Antihypertensive Drugs Inhibit Hypertension-Associated Aortic DNA Synthesis in the Rat

ALEX L. LOEB AND BARBARA L. BEAN

SUMMARY The effect of antihypertensive drug treatment on aortic DNA synthesis was examined in rats with two-kidney, one clip renal hypertension and in spontaneously hypertensive rats (SHR). In two-kidney, one clip hypertensive rats, hypertension developed over a 2-week period. Four days after clipping the renal artery, during the onset of hypertension, there was an increase in aortic DNA synthesis. Aortic DNA synthesis was also increased 3 weeks later, when hypertension had been established. Captopril, hydralazine, and verapamil were each able to prevent the increase in aortic DNA synthesis and the rise in blood pressure when given throughout the first 5 days of the developing phase of hypertension, or when given to rats with established hypertension. Drug treatment of sham-operated rats had no significant effect on DNA synthesis, although blood pressure was decreased. There were no differences in blood pressure or aortic DNA synthesis in 4-week-old SHR, as compared with age-matched Wistar-Kyoto (WKY) controls or normal Wistar rats. At 17 weeks of age, when hypertension was established, aortic DNA synthesis was significantly enhanced in the SHR. Captopril or hydralazine treatment was able to reduce blood pressure and DNA synthesis to levels seen in the WKY. At 21 weeks of age, DNA synthesis in the SHR had declined to the same levels as in the WKY. Captopril, hydralazine, and verapamil may have a common ability to reduce intracellular calcium and therefore inhibit DNA synthesis. In support of this, ouabain treatment, which increases intracellular calcium by inhibiting the Na⁺-K⁺ pump, produced a significant increase in the rate of DNA synthesis. These findings suggest that the increase in aortic DNA synthesis may be dissociated from the development of hypertension and that antihypertensive drug treatment can inhibit the increase in DNA synthesis even in animals with established hypertension. (Hypertension 8: 1135–1142, 1986)

KEY WORDS • vascular DNA synthesis • renal hypertension • spontaneously hypertensive rats • captopril • hydralazine • verapamil • ouabain

ARTERIES from hypertensive patients1-2 and from animals with experimental hypertension3-6 have a greater smooth muscle cell mass than similar vessels from normotensive controls and are hyperresponsive to pressor agents.7-9 The mechanisms by which these structural changes occur are not well understood; however, some of the consequences are clear. An increase in vessel wall thickness caused by an increase in smooth muscle mass would lead to hyperactivity of the vasculature without altered sensitivity or reactivity of the contractile elements in the muscle. Since wall contraction is greatly dependent on the outermost muscle layer, where most of the neuroeffector junctions are located,10 a given nerve stimulus would induce more contraction simply because there are more muscle cells to contract, even though each would show a normal percentage decrease in length. The increased contraction would itself increase resistance and pressure, simply because there are more muscle cells to contract, even though each would show a normal percentage decrease in length. The increased contraction would itself increase resistance and pressure, simply because there are more muscle cells to contract, even though each would show a normal percentage decrease in length. The increased contraction would itself increase resistance and pressure, simply because there are more muscle cells to contract, even though each would show a normal percentage decrease in length. The increased contraction would itself increase resistance and pressure, simply because there are more muscle cells to contract, even though each would show a normal percentage decrease in length. The increased contraction would itself increase resistance and pressure, simply because there are more muscle cells to contract, even though each would show a normal percentage decrease in length. 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the structural changes seen in hypertension may be due to an increase in the number of medial smooth muscle cells. However, evidence is accumulating that the increase in wall thickness and DNA synthesis may be due to increases in smooth muscle cell ploidy rather than increases in cell number in some models of hypertension.\(^{17-19}\)

The stimulus for the increase in DNA synthesis and vessel thickness is not well understood. Blood pressure cannot be the only stimulus, since DNA synthesis has been reported to be increased both before\(^{20}\) and during\(^{16}\) the increase in blood pressure following renal artery stenosis in the rat.

The experiments described in the present report were designed to examine some of the possible causes of increased vascular DNA synthesis in hypertension through the use of selective pharmacological tools in both renal and spontaneous hypertension in the rat. Three drugs with different primary mechanisms of action were used. Captopril (SQ 14228), an angiotensin converting enzyme inhibitor, was used to inhibit the renin-angiotensin system, since angiotensin II is known to be a tropic hormone in certain circumstances\(^{21-23}\) and because angiotensin II levels are known to be raised in renal hypertension.\(^{24}\) In addition, captopril is known to decrease blood pressure in both renal and spontaneous hypertension in the rat.\(^{25,26}\) The vasodilator hydralazine was used since it would decrease pressure by acting directly on the smooth muscle.

Hydralazine, while not directly effecting angiotensin synthesis, would be expected to increase reflex sympathetic outflow, which would increase plasma renin activity, indirectly increasing angiotensin levels. Renal hypertensive animals were also treated with verapamil to examine the possibility that decreased calcium influx could inhibit the increased DNA synthesis seen in renal hypertension. Conversely, the effect of increased intracellular calcium on aortic DNA synthesis was investigated by use of the Na\(^+\)-K\(^+\)-ATPase inhibitor ouabain.

Materials and Methods

Male and female Wistar rats (weight, 190–250 g) were obtained from Harlan (Frederick, MD, USA). Spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) were obtained from Charles River Breeding Laboratories (Wilmington, MA, USA). Rats were housed in groups of four to six in wire mesh cages. In most cases, they were fed Purina rat chow (St. Louis, MO, USA) and tap water ad libitum.

Experimental renal hypertension was induced by placing a silver clip (0.2 mm inside diameter) on the left renal artery, leaving the contralateral kidney untouched. Control animals received sham operations in which the renal artery was exposed but not clipped.

Blood pressures were measured routinely in all experiments by tail plethysmography in conscious rats using either the Doppler (Narco Bio-Systems, Houston, TX, USA) or photoelectric (ITC, Landing, NJ, USA) detection methods. With either method, rats were warmed for 20 minutes at approximately 37°C before recordings were made. At least five determinations of systolic pressure were made for each rat, and the mean of the lowest three pressures was used to calculate the daily systolic blood pressure value.

Drugs were dissolved in saline and administered intraperitoneally (captopril, gift of D. Horovitz, Squibb Institute for Medical Research, Princeton, NJ, USA, and ouabain, Sigma, St. Louis, MO, USA) or in the drinking water (hydralazine, CIBA-Geigy, Ardsley NY, USA; and verapamil, Knoll Pharmaceutical, Whippany, NY, USA). Hydralazine and verapamil were protected from ultraviolet light by wrapping the water bottles in aluminum foil. All drug solutions were freshly made each day.

The procedure for determining aortic DNA synthesis was adapted from that of Khairallah et al.\(^{22}\) Aortas were removed from the rats for biochemical analysis following decapitation. The aorta from the heart to the femoral bifurcation was removed and placed into ice-cold saline. The loose connective tissue was removed with forceps, and the aorta was rinsed in fresh saline to remove blood and debris. It was then cut lengthwise to expose the interior of the vessel and was further rinsed with ice-cold saline to remove any residual blood. The aortas were then blotted on filter paper, weighed, and placed into individual 25-ml Erlenmeyer flasks containing 20 ml of Krebs-Heinsleit buffer solution that had been gassed with 95% oxygen, 5% carbon dioxide for at least 30 minutes. Sections of sample vessels showed little, if any, remaining endothelium at the end of the procedure.

The flasks were preincubated for 60 minutes at 37°C in a shaking water bath. At the end of this preincubation period, 40 \(\mu\)Ci of \([3H]\)thymidine (1.0 mCi/ml, 50 Ci/mM; Moravek Biochemicals, La Brea, CA, USA) was added. The flasks then were incubated for 60 minutes. Incubation was followed by three rinses with ice-cold saline.

The tissue was homogenized in 2 ml of 0.2 N perchloric acid (PCA) using a glass on glass hand-held homogenizer (Model 21; Kontes, Vineland, NJ, USA). Following homogenization and centrifugation, the pellet was washed and recentrifuged twice to remove any acid-soluble radioactivity. The supernatant fraction was thought to contain free thymidine and metabolites not incorporated into DNA.

The pellet was then resuspended in 2 ml of 0.5 N PCA and heated at 90°C for 20 minutes to hydrolyze the DNA. After cooling, the hydrolysate in the supernatant was used for DNA determinations and the pellet was used to estimate protein.

The incorporation of \([3H]\)thymidine into DNA was quantified by pipetting 500 \(\mu\)l of DNA hydrolysate into counting vials containing 10 ml of scintillation fluid (Liquisint; National Diagnostics, Parsippany, NJ, USA). Aliquots of the DNA extract were also used for the assay of DNA by the method of Burton.\(^{27}\) Incorporation of thymidine into DNA was linear for up to 2 hours. Since DNA synthesis was measured as \([3H]\)thymidine incorporated into DNA per hour, the process is referred to in the text as the rate of DNA synthesis and is expressed as dpm/\(\mu\)g DNA.
Following removal of supernatant fractions for DNA determinations, the protein pellet from each sample was solubilized in 1.0 N NaOH for assay using the Bradford technique. Data are reported as group mean ± 1 SEM. Significance of differences between treated and control groups was estimated using the Student’s t test or analysis of variance followed by the Dunnett’s test for comparing a control group with a number of treated groups. A p value less than 0.05 was accepted as being statistically significant.

Results

On Day 4 after clipping, blood pressures in both clipped and sham-operated groups were not significantly different, as shown in Figure 1. Blood pressures in the rats with renal artery clips were significantly elevated thereafter. Experiments were performed 4, 5, and 21 days after clipping to examine the effect of renal hypertension on aortic DNA synthesis at developing and established stages of hypertension.

Figure 1 also shows the effect of antihypertensive drug treatment on DNA synthesis and blood pressure in sham-operated rats or rats with renal artery clips on Days 4 and 5 postoperation. Those animals given antihypertensive drugs were treated throughout the 4- or 5-day period, respectively. On Day 4, the blood pressure in the clipped, untreated group was not significantly different from that in the sham-operated group (131 ± 3 vs 126 ± 4 mm Hg). On Day 5, the clipped, untreated group had a significantly higher blood pressure than the sham-operated group. Those rats treated with captopril, hydralazine, or verapamil had blood pressures similar to the sham-operated control group. Synthesis of DNA was enhanced in the clipped, untreated group on Days 4 (p < 0.05) and 5 (p < 0.05) compared with that in the sham-operated control group, in spite of the fact that blood pressure was increased only in the 5-day animals. In addition, there was no correlation between blood pressure and DNA synthesis at this time (r = 0.348). Captopril, hydralazine, and verapamil were each able to prevent the increase in DNA synthesis when given throughout the first 4 or 5 days after operation, respectively. Although DNA synthesis in the hydralazine-treated clipped rats at 5 days was significantly increased when compared only with that in the sham-operated rats (t test), analysis of variance followed by Dunnett’s test for comparing multiple treated groups with a single control group (the sham-operated rats) found that only the clipped, untreated group had significantly higher DNA synthesis. The large variability in DNA synthesis among the hydralazine-treated group may have been due to the reflex increase in sympathetic outflow and angiotensin II seen after hydralazine treatment.

The effect of antihypertensive drug treatment on aortic DNA synthesis was also examined in rats with established hypertension. In this study, only clipped rats with blood pressure greater than 150 mm Hg were used. Hypertensive rats were dosed daily with saline, captopril, hydralazine, or verapamil from Day 15 until Day 21 after operation. In addition, sham-operated groups were also treated to determine drug effects in normotensive animals. As shown in Figure 2, both blood pressure and aortic DNA synthesis were elevated in the saline-treated, hypertensive rats as compared with the sham-operated controls. In addition, captopril, hydralazine, and verapamil were able to normalize both DNA synthesis rate and blood pressure. In sham-operated animals, captopril and hydralazine were able to significantly reduce blood pressure by 15% and 16%, respectively when compared with values in the saline-treated sham-operated group. In these animals, however, drug treatment did not have any significant effect on aortic DNA synthesis (data not shown).

Synthesis of DNA was also measured in the SHR. At 4 weeks of age, SHR, WKY, and normal Wistar rats had similar blood pressures and aortic DNA synthesis rates, as shown in Figure 3. At 17 weeks of age, when hypertension in the SHR had become established, aortic DNA synthesis was increased compared with that in control WKY, as shown in Figure 4. Captopril (100 mg/kg/day i.p.) or hydralazine (80 mg/L in the drinking water) for 6 days was able to reduce aortic DNA synthesis rates to levels not different from those of WKY and to significantly lower blood pressure, although not to the levels seen in WKY.

This experiment was repeated in 21-week-old SHR and WKY. In contrast to the previous experiment, the untreated SHR and WKY had similar aortic DNA synthesis rates, even though the SHR had a mean blood pressure of 201 ± 6 while WKY had a mean blood pressure of 127 ± 6 mm Hg, as shown in Figure 5. Five days of treatment with either drug depressed DNA synthesis in the SHR to a similar extent.

Since all the drugs used may be able to decrease the availability of intracellular calcium for contraction, we wanted to see whether an increase in cell calcium through Na⁺-Ca²⁺ exchange induced by Na⁺,K⁺-ATPase inhibition could increase vascular DNA synthesis and blood pressure in normotensive animals. For this purpose, ouabain was used to test the effects of Na⁺-K⁺ pump inhibition on DNA synthesis and blood pressure in normal Wistar rats. One group of rats was treated with ouabain (5 mg/kg/day i.p.), and the other group was given saline injections. Blood pressure was measured on Days 1 and 4 of treatment. The rats were killed and aortic DNA synthesis rates were estimated on Day 5 of treatment. As shown in Table 1, inhibition of Na⁺,K⁺-ATPase by ouabain for 5 days produced a small but statistically significant increase in aortic DNA synthesis rates and DNA content as compared with values in saline-treated controls. Neither blood pressure nor protein content was altered significantly by the treatment.

Discussion

The present study has demonstrated that captopril, hydralazine, or verapamil, three antihypertensive drugs with different pharmacodynamic properties, were each able to prevent the increase in aortic DNA...
synthesis seen during the early phase of two-kidney, one clip renal hypertension in the rat, before the hypertension had become manifest, and later, when the rats had developed increased blood pressure.

The finding that captopril was able to normalize the increase in aortic DNA synthesis in the rats with developing and established renal hypertension suggested that either angiotensin II or pressure was involved in increasing DNA synthesis. These results are in contrast to those of Rorive et al., who reported that captopril did not affect the increased rate of aortic DNA synthesis in one-kidney, one clip renal hypertensive rats using a 50 mg/kg daily dose despite the fact that captopril lowered blood pressure. The difference

FIGURE 1. Effect of antihypertensive treatment on DNA synthesis and blood pressure 4 and 5 days after renal artery clipping. [3H]Thymidine incorporation into aortic DNA (top panel) and systolic blood pressure (bottom panel) in the same animals. Animals were injected intraperitoneally with saline (Day 4: sham-operated, n = 5, clipped, n = 5; Day 5: sham-operated, n = 4, clipped, n = 18) and captopril (Day 4, n = 5; Day 5, n = 6), 30 mg/kg/day. Hydralazine (Day 4, n = 5; Day 5, n = 6), 50 mg/L in the drinking water, and verapamil (Day 5, n = 5), 1 g/L in the drinking water, were administered orally. Drugs were administered just before clipping and throughout the experimental period. All comparisons were made against the saline-treated, sham-operated group. Asterisks indicate a significant difference (p < 0.05) when compared with the control group.

FIGURE 2. Effect of antihypertensive treatment on DNA synthesis and blood pressure 3 weeks after renal artery clipping. [3H]Thymidine incorporation into aortic DNA (top panel) and systolic blood pressure (bottom panel) in the same animals. Drug treatments were identical to those in Figure 1. Drug administration in the sham-operated (saline, n = 14), clipped (saline, n = 18), captopril-treated (n = 11), hydralazine-treated (n = 5), and verapamil-treated (n = 4) groups was begun after the blood pressure had become hypertensive and was continued for 6 days. Asterisks indicate a significant difference (p < 0.05) when compared with the control group.
INHIBITION OF HYPERTENSION-ASSOCIATED DNA SYNTHESIS/Loeb and Bean

Figure 3. Aortic DNA synthesis and blood pressure in 4-week-old SHR, WKY, and Wistar rats. Aortic DNA synthesis (top panel) and blood pressure (bottom panel) in SHR (n = 6), WKY (n = 6), and normal Wistar rats (n = 5). There were no significant differences between groups.

Figure 4. Effect of antihypertensive treatment on DNA synthesis and systolic blood pressure in 17-week-old SHR. Aortic DNA synthesis (top panel) and systolic blood pressure (bottom panel) in the same animals. Saline and captopril, 100 mg/kg/day, were given intraperitoneally. Hydralazine was given orally in the drinking water (80 mg/L). There were five animals in each group. Asterisks indicate significant difference (p<0.05) compared with WKY.

Figure 5. Effect of antihypertensive drug treatment on DNA synthesis and systolic blood pressure in 21-week-old SHR. Aortic DNA synthesis (top panel) and systolic blood pressure (bottom panel) in the same animals. There were five animals per group. Drug treatment is the same as in Figure 4. Asterisks indicate significant difference (p<0.05) compared with WKY.

To test the possibility that angiotensin II is not responsible for the increased DNA synthesis, hypertensive SHR and other rats were treated with the vasodilator hydralazine during the established and developing phases of renal hypertension. Our observation that both captopril and hydralazine were able to decrease aortic DNA synthesis along with blood pressure in both models of hypertension suggests that the increase in blood vessel tone, and therefore wall stress, could be responsible for the increases in DNA synthesis. Alternatively, it could be speculated that captopril and hydralazine, although different pharmacodynamically, may share some other common effect, which brings about the reduction in the rate of DNA synthesis after a hypertensive stimulus. Both drugs are

Table 1. Effect of Ouabain on Blood Pressure and DNA Synthesis in Normal Wistar Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Saline (n = 5)</th>
<th>Ouabain (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>133 ± 2</td>
<td>141 ± 6</td>
</tr>
<tr>
<td>[3H]thymidine (dpm/µg DNA)</td>
<td>14.4 ± 1.0</td>
<td>19.7 ± 0.5*</td>
</tr>
<tr>
<td>DNA (µg/µg protein)</td>
<td>37.3 ± 2.6</td>
<td>38.4 ± 2.3</td>
</tr>
<tr>
<td>DNA (µg/µg wet weight)</td>
<td>2.06 ± 0.04</td>
<td>2.24 ± 0.06*</td>
</tr>
<tr>
<td>Protein (µg/µg wet weight)</td>
<td>56.1 ± 4.1</td>
<td>61.2 ± 2.1</td>
</tr>
</tbody>
</table>

Values are means ± 1 SEM. *p < 0.05, compared with saline values.
Ouabain (5 mg/kg/day i.p.) or saline was administered to normal Wistar rats for 5 days. Blood pressure and DNA synthesis were measured as described in Materials and Methods.

between their findings and those of the present study may be due to the different models of renal hypertension employed. Their model (one-kidney, one clip) is thought to be a low renin, volume-dependent hypertension whose induction is independent of the renin-angiotensin system.35
thought to prevent contraction by limiting the availability of intracellular calcium for contraction. Captopril, by inhibition of angiotensin II formation, prevents the increase in calcium entry into cells and the translocation of calcium from intracellular stores resulting from angiotensin II action. Hydralazine is thought to prevent vascular smooth muscle contraction by interfering with the release of calcium from intracellular stores. Thus, verapamil was a logical tool to use to investigate whether calcium was one of the factors controlling the increase in DNA synthesis in hypertension, since the drug acts as a vasodilator by interfering with the flux of calcium across the vascular smooth muscle membrane. Verapamil, like the other antihypertensive agents used, significantly reduced DNA synthesis in both early and established renal hypertension and lowered blood pressure to normal in the rats with established renal hypertension.

Caution must be used when interpreting these results; however. The changes seen in DNA synthesis cannot be due entirely to changes in blood pressure, as demonstrated by the dissociation between DNA synthesis and the blood pressure increase seen 4 days after clipping in developing hypertension. Since blood pressure in the clipped, non-drug-treated rats was not different from that in the sham-operated rats 4 days post clipping (131 ± 3 vs 126 ± 4 mm Hg) and was higher than that in the sham-operated rats after 5 days clipping (140 ± 5 vs 122 ± 3 mm Hg) in this study, and since DNA synthesis was elevated in both groups, it may be possible to minimize the influence of pressure in the induction of aortic DNA synthesis. Even at 5 days post clipping, it is evident that a large increase in blood pressure was not necessary to increase DNA synthesis, and, additionally, that when captopril and hydralazine were administered to the sham-operated rats, a greater blood pressure drop (15-16%) occurred in those rats in the absence of any change in DNA synthesis. Further evidence that the increase in DNA synthesis may be due to some factor other than blood pressure is provided by the observation that the DNA synthesis inhibitor cytosine arabinoside can prevent the development of experimental two-kidney, one clip renal hypertension.

The young, prehypertensive, 4-week-old SHR were studied to determine whether there were any alterations in the rate of DNA synthesis analogous to the prehypertensive stage of renal hypertension. Although we found no difference in aortic DNA synthesis rates among 4-week-old SHR, WKY, and normal Wistar rats, the rates were very high compared with those found in adult rats. From these experiments it appears that a high variability and a high rate of thymidine incorporation into aortic DNA are characteristics of young, fast-growing rats. Schwarz and Benditt have shown that the thymidine-labeling index of aortic endothelium in normal Sprague-Dawley rats is maximal at birth and subsequently declines to a constant value between 13 and 26 weeks of age. Their findings would be expected, since the growth rates in young rats is at a maximum. Any subtle differences in aortic DNA synthesis rates among Wistar rats, SHR, and WKY at 4 weeks of age may have been masked by their rapid body growth, with the result that any differences may not have been detectable by our methods.

At 17 weeks of age SHR had significantly higher blood pressures and DNA synthesis rates than the WKY. Captopril or hydralazine treatment for 6 days were equally effective in normalizing the increased DNA synthesis in the SHR. In this model of hypertension, however, these drugs could only partially reduce blood pressure to normal levels (i.e., those seen in WKY), demonstrating that complete normalization of blood pressure was not necessary to effect normalization of DNA synthesis.

These data were much different from those obtained in 21-week-old SHR and WKY. Although the blood pressure of the untreated SHR was 70 mm Hg higher than that of the WKY, both had similar aortic DNA synthesis rates. The reasons for these differences between the 17-week-old and 21-week-old SHR are unclear; however, they may be related to the rate of hypertension development in the SHR or to the length of time the hypertension had been maintained. Limas et al. reported that blood pressure in the SHR rises steadily until about 16 weeks of age, after which it levels off at about 200 mm Hg. By 21 weeks of age, the blood pressure of the rats we studied would have been stable for over 1 month. It is possible that in the SHR the drive to increase DNA synthesis was decreased at this time, since blood pressure had been stable for an extended period. Although apparently not true for two-kidney, one clip renal hypertension, it is also possible that the rate of vascular DNA synthesis in the SHR is dependent on the rate of change of blood pressure, or vice versa. Carlier et al. have recently presented data suggesting that aortic DNA synthesis in the one-kidney, one clip model of renal hypertension also follows the rate of blood pressure increase. They found that [H]thymidine incorporation into aortic DNA was highest when the blood pressure was increasing but returned toward control levels when blood pressure reached a plateau.

Since it was possible that captopril, hydralazine, and verapamil may all have had the effect of inhibiting calcium availability to the cell, we postulated that an increase in the intracellular calcium could be a stimulus to aortic DNA synthesis. This hypothesis was tested by using ouabain to inhibit Na⁺,K⁺-ATPase. Inhibition of this enzyme results in an increase in intracellular sodium. To normalize intracellular sodium levels, excess sodium is exchanged for calcium through a separate exchange system. In this way, the cell offloads sodium and is in turn exposed to a higher intracellular concentration of calcium. If intracellular calcium is raised as a result of hypertension, or as a result of some circulating initiator of hypertension that subsequently induces vascular DNA synthesis, one would expect to see an increase in DNA synthesis after ouabain administration. As can be seen in Table 1, ouabain did cause a slight but significant increase in DNA synthesis without a concurrent increase in blood pressure.
INHIBITION OF HYPERTENSION-ASSOCIATED DNA SYNTHESIS/Loeb and Bean 1141

pressure. These findings are similar to those of Atkinson et al., who have demonstrated that ouabain (10^{-11} to 10^{-7} M) can induce proliferation of cultured rat lymphocytes through calcium-dependent and magnesium-dependent reactions.

An alteration of calcium utilization has been well documented in hypertension. Wright and Rankin have demonstrated disturbances in calcium homeostasis in the SHR, as well as in renovascular and mineralocorticoid models of hypertension in rats. Each of the models studied had some degree of reduction in the in vitro binding or complexing of exogenous calcium by plasma. Webb and Bhalia have shown that calcium uptake by microsomal fractions and calcium transport were decreased in the SHR. This decreased ability to sequester calcium may create an increase in free intracellular calcium available for contraction and may be responsible for the decreased rate of aortic relaxation and altered calcium distributions they saw in the smooth muscle cell of SHR.

The effect of alterations in cell calcium in hypertension is particularly intriguing because of evidence suggesting that calcium ions influence cell proliferation in both in vivo and in vitro situations. Increasing the extracellular concentration of calcium from 1.0 to 1.5 mM for as little as 5 minutes resulted in a large increase in cyclic adenosine 5'-monophosphate and DNA synthetic activity in calcium-deprived lymphocytes in vitro. In vivo, halving the blood calcium concentration by parathyroidectomy prevented the normal increase in DNA synthesis in the liver seen after partial (70%) heptectomy and in the parotid gland after chronic isoprotenerol administration in the rat. In both studies, DNA synthesis resumed when hypocalcemia was reversed by parathyroid hormone or calcium chloride supplementation.

The effect of vascular tone in increasing DNA synthesis following ouabain treatment cannot be ruled out completely. Despite the fact that in our experiment ouabain did not cause an increase in blood pressure, one of the effects of cardiac glycoside administration is an increased vascular wall tension presumably induced by the increase in intracellular calcium. Even though blood pressure was not raised significantly in our experiments, as we expected it to be, reflex decreases in sympathetic tone or increases in vagal stimulation resulting in a decreased cardiac output may have compensated for any increase in peripheral resistance or wall tension, thus maintaining normal pressure.

Although we were unable to prove which factors are responsible for the increase in aortic DNA synthesis seen in developing and established renal hypertension and in the SHR, it is possible that a prolonged decrease in the available calcium to the smooth muscle cell during antihypertensive drug treatment could prevent the increase in both blood pressure and DNA synthesis. Further studies are needed to determine whether increases in intracellular calcium are necessary for the increased aortic DNA synthesis seen in renal hypertensive rats and SHR.

It is clear that lowering the blood pressure lessens the risk of cardiovascular pathology and that the generalized hypotrophy of the vessel wall (either cellular hypertrophy or hyperplasia) has the potential to increase that risk. Our finding that antihypertensive drug treatment in both developing and more established experimental hypertension can inhibit the increases in vascular DNA synthesis further emphasizes the beneficial effects of pharmacological intervention in hypertension.

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