Humoral Factors Trophic for Vascular Smooth Muscle During the Development of Hypertension in Rats (43874)

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Abstract. **There is evidence that a humoral factor or factors in rats with one-kidney, one-clip (1K1C) hypertension increase growth of cultured vascular smooth muscle cells. Such humoral trophic factors may contribute to the abnormal growth of arterial muscle in hypertension. To further study the longitudinal expression of this trophic factor or factors, we prepared rats with 1K1C hypertension of different durations. To determine if the factor or factors are also expressed in other forms of experimental hypertension, we additionally prepared rats with two-kidney, one-clip (2K1C) hypertension and paired two-kidney (2K) normotensive controls; we also studied Spontaneously Hypertensive Rats (SHR) plus appropriate controls. In the presence of growth stimulated by background levels (1%) of fetal calf serum, 20% platelet-poor, plasma-derived serum (PDS) from 1K1C rats 8–14 days (n = 10) and 28 days (n = 12) after clipping increased [^{3}H]-thymidine incorporation of growth-arrested cultured rat aortic smooth muscle cells more than the paired 1K PDS, by up to +67% and +40%, respectively ($P < 0.01$). However, with PDS from 1K1C rats 4 days (n = 11) and 38 days (n = 6) after clipping there was no evidence for a differential effect ($P > 0.5$ and $P > 0.1$, respectively). PDS from seven 2K1C rats (at 9 days) also increased [^{3}H]-thymidine incorporation of the assay cells more than PDS from the paired 2K rats, by up to +19% ($P < 0.05$). However, there was no evidence that PDS from SHR differentially increased cellular thymidine incorporation. Thus, evidence from this study suggests that the humoral factor or factors trophic for vascular smooth muscle are expressed in both low- and high-renin forms of experimental renovascular hypertension, but not in the very early or in the late complicated stages of the hypertension, or in genetic hypertension in rats.** [P.S.E.B.M. 1995, Vol 209]

We have provided evidence (1, 2) to support the hypothesis that a humoral factor or factors may contribute to the abnormal growth of smooth muscle of arteries and arterioles accompanying one-kidney, one-clip hypertension in rats, and thus to the decreased arterial compliance and the elevated vascular resistance. We detect the factor or factors involved in the <10,000 mol wt fraction of platelet-poor, plasma-derived serum (PDS) from hypertensive rats. The factors resist freezing, proteolysis, and charcoal absorption, but not boiling, and their levels are unresponsive to altered dietary sodium chloride, suggesting that circulating angiotensin II, catecholamines, and vasopressin are not involved.

The purposes of the present study were 2-fold: (i) to study the longitudinal expression of the trophic factor or factors; and (ii) to determine whether the trophic factor or factors are present in other forms of experimental hypertension in rats. Expression of the factor or factors in the earliest stages of the development of the hypertension would suggest their involvement in the initiation of the abnormal vascular growth. In contrast, increasing concentrations of the factors in the late, complicated stages of the hypertension would suggest that their production is secondary to the complications of the hypertension. Similar expression of the factors in high- and low-renin forms of experimen-

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tal renal hypertension would provide additional evidence that angiotensin is not involved. Expression in Spontaneously Hypertensive Rats would allow extension of the hypothesis to genetic forms of hypertension.

Materials and Methods

Materials. [^{3}H]-thymidine was obtained from ICN Biomedicals, Inc. (Costa Mesa, CA); Dulbecco's Modified Eagle's Medium (DMEM/F-12), Hanks' Balanced Salt Solution (HBSS), HEPES, and trypsin from Gibco Laboratories (Grand Island, NY); thromboplastin reagent (SIMPLASTIN) from Organon Teknika (Durham, NC); collagenase type I, and donor-defined fetal calf serum (FCS) from HyClone Laboratories, Inc. (Logan, UT); and multichamber cell culture plates from Corning Glass Works (Corning, NY). Male Sprague-Dawley rats were obtained from Zivic-Miller Laboratories (Zelienople, PA). Male Spontaneously Hypertensive Rats (SHR), Wistar Kyoto rats (WKY), and unrelated Wistar rats (UW) were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). All other supplies were the best grade commercially available.

Vascular Smooth Muscle Cell Cultures. We grew vascular smooth muscle cells (VSMC) from enzyme-digested thoracic aortas of Sprague-Dawley rats (100–150 g), by modifying our standardized methods (3). Briefly, excised aortas were placed in DMEM/F-12, cleansed of fat and blood, and then gently everted and tied with sutures at both ends. After washing them in DMEM/F-12, we placed the aortas in collagenase (1 mg/ml DMEM/F-12), incubated them at 37°C for 20 min, pipetted the mixture several times, and rinsed with DMEM/F-12. The fluid containing residual endothelial cells was discarded. The aortas were incubated in fresh enzyme solution for 20–30 min, pipetted several times, and the fluid containing cells was saved and added to medium containing 10% FCS. We repeated this process two to three times. The harvested cells were centrifuged, resuspended in DMEM/F-12 plus 10% FCS. Routinely, we obtained $>10^6$ cells per aorta, and >90% of the cells were viable (Trypan Blue exclusion). Cells were seeded in DMEM/F-12 plus 10% FCS at $1 \times 10^4/cm^2$ and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. We passaged confluent cells every 7 days and changed the medium every other day. Purity of passaged cultures was ascertained, as we have previously reported (4), by characteristic morphology (hill-and-valley) and by labeling with monoclonal antibodies to smooth muscle-specific α-actin and myosin heavy chain (>99% of cells positive). Subconfluent cells ($1–1.5 \times 10^4/cm^2$) at third passage were used in all assays.

Production of Hypertension. We prepared one-kidney, one-clip (1K1C) hypertensive, and paired one-kidney (1K) normotensive control rats as we have previously reported (1). Briefly, under ether anesthesia, male Sprague-Dawley rats, body weights 150–190 g, age 5 weeks, were unilaterally nephrectomized. Two to three weeks later, at body weights of 320–340 g, the contralateral (left) renal artery was partially constricted with a 0.44 mm i.d. silver clip. Normotensive unilaterally nephrectomized control rats, paired by body weight with the hypertensives, were sham clipped. In some 1K rats, a clip was left next to, but not occluding, the renal artery in the abdomen. For the preparation of two-kidney, one-clip (2K1C) hypertensive and paired two-kidney (2K) normotensive control rats, we partially constricted the left renal arteries of male Sprague-Dawley rats, body weights 300–326 g, under ether anesthesia, with 0.44 mm i.d. silver clips. Normotensive control rats (2K), also paired by body weight with the hypertensives, were sham clipped; these rats were not unilaterally nephrectomized. Body weights and blood pressures (conscious; Natsume Rat Tail Manometer System, model KN-009, Natsume, Tokyo, Japan) were measured in the rats five days a week. Male SHR, WKY, and unrelated Wistar rats were obtained one week before sacrifice (ages 5 weeks and 14 weeks) and blood pressures and body weights measured on 3 days. All rats were given food and water *ad libitum*. Rats ate standard rat chow (Agway Prolab R-M-H3000; Agway Inc., Syracuse, NY; 0.44% Na, 0.95% K, 0.97% Ca, 0.21% Mg). Procedures followed with these animals were in accordance with institutional guidelines.

At sacrifice all rats were either gaining weight or of stable weight and appeared healthy. We performed necropsies to verify general health, clip placement, and condition of heart, kidney(s), and lungs.

Platelet-Poor, Plasma-Derived Serum. We obtained platelet-poor, plasma-derived serum (PDS) from the paired 1K1C and 1K rats at 4, 8–14, 28, or 38 days after clipping or sham clipping. We obtained PDS from the paired 2K1C and 2K rats 9 days after clipping or sham clipping; all 2K1C rats had been hypertensive (systolic blood pressure >135 mm Hg) for at least 2 days. We obtained fresh PDS from the SHR, WKY, and UW rats at ages 6 weeks and 15 weeks. The overnight-fasted hypertensive and normotensive control rats, under light ether anesthesia, were bled by abdominal aortic puncture (laparotomy) into citrated plastic syringes (50 μl 0.2 *M* Tris-citrate/ml blood). The blood was iced immediately and all subsequent manipulations (except as stated below) were done in plasticware at 4°C. After an aliquot was taken for determination of hematocrit, the blood was centrifuged for 18 min at 850*g* and the supernatant recentrifuged at 26,800*g* for 30 min. To the resulting platelet-poor plasma in glass tubes we added 5 μl/ml of 1.0 *M* $CaCl_2$ plus 33 μl/ml thromboplastin reagent and allowed the

mixture, heated at 56°C (to inactivate complement), to clot for 1 hr. Then, the clot was broken up to obtain serum (PDS). We have verified that levels of platelet-derived growth factor are undetectable in our PDS (1). Fresh PDS was assayed within 7 hr of drawing blood. Frozen PDS (−40°C) was assayed up to 4 months after sacrifice; our previous studies indicate no detectable loss of activity with freezing up to 9 months. For all assays, the sample source was unknown to the person making measurements. In all rats, except 6-week-old SHR, WKY, and UW, plasma Na^+, K^+, and creatinine concentrations were measured by flame emission and Sigma kit 555. In six pairs of 1K1C-1K rats concentrations of calcium and magnesium in PDS were measured by colorimetric methods (Roche Biomedical Laboratories, Inc.).

[^{3}H]-Thymidine Incorporation. Confluent VSMCs, third passage, were harvested by adding 0.25% trypsin-EDTA (ethylenedinitrilo tetraacetic acid) in calcium-magnesium-free HBSS and plated in 24-well dishes at 1×10^4 cells/cm^2 in DMEM/F-12 containing 10% FCS. After 3 days' growth the medium was replaced with DMEM/F-12 containing 0.4% FCS to arrest growth. Forty-eight hours later, the medium was replaced by medium with 0.4%, 1%, or 10% FCS (controls), or by medium with 20% PDS plus 1% FCS, plus 1 μCi/ml [^{3}H]-thymidine, with total well volume 0.5 ml and three to four replicate wells for each treatment. For sterility, all PDS was filtered (0.22 μm Costar Filter; Costar Corporation, Cambridge, MA) before being added to cells. Sixteen hours later we harvested the cells and measured [^{3}H] incorporation into acid insoluble material by standard techniques (5). For data analysis, we used the calculated average incorporation into the three to four replicate wells for each treatment.

Each independent experiment involved PDS from five to seven pairs of renal hypertensive and normotensive control rats, or from four to six SHR, six WKY, and six UW rats. Additionally, to detect false negative assays each experiment included positive controls: frozen PDS from previous positive experiments.

Statistics. The purpose of the paired experimental design was to reduce experimental variation among independent experiments; this variation was attributable to differences in the growth characteristics of the assay cells used. (Coefficient of variation in the growth response to 10% FCS of the different batches of assay cells we used was about 55%). In order to pool data from these independent experiments, we expressed values as fold increases in thymidine incorporation over baseline levels evoked by 0.4% FCS (control). We used two-way analysis of variance (Number Cruncher Statistical System; Kaysville, UT) to compare effects of paired hypertensive and normotensive PDS on [^{3}H]-thymidine incorporation. We used one-way analysis of variance to compare effects of PDS from SHR, WKY, and UW rats on incorporation and to compare body weights, heart (ventricular) weights, blood pressures, and plasma creatinine and electrolyte concentrations among hypertensive and normotensive control rats. Significant differences between treatments were determined by Fisher's Least Significant Difference Test (6). We rejected the null hypothesis at $P \leq 0.05$.

Results

General. Table I lists systolic blood pressures averaged over the duration of normotension or hypertension (defined as systolic pressure >135 mm Hg), mean final body weights, heart (ventricular) weights, and serum creatinine concentrations in 1K1C rats with early (4 days after clipping), established (8–14, and 28 days), or late (38 days) hypertension and corresponding control 1K rats. The table also presents similar data in the seven pairs of 2K1C-2K rats. As we have previously reported (1), hematocrit and plasma sodium and potassium concentrations were within normal limits and did not significantly differ among groups. Calcium and magnesium concentrations in PDS from six pairs (8–14 days) were also measured (Roche Biomedical Laboratories, Inc.). Mean ± SEM calcium (mg/dl) was 28.3 ± 0.9 in 1K1C PDS, and 27.2 ± 0.7 in 1K PDS. Mean ± SEM magnesium (mEq/liter) was 1.37 ± 0.05 in 1K1C PDS and 1.28 ± 0.05 in 1K PDS. These values did not significantly differ between the groups ($P > 0.1$). Compared with the appropriate normotensive control rats, all groups of hypertensive rats (1K1C at each stage, and 2K1C) had increased blood pressures and ventricular weights ($P < 0.05$), but body weights and serum creatinine concentrations did not significantly differ. We observed ventricular hypertrophy and hypertension in the 1K1C rats as early as 2 to 4 days after clipping, and blood pressures and relative ventricular weights reached plateaus 28 days following clipping. At 38 days following clipping there were trends, not yet statistically significant, for decreases in body weights and increases in serum creatinine concentrations in the 1K1C rats; our previous experience indicates rapidly increasing morbidity and mortality in these rats thereafter.

Table II presents similar data on blood pressures and weights in the SHR, WKY, and UW rats. In SHR, at both 6 weeks and 15 weeks of age, blood pressures and relative ventricular weights were increased over values in both control normotensive strains. Body weights were lower than those in the UW rats.

Effects of PDS on [^{3}H]-Thymidine Incorporation by Cultured VSMCs. Figure 1 represents [^{3}H]-thymidine incorporation by VSMCs treated with frozen 20% PDS from the seven 2K1C hypertensive and paired 2K normotensive rats, in 1% FCS, and includes

Table I. Blood Pressures and Weights of Renal Hypertensive Rats

	n	BP (mm Hg)	BW (g)	HW/BW (mg/g)	CREAT (mg/dl)
4 Days					
1K1C	11	144.4 ± 2.3[a]	320.6 ± 6.0[a]	3.09 ± 0.07[a]	0.72 ± 0.02[a,c]
1K	11	127.5 ± 1.1[b]	325.4 ± 5.6[a]	2.73 ± 0.04[b]	0.72 ± 0.02[a,c]
8–14 Days					
1K1C	10	159.6 ± 4.1[c]	386.5 ± 14.2[b,d]	3.37 ± 0.07[c]	0.67 ± 0.05[a]
1K	10	128.6 ± 0.7[b]	371.3 ± 17.1[b]	2.68 ± 0.07[b]	0.70 ± 0.06[a,d]
28 Days					
1K1C	12	177.1 ± 2.2[d]	418.5 ± 15.6[c,d,e]	3.98 ± 0.09[d]	0.80 ± 0.02[b,d]
1K	12	130.2 ± 0.4[b]	446.3 ± 11.2[c,e]	2.57 ± 0.05[b]	0.81 ± 0.02[b,c]
38 Days					
1K1C	6	173.5 ± 1.8[d]	434.7 ± 14.3[e]	3.78 ± 0.15[d]	0.61 ± 0.04[a,e]
1K	6	127.8 ± 0.4[b]	443.3 ± 14.4[e]	2.58 ± 0.08[b]	0.55 ± 0.05[e]
2K1C	7	143.3 ± 2.3[f]	343.0 ± 3.8	3.13 ± 0.06[f]	0.58 ± 0.03
2K	7	126.6 ± 0.8	342.0 ± 4.1	2.81 ± 0.05	0.60 ± 0.06

Note. Means ± SEM. 1K and 1K1C = one-kidney normotensive control rats and paired one-kidney, one-clip hypertensive rats, respectively; 2K and 2K1C = two kidney normotensive control rats and paired two-kidney, one-clip hypertensive rats, respectively; BP = tail systolic blood pressure; BW = body weight; HW = heart (ventricular) weight; CREAT = serum creatinine concentration. For 1K1C-1K rats, values within each column sharing superscript letters ([a,b,c,d, and e]) are not significantly different ($P > 0.05$).
[f] A significant difference ($P < 0.01$) in comparison of 2K1C-2K rats.

Table II. Blood Pressures, Weights, and Thymidine Incorporations of Genetically Hypertensive Rats

	n	BP (mm Hg)	BW (g)	HW/BW (mg/g)	^{3}H-T INCORP
Age 6 Weeks					
SHR	4	142.8 ± 1.6	96.8 ± 1.5	4.16 ± 0.01	2.73 ± 0.06
WKY	6	118.0 ± 2.2[a]	104.3 ± 4.7	3.63 ± 0.16[a,b]	2.45 ± 0.04[a]
UW	6	125.7 ± 1.6[a]	180.8 ± 4.6[a]	3.30 ± 0.07[a]	2.85 ± 0.05
Age 15 Weeks					
SHR	6	189.7 ± 4.8	285.7 ± 5.4	3.56 ± 0.17	1.83 ± 0.03
WKY	6	131.0 ± 1.2[a]	292.2 ± 3.8	2.86 ± 0.08[a]	2.04 ± 0.07
UW	6	131.5 ± 0.9[a]	411.3 ± 5.8[a]	2.45 ± 0.07[a]	2.13 ± 0.14

Note. Means ± SEM. ^{3}H-T INCORP = PDS-evoked [^{3}H]-thymidine incorporation into assay VSMC (fold increase over baseline incorporation in 0.4% FCS); SHR = Spontaneously Hypertensive Rats; WKY = Wistar Kyoto rats; UW = unrelated Wistar rats; BP = tail systolic blood pressure; BW = body weight; HW = heart (ventricular) weight.
[a] A significant difference from SHR ($P < 0.05$) within each age group.
[b] $n = 5$.

control thymidine incorporations in 0.4%, 1%, and 10% FCS, no PDS. Data are expressed as [^{3}H] incorporation, CPM/well. From cells growth arrested in low concentrations (0.4%) of FCS, thymidine incorporation was stimulated more (+19%, $P < 0.05$) in VSMCs exposed to the 2K1C PDS than in cells exposed to the paired 2K PDS. Standard errors are included on bars representing responses to 2K1C and 2K PDS to demonstrate that incorporation in response to PDS plus 1% FCS was greater ($P < 0.01$) than control incorporation in response to the 1% FCS alone.

Figure 2 presents [^{3}H]-thymidine incorporation by VSMCs treated with 20% PDS from pairs of 1K1C and 1K rats at different durations of hypertension. Because the data were pooled from independent experiments, they are expressed as fold increases in thymidine incorporation over baseline levels evoked by 0.4% FCS. Control incorporations to 1% and 10% FCS were again measured and were similar to those presented in Figure 1 (data not shown). Differences in thymidine incorporations among the independent ex-

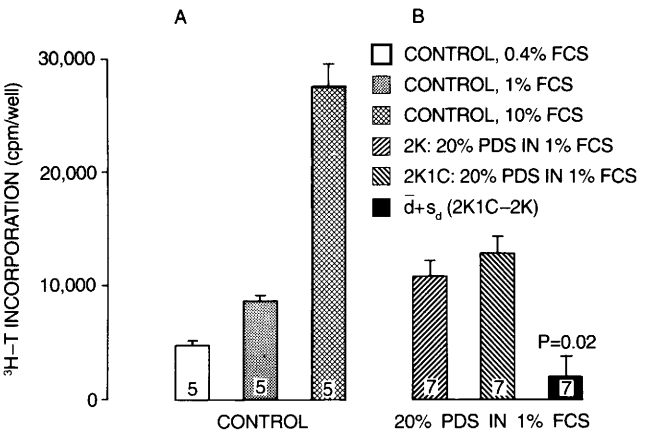

Figure 1. Effect of plasma-derived serum (PDS) from 7 two-kidney, one-clip (2K1C) and paired two-kidney control (2K) rats (means + SEM; number of observations at base of bars) on [^{3}H]-thymidine incorporation by cultured rat aortic smooth muscle cells (VSMCs). Results expressed as [^{3}H], CPM/well. (A) Control incorporations in 0.4%, 1%, and 10% FCS. (B) Incorporation evoked by 20% PDS, in 1% FCS. Significance by randomized complete block design (analysis of variance) of mean differences between pairs (2K1C-2K) represented in the solid bars.

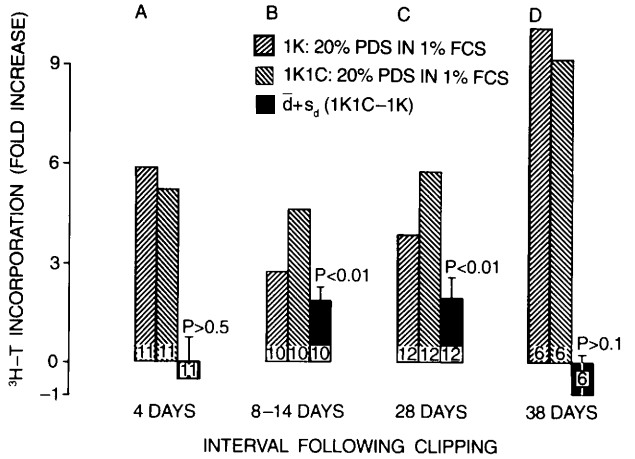

Figure 2. Effect of PDS from one-kidney, one-clip (1K1C) and paired one-kidney control (1K) rats at different intervals following clipping on [^{3}H]-thymidine incorporation by cultured VSMCs. Results expressed as fold increases in incorporation over levels in the same cells exposed to 0.4% FCS. Otherwise, as in Figure 1. (A) Incorporation evoked by 20% PDS, in 1% FCS, from rats 4 days after clipping. (B) Same, 8–14 days after clipping. (C) Same, 28 days after clipping. (D) Same, 38 days after clipping. Significance by randomized complete block design (analysis of variance) of mean difference between pairs (1K1C-1K) represented in the solid bars.

periments are attributable to variation in growth characteristics of the cultured cells used for assay. At each hypertension duration studied (exception, 38 days) results of two independent experiments each of five to six pairs were pooled for Figure 2; the results of each of these experiments individually analyzed were similar to the pooled results. As we previously reported (1), thymidine incorporation in assay VSMCs was stimulated significantly more (+67%, $P < 0.01$) by (frozen) PDS from 1K1C rats 8–14 days after clipping, than by PDS from the paired 1K rats. Similar results (+40%, $P < 0.01$) were observed in cells exposed to (frozen) PDS from rats 28 days after clipping. In contrast, (fresh) PDS from 1K1C rats 4 days, or (frozen) PDS 38 days, after clipping had no differential effects on thymidine incorporation by VSMCs ($P > 0.5$ and 0.1, respectively). Yet, in each experiment involving PDS from rats 4 or 38 days after clipping, the accompanying positive control experiments indicated a valid assay system (data not shown).

Table II presents similar data on [^{3}H]-thymidine incorporation by VSMCs treated with 20% PDS (fresh) from the SHR, WKY, and UW rats, ages 6 weeks and 15 weeks (two independent experiments). Control uptakes in 0.4%, 1%, and 10% FCS were similar to those presented in Figure 1. There was no convincing evidence in either age group that PDS from SHR rats differentially increased [^{3}H]-thymidine incorporation of VSMCs. The accompanying positive control experiments with 1K1C-1K PDS again indicated a valid assay system (data not shown).

Discussion

Considerable evidence now indicates that the abnormal vascular structure accompanying hypertension is not solely attributable to pressure-work-dependent growth (1). Neurogenic, autocrine-paracrine, blood flow-related, and humoral mechanisms have been proposed. Our laboratory has been especially interested in testing the hypothesis that humoral factors are involved.

The present study provides additional evidence that platelet-poor, plasma-derived serum from rats with established, uncomplicated one-kidney, one-clip hypertension, 8–28 days after clipping has a differentially increased effect on growth of cultured rat aortic smooth muscle cells. This observation again suggests the presence in this form and stage of hypertension of a humoral factor or factors trophic to vascular smooth muscle. However, data from the present study provides new evidence that this factor or factors may not be present in the very early developmental or late complicated stages of the hypertension (4 days or >5 weeks after clipping, respectively). Additionally, this study suggests that the trophic humoral factor or factors are present at comparable levels in one-kidney, one-clip, and two-kidney, one-clip forms of renovascular hypertension in rats, but not in Spontaneously Hypertensive Rats (SHR).

Our previous studies (1, 2) suggested the presence of a humoral factor or factors in PDS from 1K1C hypertensive rats that, in the presence of growth evoked by background levels of FCS, differentially stimulate the growth of arteriolar and aortic smooth muscle cells. The factor or factors involved are detected in the <10,000 mol wt fraction of PDS, resist freezing, proteolytic enzyme digestion, and charcoal absorption, but do not resist boiling for 10 mins. Factor levels are unresponsive to altered dietary sodium chloride.

The present study provides new evidence that the factor or factors also exist, in similar levels, in rats with established two-kidney, one-clip hypertension. However, it should be noted that these 1K1C and 2K1C experiments are not strictly comparable, because we clipped the rats at different body weights so that hypertension would develop at approximately the same interval (2 days) after clipping. Nevertheless, it seems apparent that levels of humoral factor(s) in 2K1C rats do not exceed those in the 1K1C rats. Early two-kidney, one-clip hypertension is considered a high-renin form of hypertension that may be ablated with angiotensin II blockers (7); in contrast established uncomplicated 1K1C hypertension is considered a volume-dependent, low-renin model (7). Thus, the present study provides additional evidence that the renin-angiotensin system is probably not involved in the

humoral trophic effects we have observed, and suggests that there may be a common humoral trophic factor or factors in both types of experimental renovascular hypertension.

Although the trophic factor or factors appear to be present in experimental renovascular hypertension in rats, we find no evidence for the existence of these humoral factors in the SHR strain of genetically hypertensive rats, at least at age 6 weeks when the blood pressure is rising, and at age 15 weeks when the hypertension is established. Thus, the cardiovacular hypertrophy in SHR, which is present at both ages, is apparently not attributable to the humoral trophic factor(s) that we find in rats with experimental renovascular hypertension. However, it remains possible that in SHR the factor is expressed at an earlier age, before the onset of the hypertrophy.

This seems unlikely because our studies of the development of 1K1C hypertension provide new evidence that the humoral trophic factor or factors present in the established phases may not exist in the very early (4 days) or in the late, complicated (>5 weeks) stages of this form of hypertension. At these stages, there were not even trends for greater cellular thymidine incorporation in response to 1K1C PDS. Four days after clipping, blood pressures and heart weights of the 1K1C rats as a group were significantly elevated in this study. However, there were individual differences, but we found no correlation in individual rats between level of blood pressure or heart weight and the trophic response to PDS. Thus, it appears unlikely to us that the humoral trophic factor or factors we are investigating play a role in initiating the hypertension or cardiovascular hypertrophy. In a previous experiment with a similar model of renovascular hypertension (abdominal aortic coarctation), we found that arterial hypertrophy paralleled in time that of the heart (8).

Although the humoral factor or factors apparently do not initiate the hypertrophy, their appearance at around 8 days suggests that they may serve to modulate the degree of hypertrophy that eventually develops in renovascular hypertension.

On the other hand, it is unlikely that release of the humoral factor or factors represent a complication of hypertension, because they are not present in SHR. Furthermore in 1K1C rats 5–6 weeks after clipping, when blood pressures and cardiac hypertrophy are maximal and when there are trends for falling body weights and rising serum creatinines suggesting the development of complications, the factor or factors are no longer detectable. These results also suggest that the humoral trophic factor or factors disappear in renovascular hypertension when cardiovascular restructuring is completed, as we would have expected.

We are presently investigating various stages of human forms of hypertension, renovascular and genetic, for evidence of the presence of these humoral trophic factors.

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