent the standard error of the mean. (*) Significantly different ($p < 0.05$) than the effect obtained after the administration of plasma fraction from normotensive dogs.

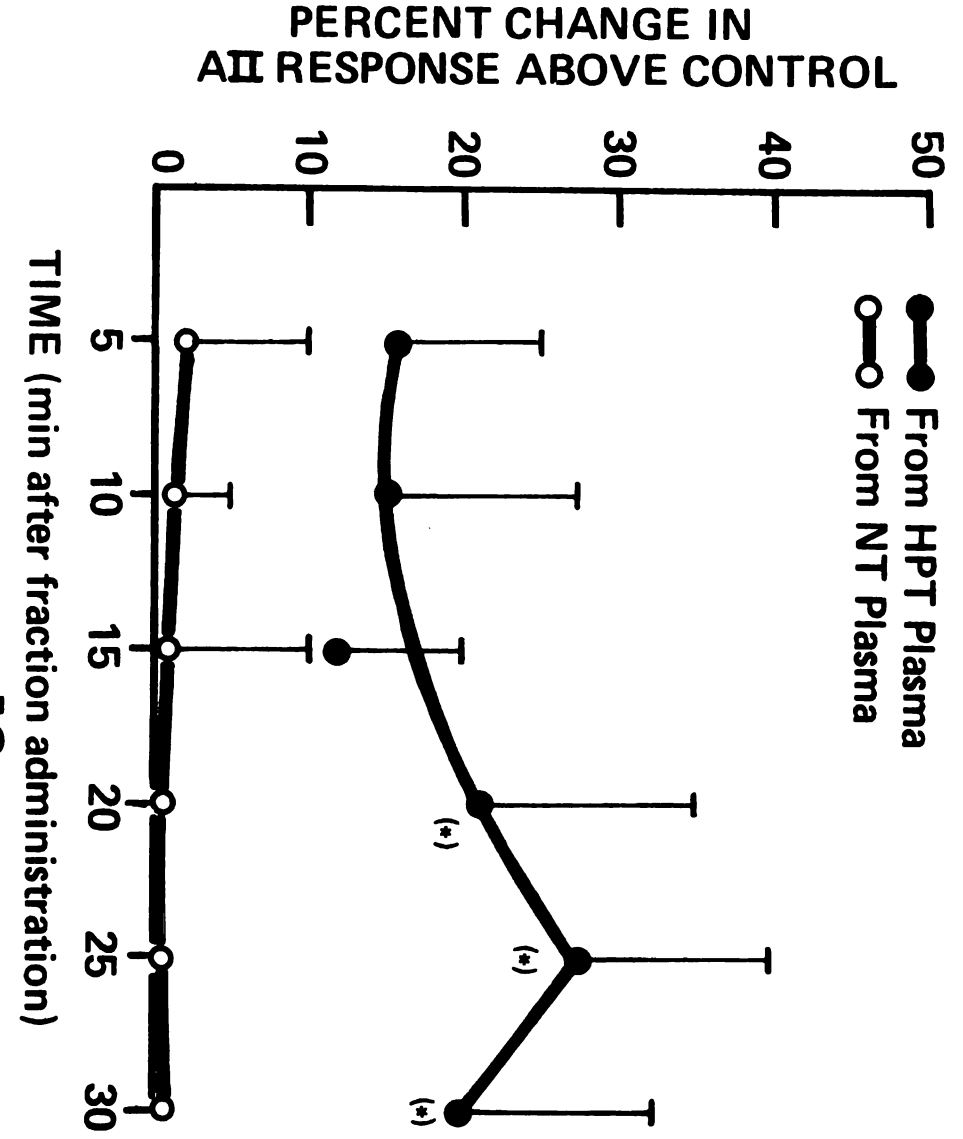

PERCENT CHANGE IN
AII RESPONSE ABOVE CONTROL
0
10
20
30
40
50
From HPT Plasma
From NT Plasma
5
10
15
20
25
30
(*)
(*)
(*)
TIME (min after fraction administration)

Figure 13

DISCUSSION

1. Relationship between renin-angiotensin-aldosterone system and one-kidney Renovascular Hypertension

It is likely that various substances or control systems are involved in the etiopathogenesis of renovascular hypertension. Several reports have suggested that 2-KH is dependent of R-A system in both the acute and the chronic stage. Thus, PRA is usually high in 2-KH (Lupu et al., 1972; Skulan et al., 1974; Leenen and de Jong, 1975) in addition, 2-KH has been reversed or prevented by use of AII inhibitors (Bumpus et al., 1973) or anti-angiotensin antibodies (Brunner et al., 1971). However, in some studies it has been found that an increased PRA may not be essential for the development of hypertension in 2-KH model (Eide, 1972; Helmchen et al., 1972a).

In chronic 1-KH model, PRA is either normal or significantly depressed (Mogil et al., 1969). Previous reports have proposed an unknown humoral factor (Fujii and Ikeda, 1971; Skeggs et al., 1975, 1976), sensitizing factors to pressor agents (Mizukoshi and Michelakis, 1972; Michelakis et al., 1975), an unknown slow acting inhibitory factor of enzymatic reaction (Overbeck et al., 1976) to explain the etiopathogenesis of 1-KH.

In the present study, we did not find any significant relationship between BP and PRA in the chronic stage of 1-KHR. Furthermore, PRA was significantly lower compared to control value in 1-KHD ($p < 0.05$) during the three month period of hypertension, and also there was a significant negative correlation between BP and PRA in those hypertensive dogs in chronic stage. Our results in one-kidney Goldblatt type of hypertension in rats and in one-kidney perinephritis hypertensive dogs do not support any significant role for renin-angiotensin in the maintenance of chronic one-kidney hypertension. This hypothesis was further supported by the fact that aldosterone concentration was significantly suppressed ($p < 0.05$).

To test the hypothesis that PRA and aldosterone concentration may be low due to water retention which may have inhibited renin formation and, consequently, decrease both the angiotensin level and the rate of aldosterone secretion in 1-KHD, we recorded the weight of dogs. However, there was no significant change in body weight for three months after hypertension had started. Alternatively, a likely elevation of renal perfusion pressure during perinephritis hypertension due to the external compression produced by the silk bag on the kidney may explain the suppression in PRA and aldosterone concentration: this possibility should be further studied.

2. Evidences for the presence of a factor which potentiates the action of angiotensin II and norepinephrine in one-kidney Renovascular Hypertension

a. Effect of plasma from normotensive and hypertensive rats and dogs on the vascular response of AII and NE: Even though several investigations have conducted studies on the effect of plasma from normotensive animals on the contraction of vascular smooth muscle (Wurzel et al., 1964; Bohr and Johansson, 1966; Wurzel et al., 1967; Bohr and Sobieski, 1968; Croxato and Diaz, 1969; Ng et al., 1971), and the pressor effect of plasma from acute renovascular hypertension (Grollman, 1970; Susic and Sparks, 1975), there are only few reports on the effect of plasma from chronically renovascular hypertensive animals on the vascular activity of vasopressor substances (Mizukoshi and Michelakis, 1972; Greenberg et al., 1974). We found that the plasma from 1-KHR showed a moderate and long lasting pressor activity in Bioassay I; 20 or 50 ml of plasma from those animals produced an increase in BP for 30 to 60 minutes in sensitive preparations. The absence of kidneys in the assay preparation may very well lengthened the biological half-life of the substance responsible for the increase in BP.

Also, we used the isolated and perfused mesenteric arteriole, a technique originally designed by Uchida et al., (1967), to evaluate the effect of plasma from one-kidney

perinephritis hypertensive dogs on the pressor activity of NE. Isolated vessels to study the vascular effect of pressor agents have been used very often (De Lande and Rand, 1965; Uchida and Bohr, 1969). The effect of plasma from normotensive animals on the pressor activity of NE has also been studied with this type of preparation. (Ng et al., 1971). Bohr and Johansson (1966) reported that 0.25 to 1.0 ml of plasma from normotensive dogs per 100 ml of perfusate potentiated the activity of catacholamines, AII and KCl on vascular smooth muscle. This potentiating effect of normal plasma has been attributed to a small molecular weight vasoactive substance (Bohr and Sobieski, 1968), or to a large protein molecule likes albumin (Wurzel et al., 1964). We used 0.5 ml of plasma (either from normotensive or hypertensive dogs) per 100 ml of perfusate to evaluate its effect on the activity of NE on resistance vessels. Dose-response curve for NE during perfusion of Krebs buffer solution containing plasma from 1-KHD was significantly increased ($p < 0.05$) compared to those values obtained during perfusion of that buffer solution containing plasma from normotensive dogs. These findings in Bioassay II strongly support the previous results in ganglion-blocked and bilaterally nephrectomized rats, suggesting that during one-kidney renovascular hypertension a plasma factor may participate in this type of hypertension by increasing the vasopressor activity of AII and NE.

b. Effect of plasma fractions from normotensive and hypertensive dogs on vascular response to angiotensin II

Amicon Diaflo Ultrafiltration Membranes were used to separate the plasma in four fractions. UM-05 retainer plasma fraction (substances with molecular weight between 500 to 1,000) from one-kidney hypertensive dogs produced a significant increase of the response of Bioassay I to AII. The fact that UM-2 retainer plasma fraction (substances with molecular weight between 1,000 to 10,000) from the same hypertensive dogs also increased the response of the assay to AII may indicate that the potentiating factor is close to the borderline fractions between UM-05 and UM-2 (M.W. 1,000). When we further fractionated UM-05 retainer on Bio-Gel P-2, 1.5 x 90 cm column, fraction No. 20 (115 to 120 ml of eluted volume) produced the maximal increase of vascular responsiveness to AII. Substances in this fraction have molecular weights close to 1,000, which is consistent with the data obtained with UM fractions.

To study the stability of the potentiating factor, we kept some gel filtration fractions No. 20 in 0.9% NaCl in normal gas environment (air) at 0°C for one week. The effect of the plasma fraction on the activity of AII in Bioassay I almost disappeared under these conditions.

The precise mechanism by which the proposed potentiating factor may enhance the vasopressor activity of

AII and NE in one-kidney renovascular hypertension is not clear. However, it may be related to a common pathway of such vasoactive substances. One possibility, which should be tested in further studies, is the effect of the humoral factor on the concentration of intracellular free Ca^{++} in the vascular smooth muscle. It has been well established that free Ca^{++} is an essential requirement for excitation-contraction coupling in smooth muscle cell (Bohr, 1973), and, therefore, it could be assumed that the humoral factor may potentiate the activity of AII and NE by increasing the process of Ca^{++} release from the membrane and/or by decreasing the process of sequestration by organelles (sarcoplasmatic reticulum, mitochondria, surface vesicles) in the vascular smooth muscle cell.

SUMMARY AND CONCLUSIONS

The results in the present study do not support the renin-angiotensin system as the main factor in the etiopathogenesis of one-kidney renovascular hypertension. The present data indicate that plasma from chronic one-kidney hypertensive rats and dogs increases the vasopressor activity of angiotensin II and norepinephrine, and that a small molecular weight substance may be responsible for the plasma effect. This mechanism may explain at least in part the etiopathogenesis of one-kidney renovascular hypertension.

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