Mechanism of Antihypertensive Action of Prolonged Administration of Hydrochlorothiazide in Rabbit and Dog

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ABSTRACT

The effect of hydrochlorothiazide on vascular reactivity to various drugs was studied in rabbit and dog. In rabbit, dose response in the mesenteric artery and vein was examined in vitro following a treatment with hydrochlorothiazide for 6 to 10 weeks. Norepinephrine produced significantly less contraction in the vein of treated than of control animals (P < 0.005), but the effect of acetylcholine, barium chloride, angiotensin, papaverine, and adenosine triphosphate (ATP) was not altered. In the hindlimb of dogs treated with hydrochlorothiazide for 6 to 8 weeks, intra-arterial norepinephrine caused substantially less pressure increment in the resistance vessels, perfused at constant flow rate, of treated than of control animals. Stimulation of lumbar sympathetic nerves also augmented pressure in the femoral artery less in treated than in control dogs. No such difference in the effect of intra-arterial ATP or angiotensin was observed between the two groups of animals. Preliminary results both in the rabbit and in the dog failed to demonstrate a significant alteration in water, sodium, potassium, and calcium content of the iliac artery or vein after hydrochlorothiazide treatment. Diminished response to norepinephrine in the vessels after hydrochlorothiazide treatment may be responsible for the antihypertensive effect after the prolonged administration of this drug.

ADDITIONAL KEY WORDS

veins vasoactive drugs electrolytes in vessels

Benzothiazides have been used for years in the treatment of high blood pressure, but the mechanism of this antihypertensive action is not yet clarified (1). Depletion of plasma and extracellular volumes can probably explain the early "hypotensive effect" of the drugs (2-4). In this stage re-expansion of plasma volume with dextran was reported to restore blood pressure to pretreatment level (5, 6). Depletion of plasma volume is unlikely, however, to be the sole cause of lowering the blood pressure after prolonged administration of benzothiazides. The plasma and extracellular volume and sodium space were found to have returned nearly to their original values after longer treatment with thiazides while the blood pressure remained lowered (1, 2). Diminished vascular activity was suggested as an explanation of the antihypertensive effect in this later stage. The evidence for the depressed vascular response is based almost entirely on experiments in which diuretics were administered in single dose or for a short term (7-11). Under such circumstances, the decreased plasma volume and the consequent increase in renin secretion may be responsible for the alteration in vascular response (12). Eckstein et al. (13) were the only investigators who examined the effect of norepinephrine after a longer treatment (5 weeks) with chlorothiazide. In those circumstances, the decreased plasma volume and the consequent increase in renin secretion may be responsible for the alteration in vascular response (12). Eckstein et al. (13) were the only investigators who examined the effect of norepinephrine after a longer treatment (5 weeks) with chlorothiazide. In those
reports our experiments with rabbits and dogs, directly on the vessels, excluding interference of prolonged administration of benzothiazide vascular effect of norepinephrine was altered. To determine how much the cardiac and the experiments, however, norepinephrine was administered intravenously and so it is difficult to determine how much the cardiac and the vascular effect of norepinephrine was altered.

We were interested in studying the effects of prolonged treatment with hydrochlorothiazide directly on the vessels, excluding interference of a possible cardiac effect. In this paper we report our experiments with rabbits and dogs, studying the effect of hydrochlorothiazide treatment on response of both arteries and veins to various drugs.

**Methods**

**Experiments in Rabbits**

White male rabbits (New Zealand) between 1800 and 2400 g were used in these experiments. Thirty animals received hydrochlorothiazide and 30 lactose mixed with ground food. The doses of hydrochlorothiazide and the duration of treatment are given in Results. The weight of the animals was recorded once a week. Following the treatment with hydrochlorothiazide or with lactose, the animals were killed by a blow on the head, the superior mesenteric vein and artery removed and placed in an organ bath. The venous segment and the helical strip of the artery, both about 20-mm long, were suspended under 1.0 g tension for 60 minutes before exposure to the drugs. The bath contained Krebs solution (in grams: 6.90 NaCl, 0.35 KCl, 0.28 CaCl₂, 2.10 NaHCO₃, 0.11 MgCl₂·6H₂O, 0.14 NaH₂PO₄·H₂O, 2.00 glucose/liter) at 37°C and was bubbled with 95% oxygen and 5% carbon dioxide. The drugs were added to the bath in increasing concentrations in 0.1-ml volumes, except for the highest concentrations given in 0.3 ml. The doses in Results are expressed as the final concentration of base in the bath. The following vasoactive materials were used: norepinephrine bitartrate (Levophed, Winthrop), acetylcholine chloride (British Drug Houses), barium chloride (Fisher certified reagent), angiotensin amide (Hypertensin, Ciba), papaverine hydrochloride (British Drug Houses), and adenosine 5′-triphosphate (Sigma Chemical Company). The effect of the drugs on vessels was recorded isometrically with Grass FT 03 displacement transducers on a Grass Model 7 recorder.

Before the removal of the mesenteric vessels, blood was sampled from the heart for analysis of sodium and potassium with a Packard Hewlett atomic absorption flame photometer, Model 5050A. Segments of the iliac artery and the iliac vein, each approximately 30-mm long, were removed for analysis of sodium and potassium concentration. After removal, the vessels were rinsed with 0.113 M MgCl₂, dried with filter paper and their wet weight was measured. They were reweighed after drying in an oven at about 110°C until constant weight was obtained. The dried samples were treated with 10% HNO₃, warmed until they were digested and then analyzed for sodium and potassium by atomic absorption flame photometry.

**Experiments in Dogs**

Mongrel dogs between 13.5 and 23.0 kg received one 50-mg hydrochlorothiazide tablet every day for 6 to 8 weeks. At the end of the treatment the animals were anesthetized with 30 mg/kg sodium pentobarbital iv. The femoral artery was cannulated and perfused with blood from the contralateral artery at constant flow rate. The rate of flow was regulated with a Sigmamotor pump to obtain pressures almost identical with those before cannulation of the artery. Vasoactive drugs were infused with an infusion pump (Harvard Apparatus Co, Model 903) into the tube leading to the femoral artery. The drugs were given in three dose levels at a rate of 1 ml/min of each concentration for 5 minutes. The following drugs were given: norepinephrine (Levophed), adenosine 5′-triphosphate (Sigma Chemical Company), and angiotensin amide (Hypertensin, Ciba). The interval between infusion of various drugs was at least 30 minutes. Following the infusion of the drugs, the left lumbar sympathetic chain was exposed by a transabdominal approach. A shielded platinum electrode was placed on the nerve and a Grass stimulator SD-5 was used for stimulation. Voltage was varied between 0.5 and 10 v, frequency between 0.2 and 15 impulses/sec, duration of impulse was 5 msec, and duration of stimulation, 15 seconds.

Pressures were simultaneously recorded in the perfused femoral artery and vein, in the small artery in the quadriceps muscle (with the polyethylene tube of 0.05 inch o.d. in wedge position) and in the femoral artery above the cannulation. Pressures were recorded by Statham P23 AC transducers on a Grass Model 7 recorder.

Arterial blood was sampled from the anesthetized dogs before the infusion of drugs for analysis of sodium, potassium, and calcium. At the end of the experiments, segments of the iliac artery and veins, approximately 30-mm long, were removed for analysis of sodium, potassium, and calcium concentration with a flame spectrophotometer (14).

Unless specified otherwise, the t-test was used for statistical analysis.

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Results

EXPERIMENTS IN RABBITS

No ill effects of hydrochlorothiazide were detected in rabbits during a treatment of 6 to 10 weeks. The increase in body weight was similar in treated and control groups.

Twenty-four rabbits received 30 mg of hydrochlorothiazide per day. In the mesenteric vein of these animals, norepinephrine produced less contraction than in the veins of control animals. Parts of an original record are shown in Figure 1. The difference in norepinephrine response of treated and control animals was variable, but quite consistent. Figure 2 demonstrates the absolute tension values using eight concentrations of norepinephrine in the mesenteric vein, when the initial tension of the vessels was adjusted to 1.0 g. Higher concentrations of norepinephrine caused consistently less contraction in the veins of treated than of control animals. Using the analysis of variance, the two dose-response curves were significantly different ($P < 0.005$). Both fast and slow components of norepinephrine-induced contractions were depressed to a similar extent in the treated rabbits. Contractions in the same veins produced by acetylcholine, barium chloride (Fig. 3), or by angiotensin were not significantly depressed in treated animals. No difference was detected in the amount of elongation required to increase tension from 0 to 1.0 g in the vessels, indicating a similar response to stretch in both groups of rabbits.

Tension produced by norepinephrine in the mesenteric vein and artery of two rabbits. The treated animal received 30 mg of hydrochlorothiazide and the control animal received lactose, both for 45 days. The same concentrations of norepinephrine, shown as the final concentrations in the bath, resulted in substantially less contraction in vessels of the treated than of the control rabbits.

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Tension after various concentrations of papaverine and ATP, given when the vessels were contracted by $10^{-6}$ g/ml norepinephrine, was quite similar in control and treated animals. Since the "initial" tension after norepinephrine was less in veins of treated rabbits, the relaxation caused by papaverine or ATP was slightly less (Fig. 3).

Tension following various concentrations of acetylcholine and barium chloride and tension loss caused by papaverine and ATP in the mesenteric vein. The numbers in parentheses refer to the number of rabbit pairs; of each pair, one was given 30 mg of hydrochlorothiazide daily and the other served as control.
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In further experiments, rabbits received 10 mg of hydrochlorothiazide for 3 to 4 weeks; one additional rabbit received 30 mg for one day, and another, 100 mg. In none of these animals was the tension increment in the superior mesenteric vein produced by norepinephrine depressed when compared to that of control animals treated with lactose for identical periods.

In the helical strips of the mesenteric arteries mean contractions caused by higher doses of norepinephrine were less after 6 to 10 weeks of treatment with hydrochlorothiazide than in control specimens. The scatter of results was pronounced in arterial strips and therefore the response to norepinephrine was not significantly different between treated and control animals.

Serum sodium and calcium remained essentially unaltered (Table 1) after 6 to 10 weeks of treatment with hydrochlorothiazide. Hemolysis could not be completely prevented in rabbits and therefore their serum potassium values were not reliable. The water content, both in the iliac artery and vein, was almost identical in control and treated animals (Table 1). The sodium and potassium concentration of the vessels was not different in the two groups when the results were expressed both as mEq per wet or dry weight of tissue. Furthermore no correlation could be detected between the efficacy of norepinephrine to produce contraction in the mesenteric vessels and the sodium or potassium concentration in the iliac vessels or in the serum of a given animal.

**TABLE 1**

Effect of Hydrochlorothiazide on Serum Concentration of Sodium, Potassium, Calcium and Water Content in the Iliac Vein and Iliac Artery of Rabbits and Dogs

<table>
<thead>
<tr>
<th></th>
<th>Serum concentration (mEq/liter)</th>
<th>H2O content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
<td>K</td>
</tr>
<tr>
<td>Rabbit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>14</td>
<td>141.3 ± 1.6</td>
</tr>
<tr>
<td>treated</td>
<td>15</td>
<td>139.3 ± 1.4</td>
</tr>
<tr>
<td>Dog</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>11</td>
<td>149.7 ± 2.7</td>
</tr>
<tr>
<td>treated</td>
<td>14</td>
<td>149.7 ± 2.4</td>
</tr>
</tbody>
</table>

**EXPERIMENTS IN DOGS**

Fifteen mongrel dogs received hydrochlorothiazide, 50 mg/day, for 6 to 8 weeks without any apparent ill effects. At the end of treatment, before the infusion of drugs, pressure in perfused femoral artery was 154.3 ± 3.9 mm Hg, compared to 143 ± 3.8 mm Hg in 15 control dogs. Pressure in the small vein was 16.4 ± 2.3 mm Hg in treated and 17.8 ± 2.0 mm Hg in control animals, and in the femoral vein 3.5 ± 0.7 and 3.2 ± 0.6 mm Hg, respectively. Figure 4 demonstrates the pressure alterations in the perfused femoral artery, small artery, and small vein of the paw by the three vasoactive materials, norepinephrine, ATP, and angiotensin, infused into the artery. Each drug was given in three gradually increased doses, for a total of 15 minutes. Pressure in the femoral vein was not altered appreciably by any of the drugs and therefore these values are not shown. Pressure in the nonperfused femoral artery was slightly elevated by the highest dose of norepinephrine and slightly decreased after the highest dose of ATP, but otherwise the effect of the drug was "localized" in the perfused hindlimb. The absence of an effect on the systemic circulation was evident also in the lack of any alteration in heart rate by the drugs. Norepinephrine (0.3, 1.0, and 3.0 μg/min) increased the pressures in the femoral artery and small artery in the quadriceps muscle substantially more in control than in treated dogs (Fig. 4). Pressure increments in the small vein of the paw were not different between the two groups. Since the blood flow in these experiments was kept constant, the increase of resistance in large
and small arteries during infusion was much less in treated than in control dogs. The infusion of angiotensin (0.3, 1.0, and 3.0 µg/min) into the femoral artery increased pressure in the perfused large and small artery and to some extent in the small vein of the paw. The effect was somewhat less in treated than in control dogs (Fig. 4). Adenosine triphosphate (0.03, 0.1, and 0.3 mg/min) diminished pressure in the perfused artery and small artery to almost identical degrees in the two groups; pressure in the small vein of the paw was not altered appreciably.

Stimulation of lumbar sympathetic nerves with 1 v and 15 impulses/sec increased the pressure in the femoral artery and small artery, but not in the small vein; the latter pressures were elevated only by stimulation with higher voltage. Pressure increments in the femoral artery caused by stimulation with increasing frequency at 10 v are shown in Figure 5. The response to stimulation of the sympathetic nerves was markedly diminished in the treated dogs. Similar results were obtained in the small arteries.

Serum potassium was significantly lower (P < 0.01) after 6 to 8 weeks of treatment with hydrochlorothiazide, but sodium and calcium were practically unchanged (Table 1). Water content and sodium, potassium and calcium concentrations, expressed per wet or dry weight, in the iliac artery and the iliac vein were not different in the vessels examined from 11 treated and 6 control dogs.

Discussion

In these experiments hydrochlorothiazide, administered for 6 weeks or longer, significantly depressed the in vitro effect of norepinephrine in the mesenteric veins of the rabbit. Similar results were obtained in vivo in the arteries of the dog. The depression of norepinephrine contraction in the mesenteric artery of the rabbit was statistically not significant. These vessels however, unlike the veins, were helically cut and so a varying number of muscle elements were severed. The greater scatter in tension increments in the artery than in the vein (as shown by the consistently higher coefficient of variation) produced by various concentrations of norepinephrine could explain the absence of signific-
Effect of sympathetic nerve stimulation (10 v, 5 msec, varying frequency) on the pressure in the femoral artery of 12 dogs given 50 mg of hydrochlorothiazide daily (——–—) and in 10 control dogs (—–—–).

Figure 2

Effect of sympathetic nerve stimulation (10 v, 5 msec, varying frequency) on the pressure in the femoral artery of 12 dogs given 50 mg of hydrochlorothiazide daily (——–—) and in 10 control dogs (—–—––).

The selective antagonism of hydrochlorothiazide towards norepinephrine is particularly noteworthy. Although some depression of vasoconstriction produced by other drugs occurred after hydrochlorothiazide treatment, this was much less pronounced and consistent than was the depression of the norepinephrine response. Other investigators who examined the effect of vasoactive drugs after a single or short term administration of hydrochlorothiazide reported a diminished vascular reactivity in general (10). In those experiments, however, the "acute" effect of thiazides on plasma and extracellular volume and sodium space with consequent increase in renin secretion may have influenced the results. Our results both in rabbit and in dog demonstrate a diminution of norepinephrine response without similar depression of contraction produced by other drugs. Thus hydrochlorothiazide does not impair the ability of vascular smooth muscle to contract, only the effect of norepinephrine. Significantly this effect could also be detected in vitro in vessels without innervation and without possible exposure to any circulating vasoactive material. The dose response to norepinephrine in the rabbit suggests a noncompetitive antagonism of hydrochlorothiazide treatment, since its antagonism was not overcome by higher doses of norepinephrine. The site at which it interferes with norepinephrine contraction is not known, but it is somewhere in coupling the receptor-drug reaction with muscular contraction.

The hypotensive effect of thiazides is usually explained by sodium depletion, consequent to increased diuresis (17). Since good evidence for electrolyte depletion in the vessels by thiazides is lacking, this explanation cannot be easily maintained. Chlorothiazide treatment in rats was reported to decrease
potassium in muscle, small intestine, and other tissues but not in vessels (18, 19). Though diazoxide was found to decrease potassium in the aorta of hypertensive rats (20), the sodium content in the vessels was not diminished by either diazoxide, chlorothiazide, or hydrochlorothiazide (18-22). Water, sodium, potassium, and calcium content of the arteries and veins were not significantly altered by hydrochlorothiazide treatment either in rabbit or in dog. These findings agree with those of other investigators (18, 19, 21, 22) in the rats as far as arterial sodium and potassium are concerned. We are unaware of any data at all on electrolytes in the veins. The development of more precise methods for measuring changes in tissue electrolytes may help to clarify the antihypertensive effect of diuretics. In addition, the understanding of the distribution of electrolytes among various cellular compartments may be necessary to appreciate the true effect of thiazides on the vessels. For example, hydrochlorothiazide may alter the noradrenephrine response by affecting the availability of functionally active calcium ions important for the contraction of vascular smooth muscle.

How does the present study help in clarifying the antihypertensive mechanism of hydrochlorothiazide? Diminished constriction in arteries and veins caused by noradrenephrine released from adrenergic nerves may explain the decrease in blood pressure. This study does not minimize the importance of the depletion of plasma volume in decreased blood pressure caused by benzothiadiazides, but suggests the presence of an additional mechanism evident only after prolonged use of hydrochlorothiazide, namely a specific depression of noradrenephrine vasoconstriction.

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