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Effects of Hydrochlorothiazide on Water, Cation, and Norepinephrine Contents of Cardiovascular Tissues of Renovascular Hypertensive Dogs[1] (38435)

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Thiazide compounds have been used extensively in the treatment of hypertension, but the manner in which they exert their antihypertensive action remains unclear. Previous work (1) has shown that hydrochlorothiazide (3.75 mg/kg/day) given orally for 45 days to normotensive dogs had no effect on blood pressure, blood and plasma volumes, or hematocrit despite significant reduction of water, potassium, and norepinephrine contents in arterial tissues. Since arterial water and cation concentrations are elevated in renovascular hypertensive animals (2, and work to be published from this laboratory) we have investigated whether hydrochlorothiazide reduces these concentrations and the blood pressure of dogs rendered hypertensive by renal artery constriction and contralateral nephrectomy.

Materials and Methods. Adult male mongrel dogs, 15–23 kg, were kept in individual metabolic cages, fed Purina dog chow, supplemented with commercial meat dog food, and given access to water *ad libitum*. During an initial period of 3 weeks, the blood pressure of each dog was recorded frequently on a Grass polygraph by means of a direct femoral arterial puncture under light thiopental (25 mg/kg) anesthesia. Thirty dogs with a mean arterial blood pressure (MABP) below 125 mm Hg (16.7 KPa) during this period were selected. One renal artery of each was constricted by means of a platinum clamp (about 75% decrease of lumen diameter), and a week later the contralateral kidney was removed.

Four weeks after the second operation, 25 dogs that had developed stable hypertension (defined as a consistent weekly reading of MABP of at least 30 mm Hg -40 KPa-above the preoperative MABP) were divided into three groups. The six dogs in group I received hydrochlorothiazide (HC) orally (3.75 mg/kg/day, in two equal doses). Those of group II received in addition 45 mg/kg KCl/day, in two equal doses. The 13 remaining animals, group III, served as controls. The blood pressure of all animals was determined weekly. Sodium and potassium were measured by emission spectrophotometry (Unicam SP 900) with lithium nitrate as internal standard. Magnesium and calcium were determined by atomic spectrophotometry (Perkin–Elmer 303) with lanthanum chloride as internal standard. Plasma renin activity was determined by a micromethod (3). Before and at the end of the experimental period we estimated blood volume using ^{131}I-human serum albumin (RISA) and a Picker Hemoliter. Tissue norepinephrine was determined spectrophotofluorometrically (4), and renal juxtaglomerular cell granularity was also measured (5).

The results were evaluated by Student's paired and unpaired t tests. Values of $P < 0.05$ were considered significant.

On the 42nd day, dogs of groups I and II were autopsied. Their myocardial and arterial tissues (branches of the mesenteric arteries; the saphenous, femoral, and carotid arteries; and the thoracic aorta) were rapidly removed for determination of their water and cation contents (6). Tissues of animals in group III were examined similarly.

Results. Blood pressure. Figure 1 shows the MABP of the 25 operated animals which de-

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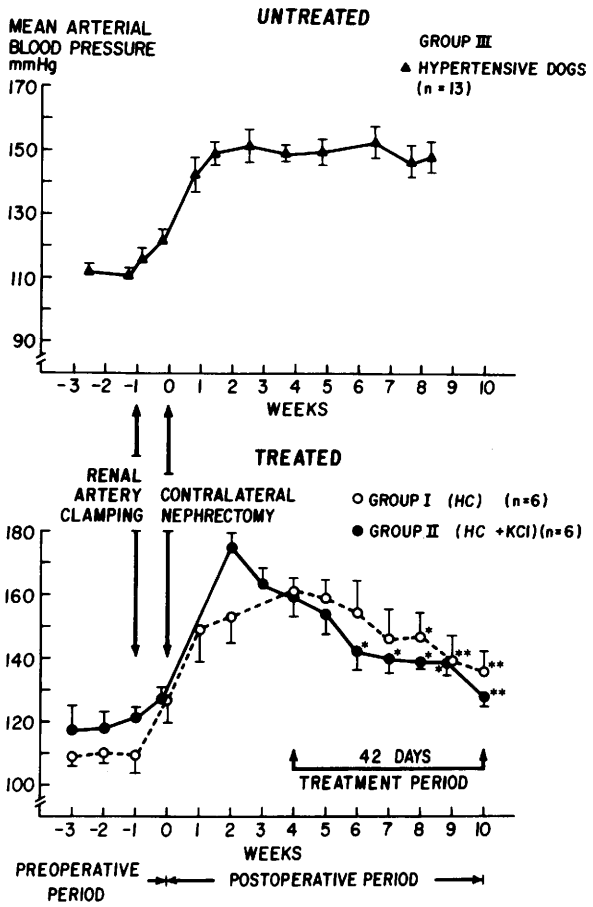

FIG. 1. Effect of hydrochlorothiazide administration on mean arterial blood pressure of operated, hypertensive dogs. Asterisks indicate the significant difference from pretreatment values (*$P < 0.05$, **$P < 0.01$).

veloped hypertension. The hydrochlorothiazide-treated dogs (below) showed a gradual decrease in blood pressure which became significant, by the paired t test after the second week of treatment. MABP values were reduced by the drug on average, 15%, 32%, and 0.1% for groups I, II, and III, respectively.

Blood volumes, hematocrit, and plasma volume. Blood volumes were reduced by the drug by 16% ($P < 0.01$) and 3% (N.S.) in groups I and II, respectively. The reduction in plasma volumes was 19% ($P < 0.01$) for group I, and 4% (N.S.) for group II. Hematocrit was increased by 9% (N.S.) and 2% (N.S.) in these groups. Two animals in group I showed a severe anaphylactic reaction to the ^{131}I-albumin, and their results were not included.

Plasma cations and plasma renin activity. All drug-treated dogs showed a consistent and significant reduction in plasma potassium. Of the other cations only magnesium changed: a decrease (−23%, $P < 0.05$) in dogs of group II in the early stage of treatment (Table I). Plasma renin activity increased in all drug treated animals, but significantly only in dogs receiving hydrochlorothiazide alone.

Cardiovascular water and cation contents. Arterial tissues from dogs of group I and group II were generally lower in sodium and potassium than those from group III dogs (Table II, Fig. 2). The sodium concentration was significantly lower in all arteries from group I, but KCl supplementation (group II) abolished this difference. The arterial water content was lower in group II than in either of the others. No striking differences were seen in arterial magnesium or calcium, except that calcium was significantly reduced in the saphenous artery of group II.

Myocardial calcium content was significantly increased in group I.

TABLE I. Plasma Cations and Renin Activity Before and During Treatment of Groups I and II.[a]

	Group (n)	Before treatment	Treatment period (day) 14th	28th	42nd
Cation (mEq/liter)					
Sodium	I (6)	143.8 ± 2.0	140.9 ± 1.1 (−2%)	142.6 ± 2.4 (−1%)	146.9 ± 2.3 (+2%)
	II (5)	143.3 ± 2.9	137.0 ± 6.2 (−4%)	145.2 ± 4.1 (+1%)	144.7 ± 1.7 (+1%)
Potassium	I (6)	4.5 ± 0.1	3.7 ± 0.1 (−18%)[c]	3.8 ± 0.1 (−16%)[c]	3.7 ± 0.1 (−18%)[c]
	II (5)	4.9 ± 0.5	3.6 ± 0.4 (−27%)[c]	4.0 ± 0.4 (−18%)[c]	4.2 ± 0.3 (−14%)[b]
Magnesium	I (6)	1.3 ± 0.0	1.4 ± 0.4 (+5%)	1.3 ± 0.1 (+3%)	1.4 ± 0.0 (+8%)
	II (5)	1.7 ± 0.4	1.3 ± 0.5 (−23%)[b]	1.5 ± 0.2 (−12%)	1.5 ± 0.2 (−9%)
Calcium	I (6)	4.7 ± 0.1	4.6 ± 0.1 (−2%)	4.6 ± 0.2 (−2%)	4.7 ± 0.0 (0%)
	II (5)	6.0 ± 0.3	5.8 ± 1.1 (−3%)	5.8 ± 0.4 (−3%)	5.6 ± 0.6 (−7%)
Renin activity (ng angio./ml/hr)	I (6)	0.29 ± 0.13	2.70 ± 0.87 (+831%)[b]	1.75 ± 0.23 (+503%)[c]	1.42 ± 0.29 (+390%)[c]
	II (5)	0.23 ± 0.12	0.30 ± 0.16 (+24%)	0.42 ± 0.22 (+45%)	0.44 ± 0.18 (+52%)

[a] Values are means ± SE. Figures in parenthesis are the percentage change from the values before treatment.
[b] $P < 0.05$.
[c] $P < 0.01$.

TABLE II. Cardiovascular Tissue Water, Cations and Norepinephrine of Groups I-III.[a]

Tissues	Group	Water % of wet wt	Cations (μEq/g dry weight)				Norepinephrine μg/g wet wt.
			Sodium	Potassium	Magnesium	Calcium	
Branches of mesenteric arteries	I (n = 6)	71.9 ± 0.3	328 ± 5.1	140 ± 3.9	28 ± 0.6	25 ± 0.6	6.12 ± 0.83
	II (n = 6)	69.8 ± 0.5	354 ± 27.4	139 ± 6.1	27 ± 1.1	24 ± 0.7	5.54 ± 0.62
	III (n = 13)	71.9 ± 0.3	353 ± 7.1	154 ± 5.9	29 ± 0.8	24 ± 1.0	6.39 ± 0.59
Saphenous artery	I	67.7 ± 0.5	248 ± 7.2	92 ± 5.5	22 ± 1.2	20 ± 0.5	1.63 ± 0.40
	II	66.1 ± 1.3	258 ± 17.2	108 ± 14.9	20 ± 1.0	16 ± 0.9	1.30 ± 0.07
	III	67.4 ± 0.5	268 ± 3.8	104 ± 5.2	21 ± 0.6	19 ± 0.6	1.46 ± 0.16
Femoral artery	I	68.5 ± 0.5	272 ± 14.6	95 ± 5.6	18 ± 1.0	23 ± 1.0	0.52 ± 0.09
	II	68.4 ± 0.4	291 ± 20.7	93 ± 6.0	21 ± 1.8	23 ± 2.5	0.52 ± 0.08
	III	69.8 ± 0.3	307 ± 7.8	101 ± 2.6	19 ± 0.4	21 ± 0.6	0.39 ± 0.05
Carotid artery	I	70.5 ± 0.5	301 ± 4.8	116 ± 3.9	23 ± 0.5	21 ± 1.0	1.24 ± 0.20
	II	70.5 ± 0.4	330 ± 9.2	111 ± 7.3	22 ± 0.8	21 ± 0.4	1.35 ± 0.12
	III	70.3 ± 0.2	333 ± 7.3	131 ± 3.7	22 ± 0.8	22 ± 0.9	1.47 ± 0.14
Thoracic aorta	I	72.8 ± 0.2	287 ± 7.1	136 ± 6.0	24 ± 0.5	25 ± 0.8	1.22 ± 0.09
	II	71.6 ± 0.6	290 ± 9.5	130 ± 3.7	22 ± 0.9	27 ± 2.3	1.39 ± 0.23
	III	73.5 ± 0.3	329 ± 6.6	154 ± 2.9	24 ± 0.7	27 ± 1.1	1.40 ± 0.17
Left myocardium	I	78.2 ± 0.2	172 ± 5.5	333 ± 1.9	91 ± 1.4	11 ± 0.6	0.31 ± 0.07
	II	77.8 ± 0.6	166 ± 25.6	342 ± 13.9	84 ± 1.1	10 ± 1.6	0.28 ± 0.01
	III	77.2 ± 0.5	172 ± 7.6	336 ± 4.3	86 ± 1.8	9 ± 0.3	0.40 ± 0.05

[a] Values are means ± SE. Levels of significance between group I, group II, and group III are given in Fig. 2.

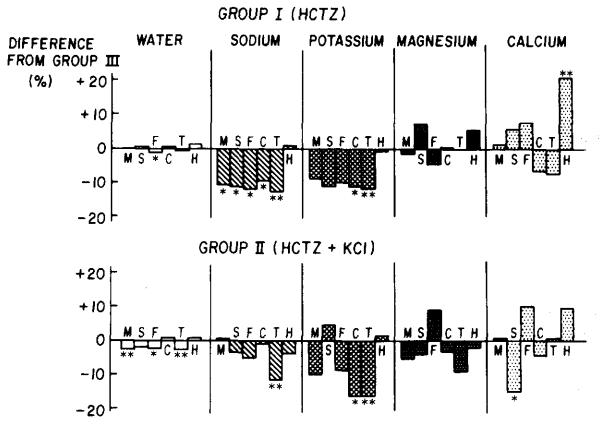

FIG. 2. Changes in water and cation contents in arteries and myocardium of experimental hypertensive dogs treated with hydrochlorothiazide alone (Group I) and hydrochlorothiazide plus KCl (Group II) for 42 days. M = branches of mesenteric arteries, S = saphenous artery, F = femoral artery, C = carotid artery, T = thoracic aorta, H = left myocardium (*$P < 0.05$, **$P < 0.01$).

Norepinephrine content. Tissue norepinephrine contents in the three groups are shown in Table II. There were no significant differences between treated and untreated hypertensive dogs.

Juxtaglomerular index. The juxtaglomerular granulation index was significantly ($P < 0.01$) higher in HC-treated dogs (mean ± SE 39.0 ± 2.7 and 42.6 ± 5.5 for groups I and II, respectively) than in untreated dogs (15.5 ± 2.9).

Discussion. In a previous study to be published we have found that dogs with renal experimental hypertension had, after 60 days but not after 20 days, more water and higher sodium, potassium, magnesium, and calcium concentrations in arterial tissues than did dogs that remained normotensive. We suggested that these raised arterial water and cation contents were the result of high blood pressure and probably were involved in its maintenance. The present study indicates that hydrochlorothiazide (with or without KCl supplement), given daily for 42 days to renal experimental hypertensive dogs, reduced their blood pressure by 30 mm Hg (4.0 KPa) on the average. The arterial sodium and potassium concentrations of animals receiving HC alone were lower than those in hypertensive untreated dogs.

Both treated groups showed decreasing plasma and arterial potassium concentrations as the blood pressure fell towards normal range. Conway and Lauwers (7), whose findings were confirmed by Villarreal and co-workers (8), have found a reduction in peripheral resistance after prolonged treatment with thiazides which they attribute to the direct action of these drugs on arteries. Our results support this hypothesis. It may be that the hypotensive action of thiazides is related directly to their ability to reduce the water, sodium and potassium contents of the vascular tissues. Decreased peripheral resistance may also be associated with a reduced responsiveness of actomyosin, which is markedly influenced by cation concentration in the vascular tissues. Orbison (9) and Weller and Haight (10) did not find the changes we report here, but they administered chlorothiazide for shorter periods. Tobian and his colleagues (11), on the other hand, have treated normal rats with chlorothiazide for 9 weeks and found no significant changes in vascular cations. It is possible that the differences in experimental designs may account for this discrepancy.

Although the amount of KCl added appears to have been insufficient to prevent the fall in plasma K^+ due to hydrochlorothiazide, it was enough to inhibit the increase of plasma renin activity seen in animals treated with hydrochlorothiazide alone. The absence of marked changes in blood and plasma volume in dogs of group II may explain the lack of change in plasma renin activity.

Renovascular hypertensive animals show a significant decrease in norepinephrine content in arterial (12, 13) and myocardial (14, 15) tissues. Our previous work has shown that HC treatment decreases arterial norepinephrine content in normal dogs (1). The results now reported suggest that hydrochlorothiazide given to renovascular hypertensive dogs has no additive

effect, *i.e.*, it does not further decrease the norepinephrine content of arterial and myocardial tissues.

Summary. Thirty adult male mongrel dogs were subjected to unilateral renal artery constriction and a week later to contralateral nephrectomy. Four weeks after the second operation, 25 animals had developed hypertension. Six of them (Group I) received hydrochlorothiazide (3.75 mg/kg/day, in two doses) orally for 6 weeks; a further six (Group II) were treated similarly but received KCl (45 mg/kg/day, in two doses) in addition; the remaining 13 animals served as controls. Mean arterial blood pressure, recorded weekly, decreased by about 30 mm Hg (4 KPa) in both treated groups. Plasma potassium also declined significantly in both groups. Plasma renin activity was increased, but significantly only in group I, which also showed decreased blood and plasma volumes. Arterial water, sodium, and potassium were in general lower in groups I and II; the decrease in sodium was more consistent in arteries of group I. The norepinephrine content of arteries from the treated groups was not significantly different from that in untreated hypertensive dogs.

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