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Passive Transfer of Pressor Hyperresponsiveness from Renal-Hypertensive to Normotensive Rats[1,2] (41748)

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Abstract. The role of serum factors in the pathogenesis of pressor hyperresponsiveness in hypertension was investigated by the passive transfer of serum from donor rats with chronic one-kidney, one clip hypertension into syngeneic normotensive recipient rats (0.25 ml iv, bid) for 3 weeks. Rats injected twice daily with the serum of normotensive rats served as controls. In rats injected with the serum of hypertensive rats there was a gradual increase in pressor responses to norepinephrine and angiotensin II and, at the end of the study, increased water content of the aorta and sodium content of the myocardium. In volume-expanded renal hypertension unidentified serum factors contribute to pressor hyperresponsiveness and increased sodium content of cardiovascular tissue.

There is evidence for unidentified circulating, slowly acting pressor, vasoconstrictor, and vascular sensitizing agents in the (blood) volume-expanded, non-renin-dependent forms of experimental and human hypertension (1). Their release is stimulated by high sodium intake. They may act by inhibition of transmembrane sodium transport (2). Previously, we have reported *in vivo* and *in vitro* evidence for a circulating humoral agent in experimental renal hypertension that causes the accumulation of excess sodium in the blood vessel wall (3–5). The long-term arterial pressor effect in the intact animal of these humoral agents has not been investigated. Their steady-state effect on vascular wall composition is not known. These long-term *in vivo* effects were investigated in the present study.

Methods. For these experiments, syngeneic male F344 rats, supplied by the Mammalian Genetics and Animal Production, Frederick, Maryland 21701, were used to prevent serum sickness during repeated injections of serum from one rat into another.

Donor rats. One-kidney, one clip hypertension was induced in 5- to 7-week-old rats according to techniques that were previously described (6). Normotensive control rats underwent sham-clipping and contralateral nephrectomy. Six-to-eight weeks later, the systolic blood pressure of rats was measured several times on different days by the microphonic manometer technique. The systolic blood pressure of the hypertensive donor rats was 182 mm Hg (mean) (range: 142–220). The rats were then bled.

For bleeding, the rats were briefly anesthetized with methoxyflurane. The jugular vein was catheterized, and the rats were injected with chloralose, 75 mg/kg iv. A tracheostomy was performed. Through a midabdominal incision, the aorta was prepared for catheterization, then the abdomen was closed. One hour after the induction of chloralose anesthesia, the aorta was quickly cannulated, and 5–7 ml of arterial blood withdrawn. The blood was allowed to clot for 5 min at room temperature and then for 20–30 min at 4°C. After centrifugation at 4°C, the serum was drawn and stored at −20°C until used.

Recipient rats. Arterial (abdominal aorta) and venous (jugular vein) catheters were implanted into 3- to 4-month-old previously unoperated F344 rats, according to the techniques developed by Dr. John Weeks, Upjohn Company, Kalamazoo, Michigan (7). Detailed updated description of the method and sample catheters were obtained from Dr. Weeks. The rats were allowed to recover for 1 week prior to the studies.

Hemodynamic measurements. The recipient rats were briefly anesthetized with me-

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thoxyflurane and wrapped in a towel. Their heads were covered with a black cloth with a small hole in it through which the arterial and venous catheter was passed. The rats awoke from anesthesia in 1–2 min and were easily trained to lie quietly for 15–20 min. Arterial pressure was detected with a P23Db pressure transducer at 10 min after the induction of anesthesia. Because of the small bore of the tip of the aortic catheters (PE 10), arterial pulse pressures were reduced to 5 mm Hg or less, and therefore, "mean" pressures are reported. Heart rate was counted by recording the arterial pressure curves at known paper speed. Arterial pressure responses to injected norepinephrine bitartrate (base) (NE) (0.05, 0.10, and 0.30 μg/kg iv) or to 5-isoleu-angiotensin II (AII) (5, 10, and 30 ng/kg iv) were then recorded. The peak rise in arterial pressure and the duration of pressure responses (seconds) were measured. At the completion of the pressure response to the highest injected dose of NE and AII, the heart rate was again counted.

Protocol. At the end of the first week, systolic pressures and heart rates of awake rats were measured on 2 different days, and arterial pressure responses to AII were recorded on 1 day and to NE on the other day. After these baseline measurements, the injection of serum (0.25 ml bid iv) collected from hypertensive (or normotensive) donor rats was begun and continued for 3 weeks. Rats injected with the serum of hypertensive donor rats will be referred to as *test* rats and the rats injected with the serum of normotensive donor rats as *control* rats. For the injection of serum, the recipient rats were restrained in a plastic box. On the day of the hemodynamic studies, the morning injection was given 90 to 120 min before the studies. Hemodynamic measurements were repeated at the end of each week of serum injections.

Cardiovascular tissue composition. At the end of the study, the rats were anesthetized with methoxyflurane and bled from the aorta. In a cold room (4°C), the thoracic aorta from the aortic valve to the diaphragm and the entire heart were removed. Defatting and measurements of the water, sodium, and potassium content of the aorta were carried out as previously described (6). The heart was dried to constant weight and was defatted. The dry, defatted heart was ground up in a mortar, mixed, and a 20- to 25-mg aliquot was taken for electrolyte measurements.

Additional studies. The blood obtained from recipient rats at the termination of the study was saved for measurements of hematocrit, plasma creatinine, and total proteins by autoanalyzer, and plasma sodium and potassium by flame photometry.

Postmortem, the kidneys were removed, cut longitudinally, and inspected for the presence of infarcts. About one out of five rats in the study had to be rejected from data analysis because of renal infarcts.

Statistical analysis. The reported values are means and standard error of the means. Systolic blood pressures, heart rates, and arterial pressure responses (peak pressure rise and its duration) to injected doses of NE and AII in test and control rats were analyzed by analysis of variance for repeated measurements on the same element (rat) (8). Tissue composition and blood chemistries in test and control rats were compared by a two-sample Student's *t* test.

Results. The weekly blood pressures and heart rates of control and test rats, injected for three weeks with the serum of normotensive or hypertensive donor rats, respectively, are shown in Fig. 1. In test rats, there was a small rise in mean blood pressure and slowing of the heart rate in the course of the study. The mean blood pressure and heart rate of

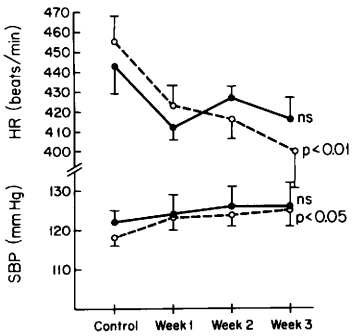

FIG. 1. Weekly systolic blood pressures and heart rates (means ± SE) in seven control (solid symbols) and eight test (open symbols) rats. Data were analyzed by analysis of variance for repeated measurements on the same elements (rats) (8). Probability values for lack of treatment effect are shown. ns, Not significant.

control and test rats, however, were not significantly different during the study.

The arterial pressure responses to NE and AII in control and test rats are shown in Figs. 2, 3, 4 and 5. During the baseline period, the magnitude and duration of the arterial pressure responses among rats was variable. The magnitude of arterial pressure responses to NE (0.30 μg/kg iv) and AII (30 ng/kg iv) among rats ranged from 16 to 48 and from 9 to 36 mm Hg, respectively. The duration of arterial pressure responses to the same doses of NE and AII ranged from 22 to 48 and from 27 to 58 sec, respectively. This variability of pressure responses among rats necessitated that the rats serve as their own control. In test rats, there was a gradual increase in the magnitude and duration of the pressure responses to NE (Figs. 2 and 4). The increase in the magnitude of pressure responses to AII in the test rats was less and more variable (Fig. 3). In some rats, after an initial increase, the pressure response to AII declined. The hyperresponsiveness of test rats to AII was manifested by doubling the duration of the pressure responses by the end of the study (Fig. 5). In control rats, the magnitude and the duration (data not shown) of the arterial pressure responses did not change (Figs. 2 and 3). In test rats, heart rates after the injection of the highest dose of NE were 418 ± 15 beats/min at the end of the baseline period and 430 ± 11, 424 ± 11,and 410 ± 26 after 1, 2, and 3 weeks of serum injections (ns). The corresponding values of heart rate after the injection of the highest dose of AII were 431 ± 13, 408 ± 12, 390 ± 11, and 414 ± 19 (ns).

At the end of the study, there were no differences in the hematocrit and plasma creatinine, sodium, potassium, or total protein concentration of control and test rats (Table I). The weight and composition of the aorta and heart in control and test rats are tabulated in Table II. Included in Table II, there are data derived from rats in which the hemodynamic studies were not completed because of aortic catheter failure, but intravenous serum injections were continued for the duration of the study. In test rats, there was increased weight and water content of the aorta, compared to controls. Expressing the weight of the aorta in terms of the body weight of rats did not abolish the difference between the two groups (data not shown). The weight and water content of the heart in the two groups was the same, but the sodium content of the heart was increased in the test rats. The numerical difference in the potassium content of the heart between the two groups did not reach statistical significance.

Discussion. Evidence for pressor and vascular sensitizing circulating humoral agents in

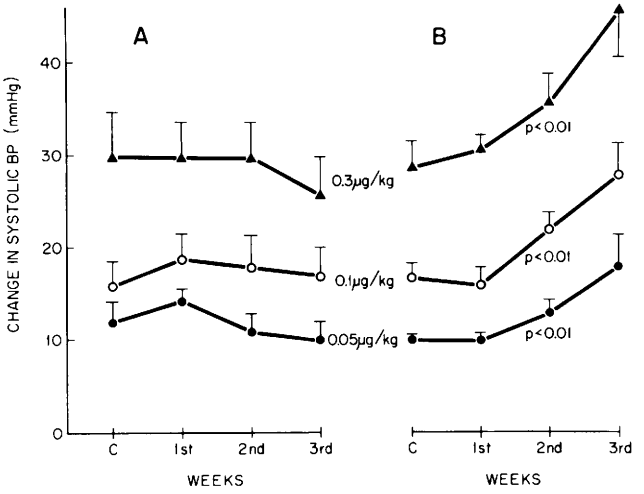

FIG. 2. Arterial pressure responses (means ± SE) to injected NE in seven control (panel A) and eight test (panel B) rats. The dose of NE is shown between the two panels. For statistical methods, see Fig. 1.

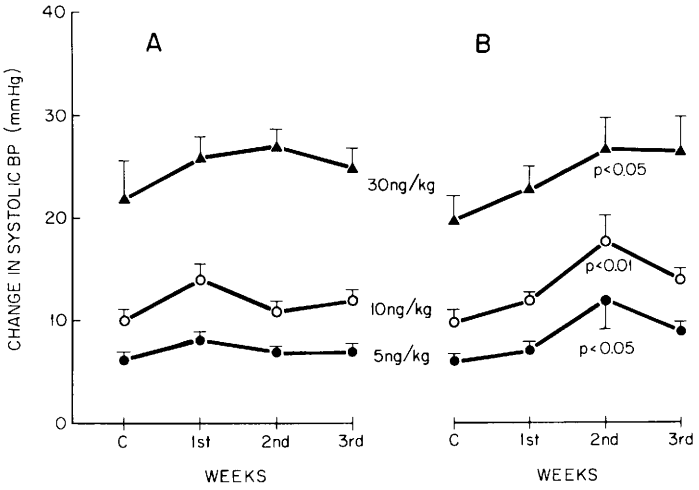

FIG. 3. Arterial pressure responses (means ± SE) to injected AII in seven control (panel A) and eight test (panel B) rats. The dose of AII is shown between the two panels. For statistical methods, see Fig. 1.

the experimental models of "volume expanded" renal hypertension has been presented by previous investigators (9–16). Michelakis and co-workers found that EDTA-treated plasma from dogs or rats with chronic one-kidney, one clip hypertension or from dogs with chronic one-kidney, one wrapped hypertension when injected into nephrectomized, pentolinium-treated rats increased blood pressure and the pressor responses to intravenous injections of NE and AII (11). The responses were slow in onset (30 min) and lasted up to 1 hr. The vascular sensitizing factor also is present in serum as was shown by Self *et al.* and Battarbee *et al.* in rats fed a high-salt diet for several months and in spontaneously hypertensive rats (12, 13). The serum factor is heat stable and stable at room temperature for up to 6 hr. More recently, Plunkett and co-workers demonstrated the presence of a vascular sensitizing and pressor substance in the plasma of saline-loaded dogs (14). The activity was found in the same chromatographic fraction of plasma that was previously shown by the same authors to contain a natriuretic-hormone-like substance (15). Evidence for pressor and vascular sensitizing humoral factors also exists in human hypertension, predominantly, in its "volume expanded" forms (9, 10). The presence of vascular sensitizing factors in human hypertension also was demonstrated *in vitro,* using an

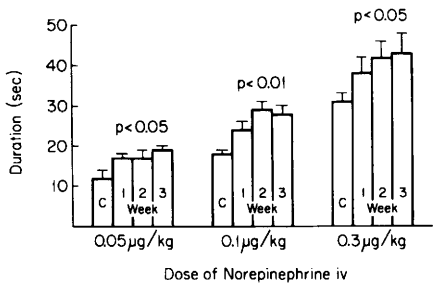

FIG. 4. The duration of pressor responses to NE in the eight test rats. For statistical methods, see Fig. 1.

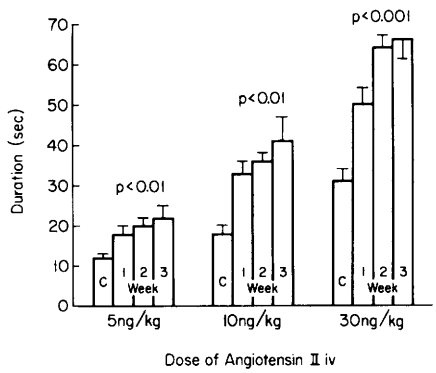

FIG. 5. The duration of pressor responses to AII in the eight test rats. For statistical methods, see Fig. 1.

TABLE I. HEMATOCRIT AND PLASMA CHEMISTRIES IN CONTROL AND TEST RATS AFTER 3 WEEKS OF SERUM INJECTIONS

Parameters	Control	*N*	Test	*N*
Hematocrit	40 ± 1	11	40 ± 1	13
Creatinine (mg/dl)	0.32 ± 0.02	9	0.34 ± 0.04	9
Sodium (mEq/l)	154 ± 2	11	154 ± 2	12
Potassium (mEq/l)	4.7 ± 0.1	11	4.7 ± 0.1	12
Total protein (gm/dl)	6.3 ± 0.1	11	6.2 ± 0.1	11

Note. Values are means ± SE.

isolated femoral artery of the rabbit as the assay organ (16).

In the present study, we investigated the long-term effects of passive transfer of serum from rats with "volume-expanded," non-renin-dependent renal hypertension to intact syngeneic normotensive rats. The passive transfer of serum from hypertensive to normotensive rats has led to the gradual development of increased arterial pressure responses to NE and AII. The gradual onset of pressor hyperresponsiveness suggests a cumulative effect of passive transfer of serum. To demonstrate the presence of vascular sensitizing serum factors in the present study, total nephrectomy and pharmacologic sympathectomy of recipient rats were not required, unlike in previous studies (9–13). The presence of intact kidneys in the recipient rats might have delayed the onset of pressor hyperresponsiveness but did not prevent it.

There were qualitative and quantitative differences in the pressor hyperresponsiveness to NE and AII in test rats. The pressor hyperresponsiveness to NE was manifested by an increase in the magnitude of the pressure rise with only minor changes in its duration. The pressor hyperresponsiveness to AII was characterized by prolongation of the pressure rise with minor changes in its magnitude. These differences were not explained by heart rate changes during injections of the two agonists, although there was a tendency for slowing of the heart rate during the injections of AII.

The transfer of serum from hypertensive to normotensive recipient rats did not produce hypertension. The increase in the mean blood pressure of rats injected with the serum of hypertensive rats amounted to only a few millimeters of mercury. The slowing of the heart rate in these rats is unexplained. The transfer of serum in larger doses and for periods longer than 3 weeks may be needed to produce hypertension. Previous investigators also failed to show a reproducible hypertensinogenic effect of vascular sensitizing factors in experimental hypertension (12, 13). In this regard, our rat model resembles human borderline hypertension and prehypertension (in normotensive relatives of hypertensive subjects), which are also characterized by increased pressor or vasoconstrictor responses to NE and AII in the absence of sustained elevation of blood pressure (17, 18).

TABLE II. CARDIOVASCULAR TISSUE COMPOSITION IN CONTROL AND TEST RATS

	Aorta				Heart			
Parameters	Control	*N*	Test	*N*	Control	*N*	Test	*N*
Water (kg/kg DDW)	2.19 ± 0.01	12	2.28 ± 0.01[a]	11	3.27 ± 0.01	11	3.27 ± 0.00	12
DDW (mg)	11.5 ± 0.2	13	12.7 ± 0.4[a]	13	138 ± 4	13	137 ± 4	13
Sodium (mEq/kg DDW)	306 ± 5	13	312 ± 6	13	175 ± 3	13	191 ± 3[b]	13
Potassium (mEq/kg DDW)	130 ± 2	13	129 ± 2	12	311 ± 6	12	327 ± 5	13

Note. Values are means ± SE. DDW, dry, defatted weight.
[a] $P < 0.05$, for comparison of values in control and test rats by a two-sample Student's t test.
[b] $P < 0.01$, for comparison of values in control and test rats by a two-sample Student's t test.

From the present study, the mechanisms responsible for the exaggerated arterial pressure responses cannot be determined. Chronic volume load is an unlikely contributing factor because pressor hyperresponsiveness did not occur in rats injected with the serum of normotensive rats. Also, pressor hyperresponsiveness in test rats did not appear until the end of the second week of serum injections. The contribution of cardiac output to pressor hyperresponsiveness is unlikely because heart rates did not change, but it cannot be ruled out. The absence of changes in heart rates is also evidence against a change in baroreceptor sensitivity as a contributing factor to pressor hyperresponsiveness. Based on our findings and those of previous investigators, the pressor hyperresponsiveness of rats injected with the serum of hypertensive rats appears to be due to vascular constriction.

Evidence for vascular constriction was provided from analysis of the composition of cardiovascular tissue in the recipient rats. A small increase in the weight and water content of the aorta was found in rats treated for 3 weeks with the serum of hypertensive rats. The increased weight of the aorta is unlikely to be secondary to the small increase in the arterial pressure of these rats and remains unexplained. The "waterlogging" of the aorta may reflect a subtle change in the sodium content of the aorta. Increased sodium content of the myocardium in rats injected with serum of hypertensive rats was demonstrated. Assuming that some of the excess sodium is intracellular, an increased sodium content of the myocardium may be easier to demonstrate than that of the aorta. In the myocardium, the relative amount of intracellular sodium is greater, therefore, a change in this compartment of tissue sodium is more easily detectable.

This is the fourth line of evidence that we have presented that indicates that circulating humoral factors in renal-hypertensive animals may play a role in the accumulation of excess sodium in cardiovascular tissue. In renal-hypertensive dogs and rats, we found increased sodium content of veins (6, 19). Because the veins are not exposed to the increased luminal pressure of arterial hypertension, the accumulation of excess sodium in veins appeared to be the result of humoral or neural stimuli. In parabiotic rats, one renal-hypertensive and one unoperated, we found increased sodium content of the vena cava in both (3). These experiments have shown that circulating humoral factors do play a direct or indirect role in the pathogenesis of increased sodium content of veins. Direct evidence for the role of humoral factors was provided from tissue culture experiments (4, 5). Rabbit aorta explants cultured in the serum of dogs with one- or two-kidney, one wrapped hypertension accumulated more sodium than explants cultured in serum obtained from the same dogs prior to the induction of hypertension.

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