Angiopathic Serum Factor in Perinephritic Hypertensive Dogs

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SUMMARY To investigate the role of circulating humoral substances in the pathogenesis of increased vascular wall water and sodium concentration in experimental hypertension, rabbit aortic media explants were cultured in tissue culture medium supplemented (10–20%) with serum obtained from the same dogs (n = 7): 1) before the induction of hypertension; 2) after wrapping one kidney in silk (two-kidney perinephritic hypertension, 2-KPHT); and 3) after contralateral nephrectomy (1-KPHT). Cultures also were run with serum of sham-wrapped and then unilaterally nephrectomized normotensive dogs (n = 4). After 3 weeks of culture, the explants were harvested, and their water, sodium and potassium concentration was measured. Compared to the composition of explants cultured in prehypertensive serum, the water concentration of explants cultured in 1-KPHT and the sodium concentration of explants cultured in 2-KPHT and 1-KPHT serum were increased (p < 0.05). The water and electrolyte content of explants cultured in sera of sham-operated normotensive control dogs was the same regardless of the type of serum used, pre- or post-sham surgery or post-nephrectomy. The effects of serum from hypertensive dogs were not explained by variations in serum creatinine, sodium and potassium levels or in plasma renin activities. The experiments provide evidence for the role of serum factor(s) in the pathogenesis of abnormal vascular wall water and sodium concentration in experimental hypertension. (Hypertension 1: 197-201, 1979)

KEY WORDS • perinephritic hypertension • serum factor • vascular wall sodium

INCREASED arterial wall water and sodium concentration in experimental and human hypertension has been known for many years and has been generally ascribed to the effects of increased intraluminal pressure. Recent reports of similar changes in the veins of experimental animals with hypertension raised the possibility that humoral or neural stimuli may also play a role in the pathogenesis of abnormal vascular wall composition in hypertension. Evidence for the role of humoral factors was found in parabiotic rats, one of which was made hypertensive by partial constriction of one renal artery. There were similar increases in the water and electrolyte content of the vena cavae of hypertensive rats and their normotensive parabiotic mates. From these experiments it could not be determined whether the unknown humoral factor, originating from the hypertensive rat, affected venous wall metabolism directly or whether its action was mediated through other mechanisms. The purpose of the present experiments was to investigate the direct effect of serum from hypertensive dogs on the water and electrolyte composition of rabbit aortic media in culture.

Methods

Preparation of Dogs

Male mongrel dogs weighing 18–27 kg were trained to lie quietly during percutaneous femoral artery puncture for blood pressure measurements. A resting mean arterial pressure of less than 130 mm Hg was documented in each dog on at least one occasion. In seven dogs anesthetized with pentobarbital (30 mg/kg I.V.), one kidney was wrapped in silk to induce perinephritic hypertension. Two weeks later, the contralateral kidney was removed surgically. Four dogs underwent sham-wrapping of one kidney and then contralateral nephrectomy 2 weeks later. The operations were performed as described in a previous publication. Antibiotics were not used either pre- or postoperatively. Blood pressure measurements were repeated before nephrectomy and again 4 weeks after nephrectomy. During the entire study period the dogs were maintained on a diet of standard dog chow and water ad libitum.
Collection of Serum

Fifty ml of blood was collected from each dog, fasted for 12 hours, in the morning, once or twice weekly, during a 4-week control period, for 2 weeks after wrapping one kidney in silk or sham surgery, and for 4 weeks after nephrectomy. The blood was allowed to clot at room temperature in a sterile, capped 50-ml glass centrifuge tube. The serum was separated following centrifugation and stored at \(-20^\circ\text{C}\) until used. The serum, filtered through a 0.45 \(\mu\)L sterile disposable filter, was used to supplement the tissue culture medium for the tissue culture experiments.

Rabbit Aortic Media Explants in Culture

Male New Zealand white rabbits (3.5-4.5 kg) were killed with an overdose of pentobarbital L.V. Using sterile techniques, the thoracic aorta of the rabbits was removed and placed in M199 (GIBCO) tissue-culture medium containing penicillin (100 \(\mu\)/ml) and streptomycin (100 \(\mu\)g/ml) at 37°C. Rabbit aortic media explants were prepared for culture according to the methods of Jarmolych et al.\(^7\) Under a sterile hood, the adventitia and the outer media of the aorta were stripped and discarded. The intima and the inner media were cut into 60 to 80.5 \(\times\) 5-mm segments. Fifteen to 20 tissue fragments were placed into each of six sterile 60-mm Falcon plastic petri dishes and covered with 2 ml of fresh M199 medium. The medium was supplemented with dog serum (see below) to a final concentration of 10, 15 or 20% serum and contained penicillin (100 \(\mu\)/ml), streptomycin (100 \(\mu\)g/ml) and L-glutamine (1 ml/100 ml of a 200 mM solution). The dishes were covered and placed in a tissue culture incubator at 37°C in an atmosphere of 95% air and 5% CO\(_2\). During the first week the cultures were left undisturbed. Thereafter, culture medium was changed three times a week for 2 weeks. At the end of each week, the explants were examined with an inverted microscope for evidence of new growth.

After 3 weeks of cultivation, the explants were removed from the culture medium in a cold room (4°C), wiped once with filter paper, placed in a preweighed glass stoppered weighing jar, and weighed to the nearest 0.1 mg. The explants were oven dried at 104°C for 24 hours and reweighed. The difference between the two weights was considered water content, which was expressed as a percentage of wet weight. For measurements of ionic content, 2 ml of 0.1 N nitric acid were added to each weighing jar, and the explants were digested for 7 days. Digested specimens were placed in an oven at 104°C to evaporate the nitric acid. Tissue contents of sodium and potassium were measured by flame photometry (Instruments Laboratories). Ion contents were expressed as mEq/kg of tissue, dry weight.

The water and ion content of seven freshly removed rabbit thoracic aortas was also measured. The aortas were placed in a dry chilled plastic tube immediately after removal. The adventitia and the outer media were stripped in a cold room. The remaining endothelium and inner media were cut into small pieces and placed into glass weighing jars for measurements of water, sodium and potassium.

Experimental Protocol

Three sets of duplicate cultures of the same rabbit aorta were run with tissue culture medium supplemented with serum obtained from the seven hypertensive dogs: 1) before the induction of hypertension (prehypertension); 2) after wrapping one kidney in silk (two-kidney perinephric hypertension, 2-KPHT); and 3) after contralateral nephrectomy (1-KPHT). Duplicate cultures also were run with the serum of the four sham-wrapped (pre- and post-sham surgery) and then nephrectomized (post-nephrectomy) normotensive control dogs. The same set of tissue culture media was used for no more than two rabbit aorta cultures. For each rabbit aorta culture, the percentage of serum in the three culture media was the same; 10, 15 or 20%. A total of 38 rabbit aorta cultures were run.

Additional Measurements

Blood was drawn from dogs at the end of the control period and 2 weeks after nephrectomy for the following measurements: hematocrit by the microhematocrit technique, serum creatinine by autoanalyzer, serum sodium and potassium by flame photometry, and plasma renin activity (PRA) by radioimmunoassay for angiotensin I. Blood for PRA was collected and centrifuged at room temperature. The plasma was stored at \(-20^\circ\text{C}\).

Flame photometry was used to measure the sodium and potassium concentration of randomly selected batches of tissue culture medium after addition of serum, antibiotics and L-glutamine.

Statistical Methods

Student’s \(t\) test for paired replicates was used to compare the water and electrolyte content of explants cultured in the different sera and the blood chemistries of dogs at the end of the control period and 2 weeks after nephrectomy. Linear correlations between the sodium and potassium concentration of explants cultured in the same serum were calculated by standard statistical techniques.

Results

Mean arterial blood pressure in the seven perinephritic dogs rose from 131 ± 9 mm Hg (mean ± SEM) at the end of the control period to 169 ± 7 2 weeks after wrapping one kidney in silk, and to 191 ± 8 in six dogs 4 weeks after nephrectomy. One dog died with signs of malignant hypertension 10 days after nephrectomy. The serum obtained from this dog after nephrectomy was not used for the tissue culture experiments. The mean arterial blood pressure of the four sham-operated and then nephrectomized dogs, 135 ± 6 at the end of the control period, did not change after shan surgery, 127 ± 11, or after nephrectomy, 124 ± 13. The hematocrit of dogs at the end of
Table 1. Blood Chemistries in Hypertensive and Normotensive Dogs

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 7)</th>
<th>1-KPHT (n = 6)</th>
<th>Control (n = 4)</th>
<th>Post-nephrectomy (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>43 ± 2</td>
<td>43 ± 2</td>
<td>44 ± 2</td>
<td>43 ± 3</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.7 ± 0.1</td>
<td>1.0 ± 0.1*</td>
<td>0.8 ± 0.1</td>
<td>1.1 ± 0.1†</td>
</tr>
<tr>
<td>Serum Na (mEq/liter)</td>
<td>154 ± 1</td>
<td>151 ± 1</td>
<td>151 ± 1</td>
<td>151 ± 1</td>
</tr>
<tr>
<td>Serum K (mEq/liter)</td>
<td>4.8 ± 0.2</td>
<td>4.4 ± 0.3</td>
<td>4.6 ± 0.1</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml/hr)</td>
<td>0.8 ± 0.4 (5)</td>
<td>0.4 ± 0.1 (5)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM and comparisons made between control and 1-KPHT and post-nephrectomy values. 1-KPHT = one-kidney perinephritic hypertension.

*p < 0.05.
†p < 0.001.

the control period and 2 weeks after nephrectomy were unchanged (table 1).

After 3 weeks of cultivation, there was microscopic evidence for new growth in every petri dish. The amount of growth varied from a few cell layers at the periphery of explants to confluent growth between explants. The concentration of serum in the tissue culture medium, 10, 15 or 20%, did not seem to bear a relationship to the amount of new growth, but attempts to quantitate the amount of proliferation were not made. Considerable variations also existed in explant electrolyte concentrations from experiment to experiment, using the aorta of a different rabbit each time, amounting to as much as 80-120 mEq/kg sodium and 20-30 mEq/kg potassium. Differences in the water and electrolyte content of explants cultured in tissue culture medium containing 10, 15 or 20% serum were not found. Table 2 presents the water, sodium and potassium concentrations of explants cultured in prehypertension and in one- and two-kidney perinephritic hypertension (2-KPHT and 1-KPHT) and in pre-sham surgery, post-sham surgery and post-nephrectomy serum. Compared to explants cultured in prehypertension serum, the water content of explants cultured in 1-KPHT serum and the sodium concentration of explants cultured in 2-KPHT and 1-KPHT serum were increased. The time of collection of serum, 1 or 2 weeks after wrapping one kidney in silk or 1, 2, 3 or 4 weeks after nephrectomy, did not seem to influence the results. The potassium concentration of explants cultured in prehypertension and posthypertension (2-KPHT and 1-KPHT) serum was similar. Not shown in table 2, the dry weight of 36 explants cultured in prehypertension serum, 6.4 ± 0.43 mg (mean ± SEM), and in 2-KPHT and 1-KPHT serum, 6.0 ± 0.40, was the same. Table 2 also presents the composition of explants cultured in the serum of sham-operated normotensive dogs. The differences between the composition of explants cultured in prehypertension serum (upper half of table 2) and in pre-sham surgery serum (lower half) did not approach statistical significance (Student's t test for independent

Table 2. Rabbit Aortic Media Explants Composition After Culture in Hypertensive and Normotensive Dog Serum

<table>
<thead>
<tr>
<th></th>
<th>Prehypertension (n = 20)</th>
<th>2-KPHT (n = 20)</th>
<th>Prehypertension (n = 16)</th>
<th>1-KPHT (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (%)</td>
<td>74.2 ± 0.5</td>
<td>74.7 ± 0.5</td>
<td>74.0 ± 0.4</td>
<td>75.2 ± 0.4*</td>
</tr>
<tr>
<td>Sodium (mEq/kg dry wt)</td>
<td>518 ± 12</td>
<td>540 ± 16*</td>
<td>504 ± 15</td>
<td>543 = 16†</td>
</tr>
<tr>
<td>Potassium (mEq/kg dry wt)</td>
<td>56 ± 2</td>
<td>54 ± 3</td>
<td>53 ± 4</td>
<td>54 ± 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Pre-sham (n = 8)</th>
<th>Post-sham (n = 8)</th>
<th>Pre-sham (n = 8)</th>
<th>Post-nephrectomy (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (%)</td>
<td>73.4 ± 1.2</td>
<td>72.8 ± 1.0</td>
<td>73.5 ± 1.4‡</td>
<td>74.0 ± 1.1†</td>
</tr>
<tr>
<td>Sodium (mEq/kg dry wt)</td>
<td>490 ± 28</td>
<td>461 ± 19</td>
<td>490 ± 28</td>
<td>479 ± 27</td>
</tr>
<tr>
<td>Potassium (mEq/kg dry wt)</td>
<td>58 ± 4</td>
<td>59 ± 3</td>
<td>58 ± 4</td>
<td>60 ± 3</td>
</tr>
</tbody>
</table>

Prehypertension sera for comparison with 2-KPHT and 1-KPHT sera were obtained from the same dogs but not always on the same day. 2-KPHT and 1-KPHT = two- and one-kidney perinephritic hypertension. Values are expressed as means ± SEM and comparisons made between prehypertension and 2- and 1-KPHT values and between pre-sham and post-sham and post-nephrectomy values.

*p < 0.05.
†p < 0.01.
‡N = 7.
variables), reflecting the large variations of values between experiments (see above). The water and potassium content of explants cultured in the serum of sham-operated animals was the same regardless of the type of serum used, pre- or post-sham surgery or post-nephrectomy. The sodium concentration of explants cultured in post-sham surgery and post-nephrectomy serum was numerically less than that of explants cultured in pre-sham surgery serum, but the differences did not reach statistical significance \( (p > 0.05, p < 0.1, \text{respectively}). \) Since this trend in explant sodium concentration was in the opposite direction from that found with 2-KPHT and 1-KPHT serum, additional experiments with the serum of sham-operated dogs were not carried out. Finally, there was no statistically significant correlation between the sodium and potassium concentration of explants cultured in either the control sera, prehypertension \( (r = 0.04, n = 36) \) or pre-sham surgery \( (r = 0.40, n = 8) \), or in posthypertension \( (r = 0.29, n = 36) \) and post-sham surgery \( (r = 0.02, n = 16) \) sera.

The water, sodium and potassium concentration of fresh rabbit aortic media tissue is shown in table 3. Compared to the composition of cultured explants, fresh tissue specimens contained less water, on the average 50–60 mEq/kg dry weight less sodium, and about twice as much potassium.

The results of the tissue culture experiments could not be explained by variations in the serum concentrations of creatinine, sodium and potassium or in the PRA of hypertensive dogs (table 1). Two weeks after nephrectomy, the serum creatinine concentration of hypertensive and normotensive control dogs was similarly increased compared to values obtained at the end of the control period. Serum electrolyte concentrations did not change in either group of dogs during the study. The PRA of hypertensive dogs in the control period and 2 weeks after nephrectomy was unchanged with the exception of one dog that developed malignant hypertension.

Finally, the electrolyte concentration of randomly selected batches of tissue culture medium, supplemented with 10, 15 or 20% serum, was physiological: sodium 143 ± 1 mEq/liter (mean ± SEM, \( n = 16 \)) and potassium 5.4 ± 0.3 (\( n = 16 \)).

### Discussion

The findings of this study provide evidence for the role of serum factor(s) in the pathogenesis of increased vascular wall water and sodium concentration in experimental hypertension. The findings were not explained by variations in the serum creatinine, sodium or potassium levels of hypertensive and normotensive dogs. The serum factor(s) is present in dogs with early (less than 2 weeks) two-kidney perinephritic hypertension and also in dogs with one-kidney perinephritic hypertension of 1–4 weeks' duration. Since the plasma renin activity of dogs with one-kidney perinephritic hypertension was within normal limits, the serum factor does not appear to be renin. It must be a relatively stable compound that is not destroyed by routine handling of blood samples, prolonged storage at \(-20^\circ C\) or repeated warming to \(37^\circ C\). Its identity is unknown.

This study, although in part confirmatory of previous in vivo experiments,\(^5\) used in vitro techniques, therefore, its findings have to be interpreted with caution. Several studies have indicated that isolated vascular smooth muscle cells in culture must first "dedifferentiate" to morphologically resemble fibroblasts before proliferation will occur.\(^6\) "Dedifferentiation" occurs between the first and second week in culture. After more than 1 week of culture, the cells are no longer phenotypically representative of vascular smooth muscle cells in vivo. In this study, rabbit aortic media cultures were carried for 3 weeks.

Under in vitro conditions, there were changes in the water and electrolyte composition of rabbit aortic media explants. The water and sodium concentration of explants increased, but the potassium concentration was one-half of that of fresh, excised tissue. The variability of explant electrolyte values from experiment to experiment was considerable. Previous investigators have shown that dog carotid artery media segments, incubated in physiologic saline solution immediately after dissection and stripping, lose potassium and gain sodium at a rapid rate.\(^7\) The extent of the loss of normal electrolyte gradients is a function of the severity of the mechanical trauma. Partial recovery occurs after prolonged incubation but normal gradients are never achieved. During the dissection some of the cells appear to be irreversibly damaged. The decreased potassium concentration of explants in this study must be due in part to the presence of nonviable cells. However, the potassium concentration of explants cultured in different sera was the same. Explant concentration of potassium bore no relationship to sodium concentration. If the accumulation of sodium by explants in culture was entirely due to the presence of nonviable cells and the subsequent loss of potassium gradient, an inverse correlation between explant potassium and sodium concentration should have existed. Therefore, the increased water and sodium concentration of explants cultured in hypertension serum cannot be explained on the basis of cell viability, as measured by the potassium concentration of explants.

Finally, these data are insufficient to determine the pathophysiological significance of the findings of this study. The growth characteristics of explants cultured in the various sera need further study. The question whether the excess accumulated sodium in explants...
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Cultured in hypertension serum is extracellular or intracellular, bound or in solution, also remains to be investigated. Pending further studies, the present results must be considered preliminary.

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