Altered Vascular Responsiveness: Initial Hypotensive Mechanism of Thiazide Diuretics (36639)

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The thiazide diuretics are well known for their effectiveness in the treatment of hypertension. However, the mechanisms whereby arterial pressure is reduced in hypertension remain unresolved. Implicated are those which involve contraction of intravascular volume, sodium depletion, altered vascular responsiveness and/or altered neural activity (1).

Because of the conflicting information the present study was designed to define the immediate systemic hemodynamic effects of chlorothiazide and to separate those changes related to altered vascular responsiveness from alterations in intravascular volume and electrolyte concentrations.

Methods. Fifteen mongrel dogs of both sexes averaging 18 kg in weight, were anesthetized with sodium pentobarbital (30 mg/kg). After the trachea was intubated and connected to a mechanical respirator (Harvard apparatus), a left thoracotomy was performed and a noncannulating electromagnetic flow transducer (Micron Instruments) was placed around the ascending aorta. The pericardium and chest were then closed and negative thoracic pressure was reestablished. The flow transducer was connected to an electromagnetic flowmeter (Biotronics) with the first nine dogs, and to a Micron electromagnetic flowmeter (last six dogs), and direct writing polygraph recorder (Sanborn). All flow transducers had been previously calibrated using excised dog aortas through which blood flow was regulated at varied levels equivalent to that expected in the *in vivo* preparation. The calibration of the flow probes of the first nine and last six dogs differed in magnitude, however, the quantitative directional changes were identical.

A femoral artery and vein were cannulated for recording mean arterial pressure and for intravenous injection of the pharmacological agents, respectively. Another catheter was placed in the right brachial artery for recording continuous phasic arterial pressure and heart rate. After disconnecting the respirator the animals were allowed to stabilize for 20 min. Total peripheral resistance was calculated by dividing the difference between mean arterial and central venous pressures by aortic blood flow; and stroke volume was calculated by dividing aortic flow by heart rate. Hereafter, aortic blood flow will be referred to as cardiac output even though coronary arterial flow was not included in the electromagnetically sensed aortic flow.

In nine dogs following the 20 min stabilization period, single bolus injections of 1.0, 3.0 and 5.0 μg *l*-norepinephrine base of levophed bitartrate, and 0.1, 0.3 and 0.5 μg Val-5-angiotensin (angiotensin II) were injected intravenously. Each bolus was immediately followed by a 5 ml normal saline flush injection. Approximately 5 min were allowed between each injection to allow the hemodynamic measurements to return to control levels. After obtaining these dose–response relationships, a 10 ml blood sample was withdrawn for determination of serum sodium, potassium, chloride, and total protein concentrations, using flame photometry (electrolytes) and the Biuret methods (protein). Chlorothiazide (25 mg/kg) was then injected intravenously, and after allowing another 20 min for intravascular mixing, the dose–response relationships were repeated. At completion of these measurements, a second

TABLE I. Hemodynamic Indices Obtained Just Prior (B) to Obtaining the First Set of Norepinephrine and Angiotensin Pressor Response Curves (and Following a 20 min Stabilization Period) and Then Again Before Chlorothiazide (A) Was Given (and Following Stabilization from the Pressor Response Measurements).

	Mean arterial pressure (mm Hg)		Heart rate (beats/min)		Cardiac output (ml/min)		TPR[a] (mm Hg/ml/min)	
Dog no.	B	A	B	A	B	A	B	A
1	110	105	165	174	930	930	0.115	0.110
2	98	85	123	102	1360	1430	0.070	0.059
3	85	85	180	174	1500	1430	0.057	0.059
4	105	103	108	108	1150	1220	0.085	0.081
5	108	103	148	176	1790	1650	0.057	0.061
6	103	98	156	165	1720	1790	0.059	0.054
7	120	113	174	216	1280	1280	0.093	0.088
8	95	90	152	156	1000	1220	0.094	0.081
9	78	75	114	138	1280	1220	0.061	0.061
Av	100	95	147	157	1334	1352	0.077	0.073

[a] TPR = total peripheral resistance.

blood sample was obtained for the above chemical determinations. For each dose level of the pressor agents before and after chlorothiazide administration the integrated arterial pressure response curve was measured by planimetry. The same procedure was followed in an additional six dogs, except that isotonic saline rather than chlorothiazide was administered. Thus, the integrated pressure responses to the pressor substances in these animals served as the controls for the above (Table II) observations before and after chlorothiazide administration.

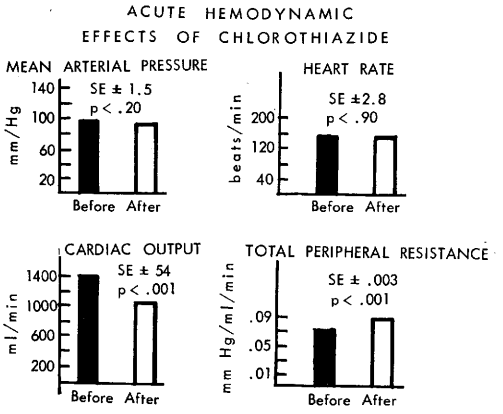

FIG. 1. Immediate hemodynamic effects of intravenous chlorothiazide (25 mg/kg). Solid bars represent the average values of the nine dogs for each hemodynamic index recorded just prior to chlorothiazide administration; the open bars represent the average values recorded 20 min following the diuretic. Also presented are the respective standard error (± 1) of the mean and the probability value for each hemodynamic index.

Statistical analysis of all data was accomplished by paired data analysis (2).

Results. Acute hemodynamic effects of chlorothiazide. Cardiac output before obtaining the pretreatment dose–response studies of the pressor agents was compared with output recorded just prior to chlorothiazide administration and following stabilization and recovery from these studies. Output failed to change significantly (1334 ± 99 and 1352 ± 86 ml/min, respectively, for the control and prechlorothiazide values) (Table I). Mean arterial pressure and heart rate did not change following chlorothiazide. However, cardiac output was reduced (1352 ± 86 to 1061 ± 52 ml/min; $p < .001$), thereby accounting for the significant increase in total peripheral resistance following chlorothiazide ($p < .001$) (Fig. 1; Table II).

Dose–pressor response relationships. Increases in mean arterial pressure and total peripheral resistance without significant change in cardiac output were consistently observed following each dose of norepineph-

TABLE II. Hemodynamic Indices Recorded Just Before (B) and Then 20 min After (A) the Intravenous Administration of Chlorothiazide.

	Mean arterial pressure (mm Hg)		Heart rate (beats/min)		Cardiac output (ml/min)		Stroke vol (ml/beat)		TPR[a] (mm Hg/ml/min)	
Dog no.	B	A	B	A	B	A	B	A	B	A
1	105	105	174	180	930	790	5	4	0.110	0.129
2	85	80	102	116	1430	1220	14	11	0.059	0.064
3	85	88	174	159	1430	1220	8	8	0.059	0.072
4	103	98	108	108	1220	1010	11	9	0.081	0.096
5	103	95	176	176	1650	1220	9	7	0.061	0.077
6	98	90	165	164	1790	1150	11	7	0.054	0.078
7	113	115	216	210	1280	930	6	4	0.088	0.123
8	90	93	156	160	1220	1080	8	7	0.081	0.085
9	75	75	138	132	1220	930	9	7	0.061	0.080
Av	95	93	157	156	1352	1061	9	7	0.073	0.089
± 1 SE	4.1	4.1	12	11	86	52	0.9	0.7	0.006	0.008
± 1 SE (paired data)	1.51		2.8		54		0.4		0.003	
$p <$	.20		.90		.001		.001		.001	

[a] TPR = total peripheral resistance.

rine and angiotensin before and after chlorothiazide. A typical presentation of the pressor response curve is presented in Fig. 2. No significant attenuation of the peak pressor responses was demonstrated following chlorothiazide. However, when the integrated areas of the pressor curves were compared, a significant attenuation of the pressor responses were demonstrated for each dosage level of both norepinephrine and angiotensin (Fig. 3).

These findings are in contrast with those obtained in the six control animals in which cardiac output failed to change significantly following administration of the saline injection (as it did following chlorothiazide). In addition, the pressor area responses to intravenous bolus injections of norepinephrine and angiotensin failed to become significantly attenuated (except at the highest norepinephrine dosage) (Table III).

Serum electrolytes and protein concentration. No significant changes were demonstrated in the serum following the chlorothiazide pressor–response studies in the concentration of sodium, potassium, chloride, and total protein concentration (Fig. 4).

Discussion. The present study demonstrates that immediately following (20 min) intravenous chlorothiazide there is a significant reduction of cardiac output; however, arterial pressure remained unchanged as a result of an increased peripheral vascular resistance. Associated with these hemodynamic changes was a significant attenuation of the pressor area responses to increasing doses of norepinephrine and angiotensin which was independent of intravascular volume and serum electrolyte concentration.

Several studies have indicated that the initial antihypertensive effects of the thiazide diuretics result from the contraction of intravascular volume brought about by natriuresis (3–7). This reduction in arterial pressure has been related to a fall in cardiac output rather than in total peripheral resistance (5, 8); but other workers found the same decrease in cardiac output and arterial pressure 70 min after chlorothiazide, even before plasma volume reduction could have participated significantly (9). This suggests that chlorothiazide produced venodilation and decreased right atrial return. Moreover, others observed equivalent diuresis within 3 hr in

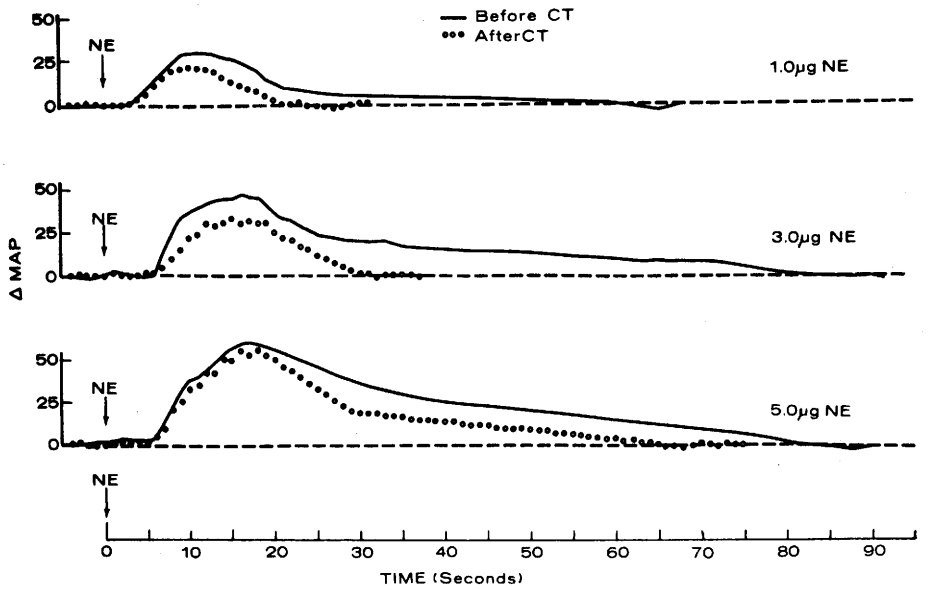

FIG. 2. A typical mean arterial pressure–time curve obtained before (—) and after (○) chlorothiazide (25 mg/kg) for 1.0, 3.0, and 5.0 μg (iv) doses of norepinephrine (NE). (arrow) time of injection. These curves were replotted from one animal's records to present in one figure the chlorothiazide-induced attenuation of the pressor–response curves.

two groups of hypertensive patients who did and did not respond to chlorothiazide with a decrease in cardiac output and arterial pressure (10). These findings indicated to the authors that the vasodepressor effect may be independent of those attributable to oligemia and that a redistribution of intravascular volume into dilated veins might be the means whereby cardiac output is reduced. The results from the present study tend to confirm most of these observations, but further indicate that altered vascular responsiveness and reduced cardiac output may even precede the hypotensive effect of the drug. Thus, initially chlorothiazide could reduce cardiac output by means of redistributing blood into dilated peripheral veins without significantly reducing arterial pressure. Moreover, these hemodynamic alterations take place before intravascular volume is changed by the diuretic effect of chlorothiazide.

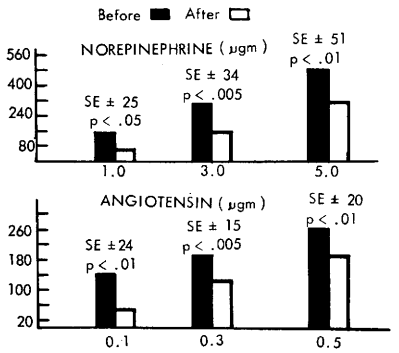

FIG. 3. Dose–pressor response relationships at three different dosages of norepinephrine and angiotensin before (solid bars) and after (open bars) chlorothiazide administration (25 mg/kg). Above each pair of bars, representing average pressor–response areas for the nine dogs, are the paired data analyses (± 1 standard error of the mean and its respective probability level).

Until recently, evidence seemed to indicate that the prolonged antihypertensive effect of the thiazide diuretics might be achieved by a depressor mechanism other than contracted intravascular volume. Thus, within 15 days plasma volume and cardiac output returned towards control values, although arterial pressure remained reduced (11–13). Hansen (7), however, demonstrated significantly reduced total blood volume after 3 months of hydro-

TABLE III. Integrated Arterial Pressor Area Responses (mm²) Following Norepinephrine and Angiotensin in the Control and Chlorothiazide-Treated Dogs.[a]

	Norepinephrine (μg)						Angiotensin (μg)					
	1.0		3.0		5.0		0.1		0.3		0.5	
Dog no.	B	A	B	A	B	A	B	A	B	A	B	A
Control group												
1	184	304	395	441	744	527	341	170	453	335	918	462
2	265	186	524	305	732	522	148	141	264	240	376	478
3	148	128	392	337	542	437	217	176	342	340	568	594
4	270	119	356	500	665	702	332	144	558	308	680	402
5	191	176	634	404	856	577	546	468	954	1178	1437	1280
6	364	173	742	546	1270	1021	486	62	770	300	693	660
Av	237	181	507	422	802	631	345	194	557	450	779	646
SE	45		64		44		62		97		85	
$p <$	.30		.30		.01		.10		.40		.20	
Attenuation (%)	24		17		21		46		19		17	
Chlorothiazide group												
1	81	11	270	87	402	245	186	0	159	93	197	60
2	283	176	483	247	728	428	300	182	278	211	430	398
3	147	31	83	83	129	106	107	20	232	228	304	289
4	222	—2	558	468	828	779	94	—22	338	300	422	420
5	108	44	111	102	203	152	73	60	158	120	151	136
6	45	32	377	77	634	220	101	7	203	34	256	140
7	190	124	346	81	548	182	217	132	120	50	167	109
8	154	115	170	168	404	272	106	0	140	82	309	139
9	44	22	352	134	471	452	85	68	134	61	165	67
Av	142	61	306	161	483	315	141	50	196	131	267	195
SE	22		34		51		18		15		20	
$p <$	.005		.005		.01		.001		.005		.01	
Attenuation (%)	57		47		35		65		33		27	

[a] In both groups the after "treatment" observations were performed 1 hr after the original control.

chlorothiazide therapy, and Tarazi, Frohlich and Dustan (14) reported that plasma and extracellular fluid volumes were reduced and plasma renin activity elevated for periods as long as 2 years after therapy had been initiated. The results from this study in no way suggest that the prolonged hypotensive effect of the thiazide diuretics is not achieved by reduction of intravascular volume. To the contrary, arterial pressure was not significantly reduced in the immediate period under investigation; these results support the concept that the hypotensive effect is not correlated with the reduced cardiac output but may operate by the later effect of contracted intravascular volume.

With respect to local mechanisms, two possibilities exist; one may be a direct vasodilating effect, and the other a direct inhibition of the pressor effect of endogenously secreted pressor substances. In two earlier studies with anesthetized dogs, arterial pressure failed to change significantly during an 80 min intravenous infusion of chlorothiazide (15) or when chlorothiazide was infused locally into constantly perfused regional circulations (15, 16). Therefore, a direct vasodilating effect of the drug seems to be excluded. Several studies indicated that the thiazides might also reduce arterial pressure by altering vascular responsiveness. Thus, Freis *et al.* (4) demonstrated diminished pressor responses to norepinephrine and augmented depressor responses to trimethaphan after chlorothiazide

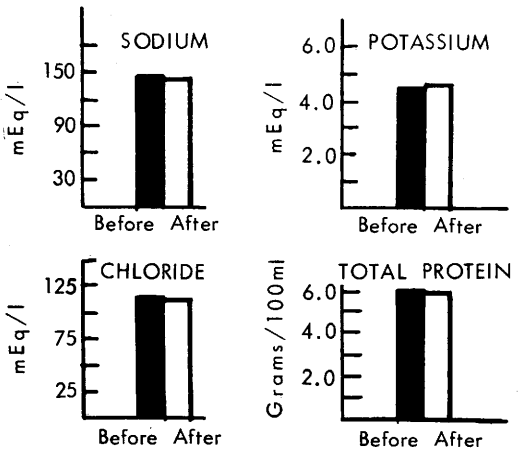

FIG. 4. Average concentrations of the serum electrolytes and total protein concentration obtained before (solid bars) and after (open bars) chlorothiazide administration (25 mg/kg). No changes in concentrations were measured.

treatment; restoration of pretreatment responsiveness was accomplished with repletion of plasma volume using salt-free dextran. The authors concluded, therefore, that the decreased response to norepinephrine was dependent upon the plasma volume reduction and that the natriuretic effect of the drug was only a permissive factor. Other groups subsequently confirmed the findings that the chlorothiazide congeners alter the vascular responses to a variety of pressor and depressor agents as well as the responses to adrenergic nerve stimulation (17–24). The results of the present study, therefore, suggest that the attenuated pressor responsiveness does not apparently relate to the same mechanism reducing arterial pressure since the depressed pressor responses occurred without a simultaneous actual reduction in arterial pressure.

Similar observations have been made using the same pressor agents and technique of integrating the areas of the pressor responses in the anesthetized dog (16). In that study and the present, chlorothiazide attenuated the areas of the arterial pressor response curves. In the former study, however, blood flow to the kidney was held constant, thereby eliminating one hemodynamic variable of the present study. In both studies the factor of time, *per se,* attenuating the vascular responsiveness was eliminated by appropriate control groups. It may be argued, that a diuresis could have existed following chlorothiazide administration thereby altering serum electrolyte concentration and intravascular volume. However, the present study indicates that no changes in intravascular volume occurred during these acute studies since measurement of total protein concentration before chlorothiazide was administered, and once again at the conclusion of the study, showed no significant change. In addition, other workers have shown no change in plasma volume even during the first 6 hr following thiazide administration (10, 25). Another possible mechanism for the altered responsiveness might be as a result of altered baroreceptor response; however, a plot of the change in heart rate with respect to the change in systolic, diastolic, or mean arterial pressure produced by the pressor agents failed to show any difference before and after chlorothiazide. Moreover, since there was no difference in the peak pressor responses to these agents before and after chlorothiazide altered vascular responsiveness must have occurred. Unresolved, is the remote possibility that chlorothiazide has altered baroreceptor control of arterioles. Nevertheless, the most plausible explanation for the altered vascular responsiveness resides in a direct effect of chlorothiazide on arteriolar smooth muscle.

Conclusion. In order to separate electrolyte and intravascular volume mechanisms of the thiazide diuretics from hemodynamic, local vascular, and neural factors mongrel dogs were studied before and 20 min after chlorothiazide (25 mg/kg, iv). Dose–response relationships of the mean arterial pressure curve following single bolus injections of norepinephrine and angiotensin were also obtained before and after chlorothiazide. Serum electrolytes, hematocrit, and plasma protein concentrations remained unchanged. Arterial pressure did not change, whereas cardiac output fell ($p < .001$). At each dose level the integrated pressure response to both pressor agents became significantly attenuated. These data indicate that the major immediate effect of the thiazide is local alteration in

vascular responsiveness. Later, with natriuresis, electrolytic, volume, and possibly neural factors also participate.

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