Evaluation of Medial Hypertrophy in Resistance Vessels of Spontaneously Hypertensive Rats

GARY K. OWENS, STEPHEN M. SCHWARTZ, AND MARY MCCANNA

SUMMARY The role of smooth muscle cell hypertrophy, hyperploidy, and hyperplasia in medial hypertrophy of mesenteric resistance vessels of 107- to 111-day-old spontaneously hypertensive rats (SHR) was examined using a combination of morphometric, biochemical, and immunological techniques. Mesenteric arteries were classified on the basis of branching order for comparative purposes. Branch level I vessels were those that directly enter the jejunal wall, while Branches II to IV represented more proximal vessels; Branch IV vessels were those that branch from the superior mesenteric artery. Medial hypertrophy was assessed in perfusion-fixed vessels by morphometric evaluation of medial cross-sectional area and smooth muscle content. Medial cross-sectional area and smooth muscle content were significantly increased in larger (Branches III and IV) but not smaller (Branches I and II) mesenteric resistance vessels of SHR compared with control normotensive Wistar-Kyoto rats (WKY). Smooth muscle cell hypertrophy and hyperploidy were evaluated in isolated cells obtained by enzymatic dissociation of mesenteric resistance vessels. Approximately 80% of the cells in these preparations were identified as smooth muscle cells using a smooth muscle-specific isoactin antibody. Feulgen-DNA microdensitometric evaluation of isolated cells showed that polyploid cells were present in mesenteric resistance vessels but at very low frequencies, and no differences were apparent between SHR and WKY. Likewise, no differences in cellular protein content or relative smooth muscle cell size (i.e., area profile) were observed between cells obtained from SHR and WKY vessels. These results demonstrate that the increase in medial smooth muscle content observed in larger mesenteric resistance vessels of SHR cannot be accounted for by smooth muscle hypertrophy and hyperploidy, inferring that hyperplasia must be present. Results indicate that studies of the initiating mechanisms for medial smooth muscle hypertrophy in SHR resistance vessels, at least relatively early in hypertension, should focus on examination of factors that induce true cellular proliferation rather than hypertrophy and hyperploidy. (Hypertension 11: 198-207, 1988)

KEY WORDS smooth muscle growth • smooth muscle cell • polyploidy • cellular hypertrophy • cellular hyperplasia • hypertension • resistance vessels

FOLKOW et al.1 and others2 have suggested, based on hemodynamic evidence, that an increase in vascular smooth muscle mass in resistance vessels may play an important role in the maintenance of increased peripheral resistance in chronic hypertension. Results of morphometric studies have demonstrated an increase in vessel and smooth muscle mass in both large and small vessels (> 100 μm in diameter) of chronically hypertensive animals3-5 and humans6 compared with their normotensive counterparts. There is considerable debate as to whether this increase in medial mass, which we will call medial hypertrophy, is a cause or the result of hypertension. There is even uncertainty about whether changes in the hypertensive media encroach on the lumen diameter at maximal vasodilatation.3,7,8 Nonetheless, there is good evidence that increased smooth muscle mass confers a functional advantage by 1) increasing maximal force-developing capability,1 although the force development per unit contractile mass appears to be unaltered,9,10 and 2) conferring a geometric advantage, since thick-walled vessels undergo proportionately larger diameter changes than thin-walled vessels for a given level of smooth muscle activation.11,12 Whether this functional advantage would itself result in an increase in vascular resistance, as suggested by Folkow...
et al. or whether it is an adaptive response to normalize wall stress, is controversial. In either case, it would be an extremely important component of a chronic elevation in vascular resistance.

These studies underscore the importance of understanding the cellular mechanisms responsible for accelerated smooth muscle cell (SMC) growth in resistance vessels of hypertensive animals. It may be particularly important to understand the relative role of processes involving cell replication (i.e., an increase in cell number or hyperplasia) versus processes that involve enlargement of existing SMCs (i.e., cellular hypertrophy) without replication since there is good evidence suggesting that the signals for these processes are different. Smooth muscle replication has been studied extensively in vitro, suggesting that possibly important mechanisms in replicative responses in vivo. Perhaps more importantly, increases in DNA content of a tissue, unlike changes in other macromolecules, are likely to be irreversible unless cell death occurs. This factor could be important if an increase in DNA content increases the capacity of the cell to synthesize contractile proteins.

Our previous studies and those of Olivetti et al. showed that the increased mass of smooth muscle in aortas of spontaneously hypertensive rats (SHR) or Goldblatt hypertensive rats compared with normotensive controls was due principally to SMC hypertrophy rather than to hyperplasia. Aortic SMC hypertrophy was accompanied by an increase in DNA ploidy, implying that DNA replication was a requisite part of a change in mass. However, results in a large conduit vessel may not be relevant to changes in smaller vessels responsible for control of vascular resistance. Lee et al. have presented evidence that the nature of the growth response varies in different size mesenteric resistance vessels, with cellular hypertrophy predominating in large vessels and cellular hyperplasia in smaller vessels, although their morphometric technique may be compromised by untested assumptions regarding SMC size, shape, and orientation. The studies by Mulvany et al. present the most convincing evidence for SMC hyperplasia in resistance vessels. However, these studies did not address the possibility of SMC polyploidy and were limited to examination of one branch level of mesenteric vessels. In addition, their morphometric method for determination of numerical density, although clearly superior to most methods, is nevertheless subject to limitations relating to small sample sizes and relatively large experimental variances (see Discussion and the report by Mulvany et al. for a further discussion).

The principal objectives of the present study were 1) to study the distribution of medial smooth muscle hypertrophy in intermediate size mesenteric resistance vessels of SHR and Wistar-Kyoto rats (WKY), and 2) to explore the relative role of SMC hypertrophy and hyperplasia versus hyperplasia in medial hypertrophy of mesenteric resistance vessels of SHR. We studied 107- to 111-day-old SHR and WKY since animals are hypertensive at this time and others have reported the presence of marked medial hypertrophy in mesenteric arteries at this age. We chose to examine mesenteric arteries between 70 and 250 μm in diameter since these vessels contribute to vascular resistance and are readily accessible for isolation, a necessary requirement for technical reasons in many of the studies described here.

**Materials and Methods**

**Animals**

Male SHR and WKY used in this study were supplied by the Core Breeding Colony (National Institutes of Health Grant Program Project P01 HL 19242) of the University of Virginia. This colony was derived from stock provided by the National Institutes of Health, and the animals have growth characteristics and pressure relationships comparable to those of other SHR colonies. All rats were between 107 and 111 days of age. A total of 20 SHR and 21 WKY were used in these studies. All animal use protocols were approved by the University of Virginia Animal Research Committee.

Systolic blood pressure was determined in conscious rats by a photoelectric tail-cuff pulse detector (ITC, Landing, NJ, USA). Ambient temperature was 27°C. Animals were conditioned to restraining cages before pressure measurements were attempted. Animals were identified by number, and the blood pressure technician was not aware of experimental groupings. A minimum of three blood pressure measurements were obtained before the rats were killed. In addition, systolic and diastolic blood pressures as well as heart rates were measured in anesthetized rats with an indwelling catheter just before perfusion fixation in morphometric studies.

**Evaluation of Medial Hypertrophy**

Rats were anesthetized with sodium pentobarbital (25 mg/kg), and the left carotid cannulated for pressure and heart rate determinations using a Beckman Type RB dynagraph (Schiller Park, IL, USA) equipped with a Statham pressor transducer (Hato Rey, Puerto Rico). Animals were then perfusion-fixed at their mean pressure through the carotid cannula using 2% glutaraldehyde/1% paraformaldehyde in Hanks balanced salt solution (HBSS; pH 7.40). The femoral vein was cut to provide a venous outflow. Rats were perfusion-fixed for 5 minutes with close monitoring of pressure. After perfusion fixation, the abdominal aorta, the mesenteric artery, its branches, and the small intestine were removed and immersion-fixed for an additional 12 hours and then stored in HBSS at 4°C until used (i.e., <48 hours).

An important consideration in these studies was to have a systematic means of classifying vessels for comparative purposes. Furthermore, our classification scheme had to be applicable to nonfixed vessels, which were needed for a number of the studies to be described. We chose to do this on the basis of branching order, as illustrated in Figure 1. A limitation of this classification scheme was that not all vessel arcades were identical (i.e., some arcades had only three...
branch levels, while others had four or more). Since the majority of arcades had four branch levels in both SHR and WKY, we chose to sample vessels only from these. Branch I vessels corresponded to those that directly enter the intestinal wall; Branches II to IV represented the increasing size of vessels. Branch IV vessels represented the first branch from the superior mesenteric artery.

Four to eight vessels of each branch level were dissected from four different arcades from each animal under a stereomicroscope. To facilitate handling of these small vessels, dissected vessels were processed in Flo-Thru specimen capsules (Normco, Silver Spring, MD, USA), which make transfer of vessels much easier and eliminate the possibility of loss, because specimens remain in the capsules while solutions are changed. Vessels were processed and embedded in Epon for transverse sections, as previously described. One-micrometer-thick sections were cut and stained with toluidine blue for morphometric evaluations.

Medial cross-sectional area was determined from transverse sections using a Zeiss videoplan system (Thornwood, NY, USA) as previously described. The major advantage of cross-sectional area determinations, as compared with measurements of wall thickness to lumen ratios, is that measurements are not influenced by changes in the activation state of the vessels. Between four and six vessels of each branch level were analyzed per animal. Vessels that were not sectioned transversely (i.e., wall thickness was asymmetrical) were not analyzed. At least two sections were analyzed from each vessel. The coefficient of variation for determinations on different sections of the same vessel was less than 5%. Representative micrographs of vessels are shown in Figure 2. The volume fraction of smooth muscle ($V_{vsmc}$) in vessels was determined from electron micrographs by standard point counting, as previously described. A minimum of six electron micrographs (magnification $\times 8000$) were analyzed per vessel (this resulted in a coefficient of variation of $<10\%$). An estimate of smooth muscle content was then calculated as the product of $V_{vsmc}$ and vessel cross-sectional area.

An estimate of vessel size for each branch level was obtained in two ways. First, luminal area was determined with a Zeiss videoplan system, and lumen diameter was calculated as: diameter = $\sqrt{\text{area}/\pi}$. Note that this evaluation is only valid with circular sections, and therefore all collapsed (i.e., noncircular) vessels were excluded from analysis. Second, the length of the internal elastic lamina was measured by planimetry, and vessel diameter was calculated as diameter = circumference/$\pi$. The latter measurement provides a relative index of vessel size that is not profoundly influenced by the activation state of the vessel, but it is not necessarily relevant for hemodynamic purposes.

Isolation of Cells from Mesenteric Resistance Vessels

Animals were killed by CO$_2$ asphyxia, and four to eight vessels of each branch level were immediately dissected as already described. Care was taken to remove as much adhering connective tissue as possible without damaging the vessels. Cells were then dissociated from pooled vessels of each branch level from each rat using collagenase and elastase digestion as previously described. Dissociation was monitored by phase microscopy. Identical digestion times were used for SHR and WKY vessels from the same class, but slightly different times were necessary for isolation of sufficient cells from the different classes of vessels. Freshly dispersed cells were cytotoxic (150 g; 5 minutes) onto glass slides. Cell smears were then fixed either in 4% paraformaldehyde in HBSS for subsequent DNA and protein determinations or in cold methanol for staining with a smooth muscle–specific isoactin monoclonal antibody for assessment of the fraction of SMC and relative cell area. Cell viability, determined by trypan blue exclusion, was greater than 90% in all instances.

Cellular Ploidy Measurements

Cellular DNA determinations were done on cell smears by Feulgen-DNA microdensitometry, as previously described. Measurements were made using either a Zeiss-Zonax scanning microdensitometer or a custom-designed digital microdensitometer (Leitz, Rockleigh, NJ, USA). Between 200 and 400 cells were evaluated per branch level per animal. Chicken erythrocytes were used as standards. Lysed cells were excluded from analyses.

Cellular Protein Evaluations

Cellular protein determinations were done as previously described using naphthol yellow S–stained cell smears. Absorption measurements were made at a wavelength of 460 nm. A random sample of at least 50 cells were evaluated per branch level per animal. All measurements were made using the Zeiss-Zonax microdensitometer.

Identification of Smooth Muscle Cells in Cell Smears

The frequency of SMCs and non-SMCs in isolated cell preparations was determined using a smooth muscle–specific isoactin monoclonal antibody provided by Drs. Allen Gown and David Gordon (University of Washington). This antibody shows specific reactivity with both the $\alpha$–smooth muscle and $\gamma$–smooth muscle isoactin forms but does not react with $\alpha$–skeletal muscle actin, $\alpha$–cardiac muscle actin, or with $\beta$–nonmuscle and $\gamma$–nonmuscle isoactins. It has been shown to stain SMCs from a variety of sources. Of most importance for this investigation, it does not stain endothelial cells or fibroblasts, which are the most likely nonmuscle cells in our isolated cell preparations.

Methanol-fixed cell smears were stained using an avidin-biotin-peroxidase procedure (Vectastain, Vector Laboratories, Burlingame, CA, USA). The smooth muscle isoactin antibody was used as ascites fluid diluted 1:250 in phosphate-buffered saline (pH 7.40). Controls were stained with nonimmune mouse serum or with an antineurofilament monoclonal antibody unreactive to SMC. In addition, some smears were
stained with a rabbit anti-chicken gizzard polyclonal actin antibody, which stains both muscle and nonmuscle isoactins as a positive control. At least 200 cells were evaluated per animal per branch level for frequency determinations.

Assessment of Relative Smooth Muscle Cell Size: Area Profile Determinations
The relative size of SMCs in isolated cell preparations was evaluated by determining the area of cells stained with the smooth muscle isoactin antibody as previously described. Cell area measurements were determined at a magnification of 1000X using a Leitz microscope equipped with a video camera and interfaced to a Zeiss videoplan digitizing board. Cell parameters were traced with an electronic pen. A minimum of 50 SMCs were evaluated per branch level per animal.

Statistical Analysis
Since multiple vessels were analyzed from each rat in determination of medial cross-sectional area, VVSMC, vessel diameter, and smooth muscle content, comparison of differences between SHR and WKY for these parameters was done by analysis of variance with a repeated-measures term that takes into account both between-animal and within-animal variance for intergroup comparisons. This was done as a single hierarchical analysis of variance (including a term for branch level), followed by a multicomparison test using a t statistic on least-square means (General Linear Models Procedure, SAS Institute, Cary, NC, USA). Note that the repeated-measures term was used since it is not appropriate to use a single mean value for these parameters for each branch level for each animal since such a test would not account for the variation between different vessels within an animal and since preliminary analyses showed that this intra-animal variation was as great as the interanimal variance. Statistical evaluation of cellular protein content and area profile was done in a similar manner but without the repeated-measures term. An \( \alpha \) level of 0.05 was considered significant.

A simultaneous combined linear model (General Linear Models Procedure, SAS Institute) was used for regression analyses. This model provides a more precise estimate of the relationship between variables than can be obtained by separate linear regression analyses in SHR and WKY. The model yields separate slope and y intercept terms for SHR and WKY respectively, but by definition the simultaneous combined model provides a single \( r^2 \) value for both lines that reflects the overall fit of the data to the respective lines. The significance of the slope and intercept (i.e., testing the null hypothesis that they equal zero) was evaluated using a t statistic (General Linear Models Procedure, SAS Institute). Differences in slopes were compared by analysis of covariance (General Linear Models Procedures, SAS Institute).

Results

Blood Pressures and Heart Weights
The mean (± SE) conscious systolic blood pressures of the SHR and WKY used in these studies during the 4 weeks preceding death were 186 ± 4 (n = 20) and 128 ± 2 (n = 21) mm Hg, respectively. Heart weights and heart weight/body weight ratios were also significantly increased (p < 0.001, by analysis of variance) in SHR compared with WKY, while body weights were not different between groups.

Evaluation of Medial Hypertrophy
Our initial objective was to determine whether medial smooth muscle hypertrophy occurred in mesenteric resistance vessels of SHR by comparing medial cross-sectional area and smooth muscle content measurements of vessels from the same branch level (see Figure 1) between SHR and WKY. Results of initial analyses showed that the medial cross-sectional area was significantly greater in SHR vessels than in WKY vessels for Branches III and IV, but not for Branches I and II (Table 1). To determine whether the increases in medial cross-sectional area in these vessels were due to an increase in the smooth muscle or extracellular component of the wall, smooth muscle volume densities (VVSMC) were determined. Results showed no differences in VVSMC between SHR and WKY vessels within a given branch level (see Table 1), indicating that medial hypertrophy in SHR is associated with proportionate increases in the cellular and extracellular components of the vessel. Consistent with cross-sectional area data, smooth muscle content was significantly increased in vessels from Branches III (23% increase;
There are several potential limitations in assessing medial hypertrophy in vessels solely on the basis of branching level. First, based on visual observation as well as morphometric estimates of vessel size (e.g., cross-sectional area and diameter), there were marked heterogeneities in vessel sizes even within a given branch order, which increase intragroup variances and thereby compromise intergroup comparisons. Second, shifts may occur in the size of vessels present in a given branch level in SHR and WKY that might not be apparent by examination of branching patterns alone. Thus, medial hypertrophy was also assessed in these studies by determining whether SHR vessels of a given size were hypertrophied relative to the corresponding vessels in WKY. Note that this type of comparison is not identical to one based on branching order alone due to heterogeneity of vessel sizes within a given branch level. The major difficulty with such an analysis is determining an appropriate index that accurately reflects a vessel’s size rather than its activation state. Ideally one would like to maximally relax a vessel and measure the undistended vessel diameter. However, this feat would be extremely difficult to accomplish in an intact animal. Furthermore, in these studies animals were perfusion-fixed at their own blood pressure in an intact animal. Furthermore, in these studies animals were perfusion-fixed at their own blood pressure in an attempt to observe the in vivo state of vessels. Thus, we have estimated vessel size based on the length of the internal elastic lamina (IEL) in order to reduce the influence of differences in vessel tone on these analyses. Note that at diameters at which the IEL is not load-bearing, IEL length will be constant with changes in diameter, which are dramatically influenced by differences in activation state. 12 Although this method does not eliminate the influence of vessel tone on size estimates, it is more advantageous than size estimates based on lumen diameter, which are dramatically influenced by differences in activation state. We want to emphasize that our intent was to assess medial hypertrophy by some additional means other than comparison of vessels of a given branch level. Furthermore, we felt that it is inappropriate to arbitrarily divide vessels into different size classes as has been done frequently in previous studies in this area. To accomplish these goals, a simultaneous combined linear regression analyses (see Materials and Methods) was done of medial cross-sectional areas versus relative vessel size (IEL length) for all SHR and WKY vessels evaluated in these studies.

Results showed a highly significant linear relationship between medial cross-sectional area and IEL length for SHR and WKY vessels ($r^2 = 0.92$, $p < 0.001$ for the slope and $y$ intercept of the curves for both SHR and WKY; Figure 3). Significantly, analysis of covariance showed that the slope of the line for SHR was steeper than that for WKY, indicating that the differences in medial area between SHR and WKY were greater in larger diameter vessels. This supports our findings based on comparison of vessels classified by branching order.

We were also concerned that differences in activation state between SHR and WKY vessels may have influenced medial area determinations. Whereas medial area should not change with changes in diameter, it could change if vessel length is altered with changes in activation. To address this possibility, control studies were done in which rats were infused with high doses of sodium nitroprusside (160 μg/kg/min) to induce vasodilation. This procedure resulted in a rapid and dramatic fall in blood pressure. After a 5-minute nitroprusside infusion, rats were perfusion-fixed at this reduced pressure as described in Materials and Methods. Medial area was determined in Class I vessels from six SHR and six WKY. Medial areas from these animals were nearly identical ($p = 0.9$) to those obtained in non-nitroprusside-infused animals, indicating that differences in activation state did not influence medial area determinations. These observations are in agree-

### Table 1. Morphometric Analysis of Perfusion-Fixed Mesenteric Resistance Vessels of 107- to 111-Day-Old SHR and WKY

<table>
<thead>
<tr>
<th>Branch level</th>
<th>Medial cross-sectional area ($\mu m^2$)</th>
<th>Lumen diameter* ($\mu m$)</th>
<th>$V_{VSMC}$</th>
<th>SM content ($\mu m^2/\mu m$ length)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>1,788 ± 51</td>
<td>73.7 ± 2.1†</td>
<td>0.824 ± 0.009</td>
<td>1,474 ± 42</td>
</tr>
<tr>
<td>WKY</td>
<td>1,871 ± 54</td>
<td>91.4 ± 3.0</td>
<td>0.801 ± 0.007</td>
<td>1,480 ± 44</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>4,928 ± 254</td>
<td>125.3 ± 4.9</td>
<td>0.776 ± 0.009</td>
<td>3,831 ± 195</td>
</tr>
<tr>
<td>WKY</td>
<td>4,372 ± 240</td>
<td>126.9 ± 4.3</td>
<td>0.769 ± 0.013</td>
<td>3,537 ± 185</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>8,435 ± 414†</td>
<td>139.8 ± 6.1†</td>
<td>0.719 ± 0.023</td>
<td>6,105 ± 336†</td>
</tr>
<tr>
<td>WKY</td>
<td>7,436 ± 244</td>
<td>164.5 ± 6.6</td>
<td>0.668 ± 0.008</td>
<td>4,961 ± 168</td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>14,962 ± 1,005†</td>
<td>175.6 ± 9.0†</td>
<td>0.698 ± 0.023</td>
<td>10,578 ± 751†</td>
</tr>
<tr>
<td>WKY</td>
<td>11,392 ± 522</td>
<td>210.7 ± 8.1</td>
<td>0.666 ± 0.014</td>
<td>7,651 ± 356</td>
</tr>
</tbody>
</table>

* Lumen diameter was determined on cross-sections of perfusion-fixed vessels by measuring luminal area and calculating the diameter of the circle with that area.

† $p < 0.05$, compared with respective value for WKY (by analysis of variance with a repeated-measures term reflecting the fact that multiple vessels from each branch level were analyzed from each subject).
SMOOTH MUSCLE HYPERTROPHY IN SHR/Owens et al.

FIGURE 2. Light micrographs showing representative cross-sections of two mesenteric arteries evaluated in these studies. The vessel depicted in A shows much folding of the internal elastic lamina, presumably indicative of vessel contraction. Note that the wall thickness to lumen ratio is high. In contrast, in the vessel depicted in B, the internal elastic lamina is not folded and the wall thickness to lumen ratio is much smaller. Comparison of A and B illustrates the profound influence that vessel activation state can have on wall thickness or wall to lumen ratio measurements and illustrates the importance of assessing wall hypertrophy by measurement of cross-sectional area rather than wall to lumen ratios. (Magnification bars = 25 μm.)

FIGURE 3. Relationship of medial cross-sectional area to relative vessel size (i.e., length of the internal elastic lamina (IEL)) for mesenteric resistance vessels of SHR and WKY. An approximate vessel diameter can be obtained by dividing IEL length by π. The r² value reflects the overall fit of SHR and WKY data to their respective regression lines.

Analysis of Polyploidy in Smooth Muscle Cells from Mesenteric Arteries

Although ideally one would like to evaluate the frequency of polyploid SMC in tissue sections by Feulgen-DNA microdensitometric analysis, pilot studies showed that this was not possible because the considerable overlap of SMC nuclei prevented these measurements on a technical basis. Therefore, the following alternative method was used to determine whether medial hypertrophy in mesenteric resistance vessels was accompanied by increases in SMC polyploidy, as occurs in large conduit vessels. Four to eight vessels of each branch level were dissected from each rat and pooled, and cells were isolated as described in Materials and Methods. Cell smears were prepared and processed for analysis of ploidy (Table 2) or for determination of the frequency of SMCs using a smooth muscle-specific isoactin monoclonal antibody. Ploidy analyses demonstrated that tetraploid cells were present in mesenteric arteries of both SHR and WKY. However, the frequency of tetraploid cells was low in all instances, and no differences were apparent between cells from SHR and WKY for any of the branch levels studied. The percentage of SMCs in cell preparations, based on antibody staining, ranged from 78.9 ± 4.8 to 83.5 ± 4.2%, and no differences were observed between SHR and WKY. This finding indicates that 1) the non-SMC contaminants had a relatively small influence on the frequencies reported in Table...
2 and 2) differences in non-SMC contaminants cannot account for the lack of differences in ploidy levels between SHR and WKY.

Analysis of Smooth Muscle Cell Hypertrophy

The preceding analyses indicate that medial hypertrophy in mesenteric arteries of SHR is not accompanied by changes in SMC ploidy. Given the strong relationship between development of SMC polyploidy and cell hypertrophy observed in our previous studies,456 this observation alone provides evidence that cellular hypertrophy has not occurred. Nevertheless, the possible contribution of an increase in individual cell mass (or cellular hypertrophy) to medial hypertrophy was directly evaluated in two ways: 1) microdensitometric analysis of the protein content of individual cells in isolated cell preparations used in ploidy analyses and 2) planimetric evaluation of the area profile of isolated cells stained with the SMC-specific isoactin antibody. The advantage of protein determinations is that they provide a very quantitative index of cell mass or hypertrophy, since greater than 50% of the dry mass of cells is protein and since the amount of the other major cellular components such as sugar and lipids are dependent on them.22 Their disadvantage is that it is not possible to distinguish between SMC and non-SMC contaminants in isolated cell preparations. However, the influence of non-SMCs on overall protein measurements would be relatively small, since they account for only 20% of the cells present, and would only affect our comparisons of SHR and WKY if they had a quite different protein content in one strain relative to the other (see further arguments in the next paragraph). The advantage of the area profile determinations is that analysis is restricted to SMCs. However, their major disadvantage is that they are a less reliable index of cell size than are protein measurements because of uncertainties regarding changes in cell shape that occur during cell processing. In addition, comparisons of area profile data between different classes of vessels cannot be made due to inherent differences in the nature of the cell isolation from vessels of different sizes. For example, isolation of cells from Class I vessels required shorter digestion times than did those from large vessels, and the cells obtained tended to be elliptical in shape, while those from large vessels were nearly spherical. Thus, comparison of area profile data between cells from different vessel classes is invalid as a measure of relative cell size due to differences in the area/volume ratio in cells from different vessels. However, within a given vessel class, identical isolation protocols were used for SHR and WKY and no obvious differences in cell shape were apparent in the isolated cells obtained. Thus, it is reasonable to assume that differential shape changes have not occurred and that area profile measurements provide a reasonable estimate of relative cell size for comparison of SHR and WKY cells within a given vessel class. Nevertheless, small differential changes in cell shape that occurred during cell isolation may not have been detected, and for this reason these data are not as reliable as the protein data. However, area profile determinations for cells from larger vessels, because cells were nearly spherical, were largely shape-independent.

No differences in cellular protein content were observed between SHR and WKY cells for each respective branch order (Table 3). It is extremely unlikely that the presence of non-SMCs in protein assay preparations significantly influenced our comparisons of SHR and WKY in these experiments since they were present at the same frequency (i.e., 20%) and would have to have an extremely large difference in protein content in one strain relative to the other to have an appreciable affect on mean protein content. For example, in Class IV vessels we saw a 38 ± 2% increase in smooth muscle content as compared with WKY. If this increase were due to SMC hypertrophy (i.e., 38% increase) rather than to hyperplasia, non-SMCs would have to have a negative protein content (clearly an impossibility) to account for the lack of a difference in

Table 3. Evaluation of Protein Contents of Individual Cells Isolated from Mesenteric Arteries and Arterioles of 107- to 111-Day-Old SHR and WKY

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Branch I</th>
<th>Branch II</th>
<th>Branch III</th>
<th>Branch IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>135.3 ± 13.6* (6)</td>
<td>195.1 ± 16.0† (6)</td>
<td>267.2 ± 17.2 (6)</td>
<td>224.9 ± 12.9 (6)</td>
</tr>
<tr>
<td>WKY</td>
<td>178.2 ± 20.7† (7)</td>
<td>228.7 ± 28.2 (5)</td>
<td>233.3 ± 28.9 (6)</td>
<td>193.3 ± 28.2 (4)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Number of animals is shown in parentheses. Cells were isolated from four to eight mesenteric arteries from each respective branch level and stained for combined protein/DNA determinations as previously described.4 Protein content was evaluated in a random sample of at least 50 cells per branch level per animal.

* p < 0.0025, compared with values for Branches II to IV for both SHR and WKY (by analysis of variance.)
† p < 0.05, compared with values for SHR Branch III (by analysis of variance.)
mean protein content in the mixed cell populations. Results of area profile determinations are consistent with results of protein determinations in that no differences were observed between SMCs from SHR and WKY for any of the branch levels evaluated (Table 4). Taken together the results of these two assays provide good evidence that SMC hypertrophy cannot account for the increase in medial smooth muscle content in large (i.e., Branches III and IV) mesenteric resistance vessels of SHR compared with WKY.

Although there were no differences in either protein content or area profile between cells from SHR and WKY within a given branch level, Class I cells from SHR did have a lower protein content than cells from Classes II to IV from either SHR or WKY (see Table 3). Class I WKY cells had a significantly lower protein content than Class III and IV cells from SHR, but they were not significantly different from those from other WKY classes. The importance of these observations is unclear.

Discussion

Our major objective in these studies was to explore the nature of the SMC growth response that is responsible for the increased content of smooth muscle in mesenteric resistance vessels of SHR versus WKY. Since there was some controversy as to the nature of hypertrophic changes in resistance vessels, our initial aims were to define if medial hypertrophy occurred and what vessels were affected. We found that significant medial hypertrophy was present in large (i.e., second and third branch level vessels with mean diameters approximately \( \geq 150 \mu m \)) but not small (i.e., first and fourth branch level vessels) mesenteric arteries of SHR compared with WKY at 107 to 111 days of age. Our data are in agreement with those of Mulvany and co-workers, 3, 5 who reported medial hypertrophy in 150-\( \mu m \) to 200-\( \mu m \) (i.e., second and third branch order) vessels from SHR as compared with WKY in animals between 12 and 24 weeks of age, and are consistent with those of Bohlen and Lobach, 7 who found no evidence of medial hypertrophy in small mesenteric resistance vessels of SHR that were approximately 80 to 130 \( \mu m \) in internal diameter. Likewise, our findings are consistent with those of Furuyama, 6 who studied medial hypertrophy in human vessels using a morphometric technique that corrects for the lack of distention in immersion-fixed autopsy material. She found that medial hypertrophy was prominent in vessels larger than 100 \( \mu m \) in lumen diameter from subjects with essential hypertension but not present in vessels smaller than this. Lee et al. 8 also observed marked medial hypertrophy in large mesenteric arteries of SHR versus WKY. Consistent with previous observations, results of the present study showed that medial hypertrophy was due in part to increased smooth muscle content. In contrast to our observations, however, Lee et al. 8 reported that medial hypertrophy was present in first branch order vessels from 12-week-old SHR (see Table 1). Closer comparison of our data with theirs showed that medial cross-sectional area values were nearly identical for SHR vessels but their values for WKY vessels were lower than all other experimental groups. The reason for this difference is not clear, but it may relate to the fact that Lee et al. 8 obtained their WKY from a commercial supplier, while their SHR were from a colony raised and maintained in house. Note also that we have not cited studies in which wall thickness or wall thickness to lumen diameter ratios alone were measured since such measurements are profoundly influenced by the activation state of the vessel and may or may not reflect medial hypertrophy. Taken together the cited studies provide good evidence that medial hypertrophy is present in larger but not smaller mesenteric resistance vessels of SHR as well as hypertensive humans compared with normotensive controls.

This increase in medial mass apparently is attributable to SMC hyperplasia. Because of the small size of vessels, we could not directly estimate cell number from DNA content as we have done previously with larger vessels. 1, 13, 14, 20 Cellular protein content and relative SMC size, however, could be measured on isolated cells. No differences in either were observed between cells derived from mesenteric arteries of SHR and WKY for any of the branch levels examined. Since the increase in smooth muscle mass observed in third and fourth level branches in SHR in these studies (i.e.,

<table>
<thead>
<tr>
<th>Table 4. Relative Size of Smooth Muscle Cells from Mesenteric Arteries and Arterioles of SHR and WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat strain</td>
</tr>
<tr>
<td>SHR</td>
</tr>
<tr>
<td>WKY</td>
</tr>
</tbody>
</table>

Values shown represent least-square means from three separate replicate experiments done on separate days. Our initial analysis of variance showed a significant effect of the day of experiment on values obtained that was the same for SHR and WKY. No significant differences between SHR and WKY cells were observed in any one experiment for any of the branch levels (by analysis of variance), but the sample sizes were quite small because of methodological limitations in the number of animals that could be done on a given day. Least-square means were calculated using General Linear Model Procedure (SAS Institute) to account for day of experiment effects. The number of animals is shown in parentheses.

Only values for SHR and WKY within a given class should be compared since slightly different cell isolation protocols were used for different classes of vessels, which may have influenced area profile measurements (see text). See Materials and Methods for details regarding cell isolation and analysis.

...
23 and 38% increases in smooth muscle content) cannot be accounted for by an increase in the mass of individual cells, we conclude that the SHR vessels must have more cells (i.e., cellular hyperplasia). Our findings are in agreement with those of Mulvany et al., who found, using a three-dimensional dissector method for determining numerical density, that SMC hyperplasia, not hypertrophy, was responsible for medial hypertrophy in Branch III mesenteric arteries of SHR compared with WKY. The mutual confirmation of our results and theirs, using totally different experimental approaches, is important, given the potential limitations of methods employed in each of the studies. Their methods have the major advantage of directly counting cell number in the intact vessel but the disadvantage that the method is tedious, producing a limited sample size (<0.2% of the aggregate volume of the vessel was analyzed from each vessel in their studies, and in most instances a single vessel was analyzed from a given animal). Furthermore, the large variances associated with their method are of some concern since their conclusion that medial hypertrophy is due to hyperplasia was based on observations that SMC numerical density was not different between SHR and WKY while smooth muscle content was increased in the SHR. Our methods have the advantage of directly evaluating cell hypertrophy or mass but only indirectly address the question of hyperplasia. Likewise, because of technical limitations, the number of cells that could be obtained from microvessels for protein and size analysis was quite small, resulting in relatively large experimental variances. Taken together, however, the results of Mulvany et al. and those from the present studies provide very strong evidence that medial hyperplasia in large mesenteric resistance vessels of SHR is due to SMC hyperplasia rather than to hypertrophy.

These studies are the first, to our knowledge, to explore SMC hyperplasia in resistance vessels. The data demonstrated that, whereas polyploid cells were present in mesenteric arteries, no differences in the frequency of polyploidy were observed between vessels from SHR and WKY. This observation is important because it suggests that changes in SMC ploidy do not play a major role in the etiology of SHR hypertension, although it remains to be determined whether longer periods of hypertension in the SHR might be associated with ploidy changes in microvascular SMCs.

The present studies do not define what role medial hypertrophy might play in SHR hypertension. Indeed, since our animals were already hypertensive, these studies do not distinguish whether medial changes were the cause or the result of the hypertension. Our observations that lumen diameter was significantly reduced in SHR vessels that were fixed at their own blood pressure is consistent with hemodynamic studies showing increased resistance in this vascular bed. However, in our studies lumen diameter was reduced even in Branch I vessels, where there was no medial hypertrophy. Although this finding indicates that medial hypertrophy was not the cause of the reduced diameter in Branch I vessels, it does not exclude the possibility that other types of structural changes (e.g., an anatomically smaller vessel, changes in passive elastic properties) may play a role. It is also possible that active contractile changes occur first but become fixed by structural changes occurring with chronic exposure of vessels to the hypertensive stimulus. Consistent with this idea, Joshua et al. reported that structural changes in cremaster vessels of rats with one-kidney, one clip hypertension gradually extend to smaller and smaller arterioles with increased duration of hypertension. Our studies in this model of chronic hypertension provide little insight as to the contribution of medial hypertrophy to the diameter reductions observed in Branch III and IV vessels, and the influence of "structural" versus "functional" reductions in lumen diameter in resistance vessels is highly controversial. Structural encroachment is supported by hemodynamic studies showing increased resistance in SHR even at "maximal" vasodilatation. Consistent with this, Warshaw et al. reported a reduced lumen diameter of Branch II and III vessels under conditions of maximal relaxation in their in vitro studies. In contrast, Lee et al. found no evidence for structural encroachment of lumen diameter in their studies of SHR. In summary, our studies and those of others provide morphological evidence supporting the contention of Folkow et al. that medial hyperplasia occurs in resistance vessels of hypertensive animals and provide a possible explanation for the increased pressor responsiveness observed in the studies of Folkow et al. It remains unclear whether this medial hyperplasia contributes to the initial cause of the hypertension or whether it is an adaptive response involved in the maintenance of the hypertension. However, in either case, it would be an extremely important component of a chronic elevation in vascular resistance.

The demonstration in this and previous studies that medial smooth muscle hypertrophy in hypertension vessels of SHR occurs by cellular hyperplasia as opposed to cellular hypertrophy and hyperplody will be important in attempts to identify the initiating mechanisms for accelerated SMC growth in resistance vessels. The growth response of aortic SMCs depends on the mode of growth stimulation in that cells undergo hypertrophy and hyperplasty in several chronic models of hypertension but they undergo proliferation in response to traumatic injury with a balloon catheter or induction of acute severe coarctation hypertension. It is intriguing in this regard to note that endothelial denudation and platelet deposition have been described in hypertensive microvessels but not in larger arteries. One interesting possibility consistent with the hypothesis of Folkow et al. is that transient spikes in blood pressure, which precede development of established hypertension, may elicit a SMC proliferative response in a manner similar to that seen when large vessels are injured with a balloon catheter. Indeed, Reidy and Schwartz and others have demonstrated that, following acute increases in blood pressure by infusion of vasoactive substances, transient breaks in the endothelial lining of resistance
vessels occur and platelets are observed attached to the injured surface. In contrast, no breaks were observed in large vessels. The endothelium, suggesting that small vessels are more sensitive to pressure-induced injury. A similar phenomenon may occur in resistance vessels of SHR, and the SMC growth response in these vessels may resemble the “response to injury,”12 that occurs in large vessels, where it is hypothesized that injury-evoked increases in platelet-derived, macrophage-derived, and endothelium-derived growth factors play a role in SMC growth initiation. This speculation obviously remains to be tested, and one must also consider the possibility that there is a genetically determined abnormality in the growth control mechanisms in SMCs from SHR or that other growth mediators are involved. In any case, studies of growth control in microvessels, at least in early hypertension, should focus on factors that induce SMC proliferation rather than hypertrophy, and studies of growth control mechanisms in SMCs from large vessels may provide useful insight into the possible cellular growth control mechanisms operating in small vessels.

Acknowledgments

The authors thank Betty Ferguson for typing the manuscript; Sharon Coleman, An-Thu Phan, Susan Purdy-Ramos, and Donna Lombardi for their technical assistance; the Physiology Electron Microscopy Core Laboratory for preparation of morphology specimens; Drs. Allen Gown and David Gordon (University of Washington) for the CGA7 antibody; and Dr. Donald Kaiser (Statistical Computing Laboratory, University of Virginia) for his advice regarding statistical analyses.

References

3. Mulvany MJ, Hansen PK, Aalkjaer C. Direct evidence that the greater contractility of resistance vessels in spontaneously hypertensive rats is associated with a narrowed lumen, a thickened media, and an increased number of smooth muscle cell layers. Circ Res 1978;43:854–864
The pulse of progressive cardiology...

...monitors the full range of scientific studies devoted to hypertension — critical information demanded by specialists in

- **Hypertension Research**
- **Cardiology**
- **Internal Medicine**

*Hypertension*, a highly respected monthly journal, is dedicated to timely publication of original reports of clinical and laboratory investigations in hypertension. Designed for clinicians, clinical investigators, and laboratory scientists.

The American Heart Association is committed to reducing premature death and disability from cardiovascular disease and stroke. As part of this commitment, articles in *Hypertension* are published after extensive peer review. The published science is the highest quality of the submitted articles.

See why *Hypertension* will have significant impact on your continuing education program, as well as on your daily practice.

---

**Subscribe Today!** Mail the coupon below to the American Heart Association, 7320 Greenville Avenue, Dallas, Texas 75231.

1988 Subscription Information Volumes 11-12.

Annually --- subscriptions accepted at any time.

$69 U.S., $95 Elsewhere except Japan and Europe. Postage included. Airmail rates available upon request. Special discount 50% available to research fellows, interns, residents, medical students in U.S., Canada and Mexico. Note: Letter from chairperson stating post and completion date is required to qualify for special discount. Single copies $15 U.S., $18 Elsewhere prepaid. Orders for JAPAN must go through Nankodo Co., Ltd., 42-6, Hongo 3-chome, Bunkyo-ku Tokyo Japan. Orders for EUROPE must go through Harcourt-Brace-Jovanovich, Foots Cray High Street, Sidcup Kent DA14 5HP ENGLAND. Remittances should be made in U.S. funds by check, draft, post office or express money order payable to American Heart Association, 7320 Greenville Avenue, Dallas, Texas 75231.

---

Please Print

Name

Address

City State Zip

Country Telephone

---

Please enter my subscription to Hypertension

New subscriptions will begin with current issue unless otherwise requested

Payment enclosed □ Bill me □
START HIS DAY OUT RIGHT
FOR MANY HYPERTENSIVE PATIENTS
Start with Once-a-Day

VASOTEC®
(Enalapril Maleate | MSD)

Certain CNS effects, such as impairment of memory, nightmares, or depression, have not been characteristic of VASOTEC.

Little or No Interference with Physical or Mental Activity
Certain subjective symptoms such as malaise and drowsiness, which may interfere with physical or mental activity, have not been characteristic of VASOTEC—a fact that may be related to the specificity of action of VASOTEC on the renin-angiotensin-aldosterone system.

VASOTEC is contraindicated in patients who are hypersensitive to this product.
Angioedema of the face, extremities, lips, tongue, glottis, and/or larynx has been reported in patients treated with angiotensin-converting-enzyme (ACE) inhibitors, including VASOTEC (0.2% of patients treated with VASOTEC in clinical trials). In such cases, VASOTEC should be promptly discontinued and the patient carefully observed until the swelling disappears. Angioedema associated with laryngeal edema may be fatal. Where there is involvement of the tongue, glottis, or larynx, likely to cause airway obstruction, appropriate therapy, e.g., subcutaneous epinephrine solution 1:1000 (0.3 mL to 0.5 mL), should be promptly administered.

Excessive hypotension was rarely seen in uncomplicated hypertensive patients but is a possible consequence of enalapril use in severely salt/volume-depleted persons, such as those treated vigorously with diuretics or patients on dialysis. In using VASOTEC, consideration should be given to the fact that another ACE inhibitor, captopril, has caused agranulocytosis, particularly in patients with renal impairment or collagen vascular disease, and the available data are insufficient to show that VASOTEC does not have a similar risk.

For a Brief Summary of Prescribing Information, please see the last page of this advertisement.

It May Change the Way Your Patients Feel on Antihypertensive Therapy
For a Brief Summary of Prescribing Information, please see the last page of this advertisement.
Contraindications: VASOTEC® (enalapril maleate, MSD) is contraindicated in patients who are hypersensitive to enalapril maleate.

Warnings: Angioedema: Angioedema of the face, extremities, lips, tongue, glottis, and/or larynx has been reported in patients treated with ACE inhibitors, including VASOTEC. In such cases, VASOTEC should be discontinued and appropriate supportive therapy provided. Angioedema may be fatal. Where there is involvement of the tongue, glottis, or larynx, cause airway obstruction, appropriate therapy, e.g., subcutaneous epinephrine solution 1:1000 (0.3 mL to 0.5 mL) should be promptly administered. See ADVERSE REACTIONS.

Hypotension: Hypotension was rarely observed in hypervolemic patients but is a possible consequence of enalapril use in severely salt-volume-depleted persons such as those treated vigorously with diuretics or patients on dialysis. See PRECAUTIONS. Drug Interactions: Drug interactions should be considered in patients treated with VASOTEC. See DOSAGE AND ADMINISTRATION.

Precautions: General: Impaired Renal Function: As a consequence of inhibiting the renin-angiotensin system, ACE inhibitors may reduce renal blood flow and precipitate oliguria or anuria, especially in patients with impaired renal function. Therefore, ACE inhibitors should be used with caution in patients with renal failure and/or edema and should be administered with caution in patients treated with diuretics. The dosage of diuretics and ACE inhibitors should be decreased in patients with renal failure. See DOSAGE AND ADMINISTRATION.

Evaluation of the hypertensive patient should always include assessment of renal function. See DOSAGE AND ADMINISTRATION in complete Prescribing Information. See also Drug Interactions.

Hyperkalemia: Elevated serum potassium (greater than 5.7 mEq/l) was observed in approximately 10% of patients in clinical trials in patients treated with VASOTEC. Elevations were usually reversible and discontinuation of enalapril and/or diuretic therapy in such patients, renal function should be monitored during the first few weeks of therapy. Some hypertensive patients with no apparent preexisting renal vascular disease have developed acute renal failure after the administration of enalapril. Renal biopsy in patients with oliguria and/or azotemia, usually irreversible, occurred during therapy with enalapril, especially when VASOTEC has been given concomitantly with a diuretic. This is more likely to occur in patients with preexisting renal impairment. Dosage reduction of VASOTEC and/or discontinuation of the diuretic may be required.

Information for Patients: Angioedema: Angioedema, including laryngeal edema, may occur especially following the first dose of enalapril. Patients should be advised to report immediately any signs or symptoms suggesting angioedema, (swelling of the face, extremities, lips, tongue, difficulty in breathing) and to take no more drug until they have consulted with the prescribing physician. Hypotension: Patients should be counseled to report lightheadedness especially during the first few days of therapy. If actual syncope occurs, the patient should be told to discontinue the drug until they have consulted with the prescribing physician. All patients should be advised that excessive sodium or fluid retention may block angiotensin formation secondary to compensatory renin release. If hypertension occurs and is considered to be due to this mechanism, it can be corrected by volume expansion.

Information for Parents: Angioedema: including laryngeal edema, may occur especially following the first dose of enalapril. Patients should be so advised and told to report immediately any signs or symptoms suggesting angioedema (swelling of the face, extremities, lips, tongue, difficulty in breathing) and to take no more drug until they have consulted with the prescribing physician. Hypotension: Patients should be counseled to report lightheadedness especially during the first few days of therapy. If actual syncope occurs, the patient should be told to discontinue the drug until they have consulted with the prescribing physician. All patients should be advised that excessive sodium or fluid retention may block angiotensin formation secondary to compensatory renin release. If hypertension occurs and is considered to be due to this mechanism, it can be corrected by volume expansion.

Information for Patients: Angioedema: including laryngeal edema, may occur especially following the first dose of enalapril. Patients should be so advised and told to report immediately any signs or symptoms suggesting angioedema (swelling of the face, extremities, lips, tongue, difficulty in breathing) and to take no more drug until they have consulted with the prescribing physician. Hypotension: Patients should be counseled to report lightheadedness especially during the first few days of therapy. If actual syncope occurs, the patient should be told to discontinue the drug until they have consulted with the prescribing physician. All patients should be advised that excessive sodium or fluid retention may block angiotensin formation secondary to compensatory renin release. If hypertension occurs and is considered to be due to this mechanism, it can be corrected by volume expansion.

Other Cardiovascular Agents: VASOTEC® (enalapril maleate, MSD) has been used concomitantly with beta-adrenergic-blocking agents, metyrapone, nitrates, calcium-blocking agents, hydralazine, and prazosin without evidence of clinically significant adverse interactions. Agents Increasing Serum Potassium: VASOTEC may attenuate potassium-sparing diuretics, potassium-sparing agents (e.g., amiloride, triamterene, spironolactone), potassium supplements, or potassium-containing salt substitutes that may lead to significant increases in serum potassium. Therefore, if concomitant use of these agents is indicated, they should be used with caution and with measurement of serum potassium. Pregnancy—Category C: There was no teratogenicity or lethality in rats treated with up to 200 mg/kg/day of enalapril (33 times the maximum human dose). Teratogenicity, expressed as a structural abnormality, was observed in the rat when given 1250 mg/kg/day of enalapril but did not occur when these animals were supplemented with saline. Enalapril was not teratogenic in rabbits. However, maternal and fetal toxicity occurred in some rabbits at doses of 1 mg/kg/day or more (15 times the maximum human dose). Where possible, measures should be taken to discontinue treatment with enalapril at or before the first trimester of pregnancy, especially since many drugs are secreted in human milk. Many experts also advise discontinuation of breastfeeding when a woman is nursing her mother.

Pediatric Use: Safety and effectiveness in children have not been established.

Adverse Reactions: VASOTEC has been evaluated for safety in more than 10,000 patients. VASOTEC has been found to be generally well tolerated in controlled clinical trials involving 2677 patients.

The most frequent clinical adverse experiences in controlled trials were: headache (4.8%), dizziness (4.8%), and fatigue (6.8%). For the most part, adverse effects were mild and transient in nature. Discontinuation of therapy was required in 6.0% of patients. In clinical trials, the overall frequency of adverse experiences was not related to total daily dosage within the range of 10 to 40 mg. The overall percentage of patients treated with VASOTEC reporting adverse experiences was comparable to placebo.

Other adverse experiences occurring in greater than 1% of patients treated with VASOTEC in controlled clinical trials were: diaphoresis (1.6%), rash (1.5%), hypotension (1.4%), cough (1.3%), asthenia (1.1%), and orthostatic effects (1.1%). Clinical adverse experiences occurring in 0.5% to 1% of patients in the controlled trials or since the drug was marketed include: Cardiovascular: Syncope, orthostatic hypotension, palpitations, chest pain.

Nervous System: Somnolence, nervousness, paresthesia, somnolence.

Cardiovascular System: Abdominal pain, angina pectoris, renal dysfunction, renal failure, oliguria.

Dosing: Oral: 1.25 mg once daily. In cases of renal or hepatic dysfunction, dosage should be reduced to 0.625 mg once daily in cases of severe renal dysfunction.

Clinical Laboratory Test Findings: Hyperkalemia: See PRECAUTIONS.

Cholestasis: In controlled clinical trials, minor increases in blood urea nitrogen and serum creatinine, reversible upon discontinuation of therapy, were observed in about 0.2% of patients with essential hypertension treated with VASOTEC alone. Increases were more frequent in patients receiving concomitant diuretics or in patients with renal artery stenosis. See PRECAUTIONS.

Hemoglobin and Hematocrit: Small decreases in hemoglobin and hematocrit (mean decreases of approximately 0.3 g/dl and 1.0 vol% respectively) occurred frequently in hypertensive patients treated with VASOTEC, but were usually of no clinical importance unless accompanied by symptoms of anemia. In clinical trials, less than 0.1% of patients discontinued therapy due to anemia.

Other (Causal Relationship Unknown): Rare elevations of liver enzymes and/or serum bilirubin have occurred. In more extensive experience, rare cases of neutropenia, thrombocytopenia, and bone marrow depression have been reported.

Doseage and Administration: In patients who are currently being treated with a diuretic, symptomatic hypotension occasionally may occur following the initial dose of VASOTEC. The diuretic should be discontinued for at least 48 hours before beginning treatment with VASOTEC to reduce the likelihood of hypotensive symptoms. See PRECAUTIONS. If the patient's blood pressure is not controlled with VASOTEC alone, diuretic therapy may be resumed.

The recommended initial dose in patients not on diuretics is 5 mg once per day. Dose should be adjusted according to blood pressure response. The usual dosage range is 10 to 40 mg per day administered in a single dose or in two divided doses. In some patients treated once daily, the antihypertensive effect may diminish toward the end of the dosing interval. In such patients, an increase in dosage or twice-daily administration should be considered. If blood pressure is not controlled with VASOTEC alone, a diuretic may be added.

Concomitant administration of VASOTEC with potassium supplements, potassium salt substitutes, or potassium-sparing diuretics may lead to increases in serum potassium (see PRECAUTIONS).

Dose Adjustment in Renal Impairment: The usual dose of enalapril is recommended for patients with renal function up to and including up to approximately 3 ml/min. For patients with creatinine clearance ≤ 30 ml/min (serum creatinine = 3 mg/dl), the initial dose is 2.5 mg once per day. The dosage may be titrated upward until blood pressure is controlled or to a maximum of 10 mg once per day. In patients with creatinine clearance of 40 ml/min or greater, the initial dose is 5 mg once per day. The recommended initial dose in patients on hemodialysis is 2.5 mg/day. Dose adjustment should be considered depending on blood pressure response.

For more detailed information, consult your MSD representative or see Prescribing Information: Merck Sharp & Dohme, Division of Merck & Co. Inc., West Point, PA 19486.
The benefit of antianginal protection plus safety...

CARDIZEM®
diltiazem HCl/Marion

A remarkable safety profile
The low incidence of side effects with Cardizem allows patients to feel better.

Protection against angina attacks
The predictable efficacy of Cardizem in stable exertional and vasospastic angina allows patients to do more.

A decrease in myocardial oxygen demand
Resulting from a lowered heart rate-blood pressure product.

Compatible with other antianginals

Safe in angina with coexisting hypertension, COPD, asthma, or PVD

CARDIZEM® (diltiazem HCl) is indicated in the treatment of angina pectoris due to coronary artery spasm and in the management of chronic stable angina (classic effort-associated angina) in patients who cannot tolerate therapy with beta-blockers and/or nitrates or who remain symptomatic despite adequate doses of these agents.

*See Warnings and Precautions

Please see brief summary of prescribing information on the next page.
Brief Summary

Professional Use Information

**CARDIZEM**
(diltiazem HCl) 30 mg, 60 mg, 90 mg, and 120 mg Tablets

**CONTRAINDICATIONS**
Cardiovascular and organic conduction in (1) patients with sick sinus syndrome (except in the presence of a functioning ventricular pacemaker, (2) patients with second- or third-degree AV block (except in the presence of a functioning ventricular pacemaker, and (3) patients with hypotension (less than 90 mm Hg systolic).

**WARNINGS**
1. **Cardiac Conduction.** Cardizem prolongs AV node refractoriness without significantly prolonging sinus node recovery time, except in patients with sick sinus syndrome. This effect may rarely result in abnormally slow heart rates, particularly in patients with sick sinus syndrome or second- or third-degree AV block (six of 1,243 patients for 0.48%). Concomitant use of diltiazem with beta blockers or digoxin may result in additive effects on cardiac conduction. A patient with Prinzmetal's angina developed periods of asystole (2 to 5 seconds) after a single dose of 60 mg of diltiazem.

2. **Congestive Heart Failure.** Although diltiazem has a negative inotropic effect, isolated animal studies, and hemodynamic studies in humans with normal ventricular function have not shown a reduction in cardiac index nor consistent negative effects on contractility (dp/dt). Experience with the use of Cardizem alone or in combination with beta blockers in patients with impaired ventricular function is very limited. Caution should be exercised when using the drug in such patients.

3. **Hypotension.** Decreases in blood pressure associated with Cardizem therapy may occasionally result in symptomatic hypotension.

4. **Acute Hepatic Injury.** In rare instances, significant elevations in enzymes such as alkaline phosphatase, CPK, LDH, SGOT, SGPT, and other symptoms consistent with acute hepatic injury have been noted. These reactions have been reversible upon discontinuation of drug therapy. The relationship to Cardizem is uncertain in most cases, but probable in some. (See PRECAUTIONS.)

**PRECAUTIONS**

General. Cardizem (diltiazem hydrochloride) is extensively metabolized by the liver and excreted by the kidneys and in bile. As with any new drug given over prolonged periods, laboratory parameters should be monitored at regular intervals. The drug should be used with caution in patients with impaired renal or hepatic function. In subjects with angina and rat studies designed to produce toxicity, high doses of diltiazem were associated with hepatic damage, especially subacute hepatic studies of doses of 125 mg/kg and higher in rats were associated with histological changes in the liver which were reversible when the drug was discontinued. In dogs, doses of 20 mg/kg were also associated with hepatic changes, however, these changes were reversible with continued dosing.

**Drug Interaction.** Pharmacological studies indicate that there may be additive effects in prolonging AV conduction when using beta blockers or digoxin concomitantly with Cardizem. (See WARNINGS.) Controlled and uncontrolled domestic studies suggest that concomitant use of Cardizem and beta blockers or digoxin is usually well tolerated. Available data are not sufficient, however, to predict the effects of concomitant treatment, particularly in patients with antiventricular dysfunction or cardic conduction abnormalities. In healthy volunteers, diltiazem has been shown to increase serum digoxin levels up to 20%.

**Carcinogenesis, Mutagenesis, Impairment of Fertility:** A 24-month study in rats and a 21-month study in mice showed no evidence of carcinogenicity. There was also no mutagenic response in in vitro bacterial tests. No intrinsic effect on fertility was observed in rats.

**Pregnancy:** Category C. Reproductive studies have been conducted in mice, rats, and rabbits. Administration of doses ranging from 5 to 10 times greater (on a mg/kg basis) than the daily recommended therapeutic dose has resulted in embryofetal lethality. In dogs, doses of 20 mg/kg resulted in embryofetal lethality. These doses, in some studies, have been reported to cause skeletal abnormalities. In the perinatal/postnatal studies, there was some reduction in early individual pup weights and survival rates. There was an increased incidence of stillbirths or deaths of 20 times the human term in rats.

There are no well-controlled studies in pregnant women. Therefore, use Cardizem in pregnant women only when the potential benefit justifies the potential risk to the fetus.

**Nursing Mothers.** Diltiazem is excreted in human milk. No alternative method of infant feeding should be instituted. (See PRECAUTIONS.)

**Dermatologic:** Petechiae, pruritus, photosensitivity, leukopenia.

**Other:** Amblyopia, dysphoric episodes, eye irritation, hyperpyrexia, nasal congestion, retroauricular pain, polypnea, sexual difficulties.

The following postmarketing events have been reported infrequently in patients receiving Cardizem: aplosia, gingival hyperplasia, erythema multiform, and leukopenia. However, a definitive causal and effect between these events and Cardizem therapy is yet to be established.

**ADVERSE REACTIONS**

Serious adverse reactions have been rare in studies carried out to date, but should be recognized that patients with impaired ventricular function and conduction abnormalities have usually been excluded. In domestic placebo-controlled trials, the incidence of adverse reactions reported during Cardizem therapy was not greater than that reported during placebo therapy. The following represent representative of reported side effects which can be of least reasonably associated with the pharmacology of calcium influx inhibition. In many cases, the relationship to Cardizem has not been established. The most common occurrences, as well as the frequency of presentation are: edema (2.4%); headache (2.1%); nausea (1.9%); diarrhea (1.5%); anorexia (1.3%); asthenia (1.2%); rash (1.1%); urinary tract infection (1.0%); nasal congestion (0.7%); dizziness (0.7%); rhinitis (0.5%); somnolence (0.5%); chest pain (0.5%); urticaria (0.4%); dysgeusia (0.4%); dyspepsia (0.3%); mild elevations of transaminase (0.3%); vomiting, dyspepsia, anorexia, diarrhea, dyspepsia, mild elevations of alkaline phosphatase, SGOT, SGPT, and LDH (see hepatic warnings); worsening weight increase.

**Nervous System:** Amnesia, gastroparesis, hallucinations, insomnia, nervousness, paresthesia, personality change, somnolence, tinnitus, tremor.

**Gastrointestinal:** Anorexia, constipation, diarrhea, dyspepsia, eructation, flatulence, nausea, vomiting, and weight increase.

**Cardiovascular:** Angina, arrhythmias, AV block (first degree), AV block (second or third degree) — see conduction warning), bradycardia, congestive heart failure, flushing, hypotension, palpitations, syncope.

**Renal:** Acute renal failure, oliguria, polyuria.

**Local:** Gastrointestinal: diarrhea, dyspepsia, anorexia, flatulence, nausea, vomiting.

**Other:** Myocardial infarction, spontaneous abortion, failure to thrive, hydrops fetalis, polyuria.

**Dermatologic:** Petechiae, pruritus, photosensitivity, leukopenia.

**Other:** Amblyopia, dysphoric episodes, eye irritation, hyperpyrexia, nasal congestion, retroauricular pain, polypnea, sexual difficulties.

The following postmarketing events have been reported infrequently in patients receiving Cardizem: aplosia, gingival hyperplasia, erythema multiform, and leukopenia. However, a definitive causal and effect between these events and Cardizem therapy is yet to be established.

**References:**
Evaluation of medial hypertrophy in resistance vessels of spontaneously hypertensive rats.
G K Owens, S M Schwartz and M McCanna

Hypertension. 1988;11:198-207
doi: 10.1161/01.HYP.11.2.198

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/11/2/198

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/