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Venous Wall Electrolytes and Hexosamines in Hypertensive Rats[1] (40926)

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Abstract. The relationship between vascular wall water, sodium, and glycosaminoglycans in experimental hypertension was investigated by measurements of the water, electrolyte, and hexosamine content of the vena cava in 43 one-clip, one-kidney Goldblatt hypertensive and in 22 spontaneously hypertensive (SHR) rats. Twenty-eight sham-operated normotensive and 21 normotensive Wistar–Kyoto rats served as controls. Compared to values obtained in normotensive control rats, the sodium content of the vena cava was increased by 12.5 and 16.4% in rats with mild (systolic BP <180 mm Hg) and severe (>180 mm Hg) Goldblatt hypertension, respectively. In contrast, an increase in the water and hexosamine content of the vena cava, 13.2 and 25.1%, was found only in the more severly hypertensive Goldblatt rats. It is, therefore, unlikely that the binding of sodium to acidic glycosaminoglycans is an important mechanism accounting for the accumulation of venous wall sodium in renal hypertensive rats. An increase in venous wall hexosamine content, however, may play a role in the waterlogging of veins. There were no significant changes in the composition of the vena cava in SHR with either mild or moderate hypertension.

Increased arterial wall water and sodium content in experimental and human hypertension has been known for many years. The excess vascular wall sodium has been shown to be primarily extracellular (1). Controversy exists as to the biochemical state of this excess sodium (2). Some investigators suggest that the excess sodium is bound, through covalent bonds, by acidic glycosaminoglycans. Others deny that the small accumulation of arterial wall glycosaminoglycans in hypertension could account for all the excess sodium. Recent findings of increased water and sodium content of veins in experimental renovascular hypertension provide an opportunity to reexamine the relationship between vascular wall water, sodium, and acidic glycosaminoglycans, while at the same time eliminating the contribution that increased intraluminal pressure appears to make to the development of these changes on the arterial side of the circulation (3–6). If the binding of sodium to glycosaminoglycans is an important mechanism underlying the accumulation of excess vascular wall sodium, then the glycosaminoglycan content of veins also should be increased in hypertension. To date, increased biosynthesis of venous wall glycosaminoglycans has been reported in spontaneously hypertensive rats (SHR), but measurements of venous wall glycosaminoglycan content in experimental hypertension are lacking (7). The purpose of this study was to investigate the relationship between venous wall water, sodium, and acidic glycosaminoglycans in two types of experimental hypertension, the chronic one-clip, one-kidney hypertensive rat and the SHR.

Methods. Goldblatt hypertension was produced in 43 male Sprague–Dawley rats (5–6 weeks old) by applying a silver clip with an inner diameter of 0.2 mm to the left renal artery. The right kidney was removed 1 week later. The rats were studied 6–7 weeks after clipping. Twenty-eight male, Sprague–Dawley rats, age-matched and sham-clipped, with contralateral nephrectomy, served as controls. Studies also were performed in 22 male SHR and 21 age- and weight-matched Wistar–Kyoto normotensive control rats. Age matching was based on the birth date of rats. The age of rats was 12–14 weeks at the time of the experiments. To achieve weight matching, some of the lighter SHR and some of the heavier

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Wistar–Kyoto rats were rejected. Taconic Farms (Germantown, N.Y.) was the source of SHR and Wistar–Kyoto rats. Hypertensive and normotensive rats were kept under the same conditions, fed regular rat chow, and were given water *ad libitum*. The systolic blood pressure of methoxyflurane-anesthetized rats was measured twice, on 2 separate days, by the microphonic manometer technique during the week of the experiments. The blood pressure values were averaged. The rats were weighed to the nearest 1 g on the day of the experiments.

The same segments of the inferior vena cava, from the iliac veins to the left renal vein, and of the superior vena cava, from the diaphragm to the right clavicle, excluding the right atrial appendage, were removed from methoxyflurane-anesthetized rats in a cold room (4°C). All specimens were cleaned of perivascular tissue, cut into 4- to 6-mm pieces, blotted once with filter paper to remove blood, and then weighed to the nearest 0.1 mg. The specimens were oven-dried at 104° for 24 hr and reweighed. The dried tissue was defatted with two changes of acetone and one change of ether over a 3-day period, oven-dried, and reweighed. Water and fat content was expressed as kilogram per kilogram dry, defatted weight. Dry, defatted tissue specimens were processed further for measurements of ionic or hexosamine content, but not both.

For measurement of ionic content, to the dry, defatted tissue 2 ml of 0.75 *N* nitric acid was added. Specimens were digested for 7 days. Digested specimens were placed in the oven at 104° for 24 hr to evaporate the nitric acid. Tissue contents of sodium and potassium were measured by flame photometry (Instruments Laboratories). Ion contents were expressed as milliequivalents per kilogram dry, defatted weight.

The hexosamine content of tissue was taken as a measure of the total acidic glycosaminoglycans. In the course of preliminary experiments, the optimal hydrolysis conditions for the release of hexosamines from rat vena cava were determined. These were found to be 5 ml of 2 *N* hydrochloric acid at 104° for 16 hr in a sealed glass hydrolysis tube. Hexosamine was measured by the method of Grant *et al.* (8). Our methods were tested by analyzing the hexosamine content of the thoracic aorta of four male, Sprague–Dawley rats (average body weight, 416 g). Our results for hexosamine, 26.1 ± 3.8 mmole/kg dry weight (mean ± SE), were in good agreement with previously published results (9).

The reported values are means with standard errors of the mean. Student's *t* test for independent variables was used to compare the measured parameters in hypertensive and normotensive rats.

Results. The body weight of 43 rats with one-clip, one-kidney hypertension, 346 ± 9 g, was less than that of 28 normotensive control rats, 388 ± 13 ($P < 0.01$). The systolic blood pressure of Goldblatt hypertensive rats was 197 ± 5 mm Hg, and that of normotensive control rats was 111 ± 4 ($P < 0.001$). During removal of vein specimens, it was difficult to separate the thin-walled veins from the adherent interstitial fat. The fat content of vein specimens was high, 0.52 ± 0.04 kg/kg dry weight in normotensive control rats ($N = 28$) and 0.42 ± 0.04 in Goldblatt hypertensive rats ($N = 43$) ($P < 0.1$). Therefore, the results of the chemical analysis of veins were expressed in terms of dry, defatted weight. The composition of the vena cava in the two groups of rats is shown in Table I. Venous tissue water and Na content was increased in the hypertensive rats. The numerical difference in the hexosamine content of the vena cava between hypertensive and normotensive rats was of borderline statistical significance ($P < 0.06$).

The body weight of 22 SHR, 322 ± 5 g, and of 21 Wistar–Kyoto control rats, 316 ± 5, was statistically the same. Systolic blood pressure was 158 ± 6 mm Hg in SHR and 108 ± 4 in the Wistar–Kyoto rats ($P < 0.001$). The fat content of the vena cava in Wistar–Kyoto rats was 0.45 ± 0.04 kg/kg dry weight ($N = 21$) and in SHR 0.42 ± 0.04 ($N = 22$). The water, electrolyte, and hexosamine content of the vena cava in the two groups of rats is shown in Table I. There were no statistically significant differences in the composition of the vena

TABLE I. VENOUS WALL COMPOSITION IN ONE-CLIP, ONE-KIDNEY HYPERTENSIVE (1-C, 1-K HT), NORMOTENSIVE CONTROL, SPONTANEOUSLY HYPERTENSIVE (SHR), AND WISTAR–KYOTO CONTROL (W–K) RATS

	Control	*N*	1-C, 1-K HT	*N*	W–K	*N*	SHR	*N*
Water (kg/kg dry weight)	2.89 ± 0.05[a]	28	3.12 ± 0.06*	43	2.94 ± 0.06	21	3.07 ± 0.05	22
Dry, defatted weight (mg)	7.2 ± 0.4	28	6.7 ± 0.6	43	6.6 ± 0.5	21	5.7 ± 0.3	22
Sodium (meq/kg dry weight)	385 ± 8	10	438 ± 6**	12	385 ± 12	9	408 ± 18	10
Potassium (meq/kg dry weight)	156 ± 5	10	168 ± 3	12	169 ± 6	9	176 ± 9	10
Hexosamine (mmole/kg dry weight)	23.5 ± 0.9	18	27.3 ± 1.4	31	28.7 ± 1.2	12	28.3 ± 1.1	12

[a] Values indicate mean ± SE of the mean.
* $P < 0.02$, for comparison of 1-C, 1-K HT rats with control rats.
** $P < 0.001$, for comparison of 1-C, 1-K HT rats with control rats.

cava in the two groups. The small numerical increase in the water content of the vena cava in SHR did not reach statistical significance ($P < 0.1$).

The effect of the severity of hypertension on vena cava composition was examined by subdividing the Goldblatt hypertensive and the SHR into two groups, one with mild, the other with more severe hypertension (Table II). The severity of the hypertension had no effect on the increased Na content of the vena cava in Goldblatt hypertensive rats. In contrast, the water and hexosamine content of the vena cava was increased in rats with more severe Goldblatt hypertension and unchanged in rats with mild hypertension. In SHR, the severity of hypertension did not have an effect on venous wall composition.

Discussion. Waterlogging and increased sodium content of veins have been previously reported in dogs with one kidney and perinephritic hypertension, in rats with one-clip, two-kidney hypertension, and in rats with coarctation hypertension (3–5). The present study extends these findings to rats with chronic one-clip, one-kidney hypertension. In rats with more severe one-clip, one-kidney hypertension, the hexosamine content of large veins also is increased, suggesting an increased venous wall glycosaminoglycan content. Since these changes occur on the low pressure side of the circulation, they are unlikely to be secondary to increased intraluminal pressure associated with arterial hypertension but must be the result of neural or humoral influences. Previously, we have reported evidence for circulating serum factors in experimental renovascular hypertension that result in the accumulation of excess vascular wall water and sodium (10).

In one-clip, one-kidney hypertensive rats, the increase in sodium content of the vena cava appeared to be independent of the severity of hypertension, but increased water and hexosamine content was found only in the more severely hypertensive rats. Because of this dissociation between the increases of venous wall sodium and hexosamine, it is unlikely that the binding

TABLE II. EFFECT OF THE SEVERITY OF HYPERTENSION ON VENOUS WALL COMPOSITION IN ONE-CLIP, ONE-KIDNEY HYPERTENSIVE (1-C, 1-K HT) AND SPONTANEOUSLY HYPERTENSIVE (SHR) RATS

	1-C, 1-K HT				SHR			
	SBP < 180	N	SBP > 180	N	SBP < 160	N	SBP > 160	N
Systolic BP (mm Hg)	164 ± 2[a,*]	19	222 ± 5*	24	144 ± 2*	10	187 ± 4*	12
Water (kg/kg dry weight)	2.93 ± 0.10	19	3.27 ± 0.07*	24	3.07 ± 0.13	10	3.08 ± 0.05	12
Sodium (meq/kg dry weight)	433 ± 8*	8	448 ± 9*	4	429 ± 38	4	393 ± 18	6
Hexosamine (mmole/kg dry weight)	23.5 ± 1.4	11	29.4 ± 1.9**	20	28.3 ± 1.0	6	28.0 ± 2.0	6

[a] Values indicate mean ± SE of the mean.
* $P < 0.001$, for comparison of hypertensive rats with appropriate normotensive control rats (Table I).
** $P < 0.02$, for comparison of one-clip, one-kidney hypertensive rats with normotensive control rats (Table I).

of sodium to acidic glycosaminoglycans is an important mechanism accounting for the accumulation of sodium in the wall of veins in this model of hypertension. Furthermore, the increases in venous wall sodium, 53 meq/kg dry weight (mean), greatly exceeded the amount of excess hexosamine, 3.8 mmole/kg dry weight. Jones and Swain have estimated that the increase in acidic glycosaminoglycan content that this small increase in hexosamine content represents could account for the binding of only a small portion of the excess sodium (2). An increase in venous wall hexosamine content, however, may play a role in the waterlogging of large veins. In this regard, Lorenzen found a direct linear correlation between the total glycosaminoglycan content of rabbit aorta and its water content (11).

Compared to Wistar–Kyoto normotensive control rats, there were no significant changes in the venous wall composition of rats with mild or moderate spontaneous hypertension, but the methods employed may not have been sensitive enough to detect small increases in the water and sodium content of the vena cava. The increased rate of incorporation of [^{14}C]glucosamine into the portal vein and vena cava of SHR, reported by Greenberg *et al.*, does not seem to result in increased venous wall content of hexosamines (7). Finally, the potassium content of the vena cava was unchanged in both Goldblatt hypertensive rats and SHR.

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